MECHANISMS OF ABNORMAL DEVELOPMENT

I. Causes of Abnormal Development in the Embryo

II. Embryonic Development of Malformations

III. Postnatal Developmental Abnormalities

PETER GRUENWALD, M.D.
NEW YORK

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MECHANISMS OF ABNORMAL DEVELOPMENT

I. Causes of Abnormal Development in the Embryo

PETER GRUENWALD, M.D.
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IN RECENT years investigation of abnormal development has gone far beyond the stage in which it consisted mainly of accumulations of descriptions of malformations and speculations as to the probable developmental stages of the anomalies under consideration. It is true that experiments were made long ago with the aim of producing malformations by means of various chemical and physical factors, but the agents were chosen arbitrarily and little of general teratologic significance was gained by this work. Only recent advances in developmental physiology and in genetics, as well as combined work in both fields, have yielded substantial and systematic knowledge of the mechanisms of abnormal development. These will be briefly discussed, and the morphology of malformations will be referred to only as far as is necessary in the course of this discussion of developmental mechanisms.

Many authors have in the past discussed the difficulties of defining malformations. Recent work has increased these difficulties, for it is now known that conditions which are generally recognized as malformations may be produced by a variety of agents which also cause diseases in postnatal life. It is certain that malformations are the result of abnormal development. Since any change of form is development, all abnormal forms might be considered as malformations. There is, for instance, no essential difference between disturbances of early embryonic development caused by chemical agents and, for instance, a tumor forming in the adult after treatment with a carcinogenic chemical. It is important to realize that teratology is a part of pathology not essentially different from the rest.

From the Department of Pathology, Long Island College of Medicine, Brooklyn.

This work was begun while the author was Fellow in Pathology at the Mount Sinai Hospital, New York.

The review of the literature was concluded in August 1946. However, many European journals of the past few years were not available at that time, on account of the interruption of communications during the war.

Knowledge gained in the study of developmental pathology of early stages may offer fundamental points of view for the study of the pathology of the adult, and vice versa. The age at which a disturbance takes place has often been considered as a criterion for malformation, and birth is set as the borderline. This cannot be strictly subscribed to, for identical malformations of teeth, for instance, may develop before and after birth, and typical inflammatory diseases which nobody would regard as malformations have been found in the embryo. No strict definition of a borderline between malformation and disease will be given here, not only because this is impossible but also because it is more profitable to stress the similarities than the differences between the two.

Several authors have discussed broad aspects of teratology in connection with their own descriptive, experimental-embryologic and genetic studies.[1] These reports are good examples of the scope and the success of modern teratology.

There are excellent recent accounts of the sciences which form the basis of developmental pathology. In the field of embryology, particularly in its physiologic aspects, the books of Spemann,[2] Weiss[3] and Needham[4] may be consulted. The last-mentioned author has gathered an impressive amount of the most modern information, including, among others, such borderline fields as embryonic metabolism

1. (*a*) Stockard, C. R.: Am. J. Anat. **28**:116, 1921; (*b*) Am. Naturalist **58**:24, 1924. (*c*) von Szily, A.: Ztschr. f. Anat. u. Entwcklngsgesch. **74**:1, 1924. (*d*) Mohr, O. L.: Ztschr. f. indukt. Abstamm. u. Vererbgsl. **41**:59, 1926; (*e*) Heredity and Disease, New York, W. W. Norton & Company, Inc., 1934. (*f*) Mangold, O.: Ergebn. d. Biol. **7**:193, 1931. (*g*) Wright, S., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1934, vol. 2, p. 137; (*h*) Physiol. Rev. **21**:487, 1941. (*i*) Mann, I. C.: Developmental Anomalies of the Eye, London, Cambridge University Press, 1937. (*j*) Landauer, W.: Proc. Seventh Internat. Genet. Cong. Edinburgh, 1939; (*k*) Bulletin 236, Storrs Agricultural Experiment Station, 1941. (*l*) Murphy, D. P.: Congenital Malformations, Philadelphia, The Author, 1939. (*m*) Dunn, L. C., in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1940, vol. 35, p. 135; (*n*) Growth (supp.) **5**:147, 1941. (*o*) Potter, E. L., and Adair, F. L.: Fetal and Neonatal Death, Chicago, University of Chicago Press, 1940. (*p*) Snell, G. D.: Biology of the Laboratory Mouse, Philadelphia, The Blakiston Company, 1941. (*q*) Hamburger, V.: Biol. Symposia **6**:311, 1942. (*r*) Grüneberg, H.: The Genetics of the Mouse, London, Cambridge University Press, 1943. (*s*) Gruenwald, P.: Am. J. Anat. **74**:217, 1944.

2. Spemann, H.: Embryonic Development and Induction, New Haven, Yale University Press, 1938.

3. Weiss, P.: Principles of Development, New York, Henry Holt & Company, Inc., 1939.

4. Needham, J.: Biochemistry and Morphogenesis, London, Cambridge University Press, 1942.

and the cancer problem. In the field of genetics, there is, in addition to the standard texts, Grüneberg's [1r] book on the genetics of the mouse, which contains much of the best founded teratologic information now at hand.

The following example will be given to illustrate the changes that have been made in the approach to teratology during the past few decades. Cyclopia, one of the best studied single problems of this field, was first examined with regard to its morphologic aspects, and a series of degrees of the defect was established. It became apparent that the obvious ocular changes are but a part of a more extensive and complex defect, and it was logical to include lesser degrees of the malformation, in which the eyes were not quite fused (synophthalmia). The next step was to trace back the defects of the eyes and the brain to the stage of the flat, open neural plate. By a process comparable to showing a motion picture backward, the tissues of various parts of the eye and the brain were followed back to their position in the neural plate (to a stage in which they are not yet differentiated), and in this map of prospective retina, optic nerve, chiasma and other structures the cyclopic defects were outlined. It was thus shown that the missing structures are comprised in a wedge-shaped symmetric area.[5] The conclusion was reached that a wedge-shaped defect of the early embryo comprising as its most important part portions of the neural plate but also some of the underlying tissue causes cyclopia. Nothing could be said of the nature of this defect beyond the assumption that it never is an open cleft, as if tissue had been cut out, but is rather an absence of parts such that the bordering eye-forming tissue of the two sides develops as one fused mass from the very beginning. By this time experiments had been performed in which cyclopia was produced by treating embryos of various classes of vertebrates with chemicals.[6] The treatment was applied to the whole embryo at early stages, and the mechanism of action was not understood. Destruction of tissue in the aforementioned wedge-shaped area by mechanical means,[7] radiation [8] or electrocautery [8a] was shown to produce cyclopia, but it soon became clear that this was not the mechanism of spontaneous or of chemically produced cyclopia. A new con-

5. Fischel, A.: Arch. f. Entwcklngsmechn. d. Organ. **49**:383, 1921.

6. (*a*) Stockard, C. R.: J. Exper. Zool. **4**:165, 1907; (*b*) Arch. f. Entwcklngsmechn. d. Organ. **23**:249, 1907; (*c*) Anat. Rec. **3**:167, 1909. (*d*) McClendon, J. F.: Am. J. Physiol. **29**:289, 1912. (*e*) Werber, E. F.: J. Exper. Zool. **21**:485, 1916. (*f*) LePlat, G.: Arch. de biol. **30**:231, 1919.

7. Lewis, W. H.: Anat. Rec. **3**:175, 1909.

8. Wolff, E.: Arch. d'anat., d'histol. et d'embryol. (*a*) **18**:145, 1934; (*b*) **18**:229, 1934; (*c*) **22**:1, 1936.

cept stems from experiments made in amphibians. It had long been known that in the early embryo the mesoderm of the notochord and nearby areas acts on the covering ectoderm and induces it to form the neural plate. Adelmann[9] and Mangold[10] were the first to show that median defects produced by excision in the mesoderm of the head caused, apparently also by way of induction, a change in the determinations within the neural plate which had been present in normal size and shape at the time of operation. This neural plate, without being directly affected by the experiment, as shown by controls, formed a cyclopic brain and eye. Thus, not the absence of tissue but abnormal determinations of its prospective parts caused cyclopia. Since induction and thus determination of one part by an adjacent tissue (in this case the mesoderm) are known to be mediated by chemicals released by the latter, one can understand that artificially introduced chemicals may interfere with this process and thus cause cyclopia and in a similar manner other malformations.

Several examples of hereditary median defects, including cyclopia, have been found.[11] This is just one of many instances in which similar if not identical malformations result from hereditary and environmental influences. An inkling of the direction in which the solution of this parallelism is to be sought is given by the increasing volume of evidence that genes act by determining the presence of enzymes and that mutations primarily affect the course of metabolism.[12] Thus the primitive idea of elimination of parts evidently missing in cyclopia has been supplanted by a physiologic concept which is far from complete, but stimulates progress. The formation of cyclopia will be discussed in more detail in part II of this review.

As was indicated by the introductory remarks and the preceding example, the present review will deal with the causes of abnormal development and with the mechanisms which follow the causes and produce the abnormal conditions commonly seen. It has already been stressed that there is no natural borderline between malformation and morbid disturbance of structure. The arbitrary limitation to embryonic disturbances which many writers have imposed on teratology is justified only so far as the principal structural pattern of the organism is determined and produced in the embryo. It is obvious that the most striking and far reaching structural abnormalities must develop in

9. Adelmann, H. B.: J. Exper. Zool. **57**:223, 1930.

10. Footnote deleted by the author.

11. Wright, S., and Eaton, O. N.: J. Agric. Research **26**:161, 1923. Wright, S.: Genetics **19**:471, 1934. Wright, S., and Wagner, K.: Am. J. Anat. **54**:383, 1934.

12. Beadle, J. W., in Harvey Lectures, Lancaster, Science Press, 1945, vol. 40, p. 179; Am. Scientist **34**:31, 1946.

these early stages. For this technical reason (and it should be realized that there is no other) the greater part of this review will also deal with the embryo. Since the close relationship of fetal diseases, such as infection, with malformations is becoming increasingly evident, fetal diseases will also be discussed. In all instances attention will be directed toward representative examples rather than complete listings of references.

The present review will appear in three parts. The first will deal with the causes of abnormal development, the second with the developmental mechanisms of embryonic malformations, and the third with examples of conditions in postnatal life which have been adequately analyzed along similar lines, including a brief discussion of the developmental aspects of tumors. Since the causes of most spontaneous developmental aberrations are not known, the second and third parts will have to treat of many conditions which have as yet not been traced to any of the causes described in the first part.

THE CAUSES OF ABNORMAL DEVELOPMENT

If one were to be exact in defining the cause of any particular malformation, it would be necessary to trace the abnormality back to the point where an external force produced the first aberration of any kind from the normal condition. In the case of a hereditary abnormality this means going back through all generations carrying the abnormal gene, to find the external cause of the mutation. This is obviously impossible with the so-called spontaneous mutations, even though one may assume that they, just as those induced in the laboratory, have external causes and are therefore not truly spontaneous in the last analysis. Even in the case of a nonhereditary malformation, in which the cause must have acted on the individual itself, investigators are usually unable to find that cause later on unless they have produced the malformation in the laboratory. This illustrates the value of the experimental production of developmental disturbances, as they are the only ones in which the mechanism can be discovered with any degree of dependability. They are models which show how similar malformations of unknown causes may have developed. However, greatest caution must be observed in applying experimental results to malformations found in nature. The previously quoted example of cyclopia shows that many different causes and even entirely different developmental mechanisms may produce very similar end results. Many more examples of this will be quoted in the following pages.

The causes of abnormal development to be discussed here may be classified as follows:

Genetic causes (including influences on the germ cells probably affecting the genotype):

- Mutations with unknown causes ("spontaneous")
- Mutations induced by { radiation / chemical treatment }
- Hybridization
- Overripeness of the egg cell
- Somatic mutations

Agents affecting the phenotype without effect on the genotype:

- Mechanical agents
- Radiations
- Chemical influences { addition of substances / deficiencies }
- Temperature changes
- Infections

The spatial relation of the causative agent to the affected part has been discussed by Gruenwald.[18] Three possibilities exist for the location of the agent: It may be (*a*) within the primarily affected part, (*b*) within the organism but outside the primarily affected part or (*c*) outside the organism.

The first-mentioned relationship prevails in genetically determined malformations and includes also normal properties of the part in question, which make it more susceptible to extrinsic influences. The second possibility exists if abnormal, or rarely normal, conditions elsewhere in the body cause or favor maldevelopment. The third possibility includes the chemical, physical and infectious agents to be discussed in later sections. It should be noted that this classification includes, in addition to frank teratogenic agents, conditions which are not harmful in themselves but which predispose a given part to abnormal development. No sharp distinction of these two types of agents or conditions is possible. In some cases each is ineffective without the other.[18]

GENETIC CAUSES OF MALDEVELOPMENT

In the case of a typical hereditary malformation an abnormal gene appears by mutation with or without an apparent cause. This gene, in homozygous or heterozygous [13] condition, alone or in combination with other genes or with extrinsic agents, causes abnormal development. The long history of attempts to modify the genetic constitution

13. In the homozygous condition the corresponding places in the two sets of chromosomes of each body cell are occupied by genes of the same quality; in the heterozygous state these two places are occupied by alleles which affect the same traits in different manners, and of which either the normal or the abnormal one may dominate over the other.

will not be reviewed. The problem of the inheritance of acquired properties is still unsolved, as is the related problem of the manner in which species are modified in nature and new species formed. There is, however, unquestionable evidence of the existence of agents by which mutations and consecutive hereditary malformations can be produced. It is generally acknowledged that the genes are located in the chromosomes, which undergo regular and complicated changes during mitotic division of the cell. It is therefore not surprising that agents which are known to have a profound influence on mitosis, namely, certain radiations,[14] are also most potent in the experimental production of mutations. Ultraviolet and roentgen rays have been used extensively in the study of mutations of such primitive organisms as bacteria and fungi,[15] and much of the recent fundamental knowledge of the manner in which genes act on metabolism is derived from this work.

An interesting group of investigations made in amphibians will be mentioned here because the effect is transmitted to the embryo by one of the germ cells, even though it is not certain that the effect is of a genetic nature. Beginning many years ago, numerous workers [16] have irradiated male or female germ cells previous to fertilization and have reported early death of the embryo or, if suitable doses were used, various severe malformations. Among these are abnormalities of gastrulation and defects of the brain and eyes. It was at first surprising that severe irradiation of spermia is followed by normal development. This happens because the chromatin of the spermia is so severely damaged that it does not take part in further development. However, the spermia are still motile and stimulate the egg cells to develop, in a parthenogenetic manner as far as the chromatin is concerned. Accordingly, the embryo has the haploid number of chromosomes, derived entirely from the egg cell.[17] Henshaw [18] confirmed these results and found, in addition to deformities of organs, anaplastic papillomatous growth of the ectoderm, similar to that resulting from development of overripe eggs (see page 407) and other experimental procedures.

The possibility of producing mutations in mammals by the action of roentgen rays has been examined in great detail. Irradiated male mice

14. Politzer, G., in Chambers, R., and others: Protoplasma-Monographien, Berlin, Verlagsbuchhandlung Gebrüder Borntraeger, 1934, vol. 7.

15. Gray, C. H., and Tatum, E. L.: Proc. Nat. Acad. Sc. **30**:404, 1944. Beadle.[12]

16. (*a*) McGregor, J. H.: Science **27**:445, 1908. (*b*) Bardeen, C. R.: Am. J. Anat. **11**:419, 1911. (*c*) Hertwig, O.: Arch. f. mikr. Anat. **77**:1, 1911; (*d*) **77**:165, 1911; (*e*) (supp.) Anat. Anz. **54**:94, 1921. (*f*) Rugh, R.: Proc. Am. Philos. Soc. **81**:447, 1939.

17. Hertwig.[16e] Rugh.[16f]

18. Henshaw, P. S.: J. Nat. Cancer Inst. **3**:409, 1943.

had offspring with hereditary malformations of brain, eyes, face, extremities and urogenital tract. These abnormalities have been followed through many generations.[19] Their embryologic aspects will be described in part II. The causal relationship between irradiation and the origin of this mutation has recently been questioned,[1r] but there are other instances, involving mostly malformations of the brain, in which the hypothesis that the mutation is caused by roentgen rays is more probably true.[20]

This work has raised the question whether or not therapeutic irradiation of the ovaries or the testes of man can be the cause of abnormal offspring by producing mutations. This has been discussed extensively by geneticists as well as by clinicians. It is obvious that damage to the offspring by mutations induced in the parental germ cells must be clearly distinguished from noninherited damage due to the embryos having been irradiated in utero. The former may not be apparent until two or more generations hence. The latter, which is definitely known to produce malformations, will be referred to in a subsequent section.

There is no direct proof that irradiation of the maternal ovaries preceding conception produces malformations in the offspring. A review of 265 cases [21] showed 5 per cent defective children, but it was pointed out that these small numbers of defects not conforming to any definite pattern of maldevelopment may well be due to other influences of the environment of the fetus, since the mothers had some pathologic conditions requiring roentgen therapy. Another writer [22] asserts with questionable logic that there is no danger for pregnancies following therapeutic irradiation, even though he has found an increased incidence of abortions as well as of retarded development of the children in later years. The possibility of roentgen ray damage of the internal genital organs of the mother with the result that the development of the ovum may be interfered with must also be taken into consideration.[23] Several authors have reviewed animal experiments, some of which were referred to in foregoing paragraphs, and have concluded that damage of the genetic constitution of the immature egg cells of the ovary is improbable if not impossible.[24] Statistical evaluation of the probability of mutations led to similar conclusions.[25] However, several authors

19. Little, C. C., and Bagg, H. J.: J. Exper. Zool. **41**:45, 1924, Little, C. C.: Am. Naturalist **65**:370, 1931.

20. (*a*) Snell, G. D.; Bodemann, E., and Hollander, W.: J. Exper. Zool. **67**:93, 1934. (*b*) Snell, G. D., and Picken, D. I.: J. Genet. **31**:213, 1935. (*c*) Snell, G. D.: Radiology **36**:189, 1941.

21. Murphy, D. P.: Surg., Gynec. & Obst. **48**:766, 1929.

22. Werner, P.: München. med. Wchnschr. **68**:767, 1921.

23. Borak, J.: Arch. f. Gynäk. **147**:304, 1931.

24. Murphy, D. P.: Surg., Gynec. & Obst. **47**:201, 1928. Borak.[23]

25. Peller, S.: Arch. f. Gynäk. **147**:360, 1931.

recommend caution and protection of the gonads from unnecessary irradiation even though the probability of ill effects may be small.[26a] Schubert [26d] points out in this connection that in the production of mutations only the total dose counts, regardless of the size and intervals of the single doses, whereas Müller [26b] gives a maximum daily dose which he considers safe. No objection to diagnostic doses as used for roentgenograms has so far been raised. Snell [20c] holds that while gene mutations (affecting single genes) are not to be expected, chromosome mutations, such as translocation of larger portions of a chromosome, are far more probable. He agrees with other writers that the only proved instance of damage of the offspring due to irradiation of the germ cells is that of damage caused by treatment of mature sperm cells. It thus appears to be the opinion of most authors that while maldevelopment has been proved to occur only when mature sperm cells have been irradiated shortly before fertilization, greatest caution should be exerted and the gonads of persons in the reproductive age protected from roentgen rays. In the male the typical irradiation effect consists of a transient period of sterility, which does not immediately follow the treatment. Only fertilization occurring during the period between treatment and this temporary sterility is definitely known to produce defective offspring.[27]

Little conclusive work has been reported on hereditary changes produced by methods other than irradiation. There are a few reports on the offspring of animals treated with chemical agents. They are important from the medical point of view because they concern substances to which human beings may be exposed. Stockard and his coworkers [28] describe in the descendants of alcoholized guinea-pigs a hereditary inferiority resulting in a reduced number of offspring and early death of many young. Pearl [29] exposed chickens to vapors of ethyl alcohol, methyl alcohol or ether and found a reduction in the number of fertile eggs but a lower prenatal and postnatal mortality of the chickens derived from fertile eggs as compared with his controls. There was also a higher mean adult body weight, and no increase in the incidence of malformations. In order to explain the discrepancy of his own results and those of Stockard and others not mentioned

26. (*a*) Murphy, D. P., and Goldstein, L.: Am. J. Roentgenol. **22**:207, 1929. (*b*) Müller, J. H.: Monatschr. f. Geburtsh. u. Gynäk. **109**:105, 1939. (*c*) Muller, H. J.: Nature, London **147**:718, 1941. (*d*) Schubert, G.: Röntgenpraxis **13**:1, 1941.

27. Borak.[23] Snell.[20c]

28. Stockard, C. R., and Craig, D. M.: Arch. f. Entwcklngsmechn. d. Organ. **35**:569, 1912. Stockard, C. R., and Papanicolaou, G. H.: Am. Naturalist **50**:144, 1916.

29. Pearl, R.: J. Exper. Zool. **22**:241, 1917.

here, Pearl proposes the following hypothesis: The germ cells of a given species differ in their capacity to produce normal, sturdy offspring and also in their resistance to damage caused by alcohol or other means. These two properties are coupled so that in some species and under certain conditions alcohol will kill those cells which would otherwise produce weak offspring and leave the others unharmed, and in other species alcohol will also damage the remaining cells. The former would explain Pearl's own results with chickens, and the latter, Stockard's findings in guinea pigs. In this manner the seemingly conflicting results may be explained. In a more recent review of the subject, P. Hertwig [30] cites many articles accepting or denying an effect of alcohol on the progeny, and concludes that the final answer has yet to be found.

Landauer [31] published a preliminary report on the offspring of cocks treated with thallium. There was a high mortality during a narrowly limited period of time within the first two weeks after hatching, which varied slightly with the intensity of the fathers' treatment. These experiments have not been continued on a satisfactory scale, and Landauer himself [32] considers his results as significant but not conclusive.

The offspring of guinea pigs affected by lead poisoning have been examined.[33] They showed a reduced birth weight, an increased death rate during the first postnatal week and general retardation of development. When the lead treatment ends, the gonads recover, and the new progeny is normal. There is no mention of offspring of the retarded young.

Somewhat doubtful is the interpretation of hereditary malformations of the eye, of the progeny of rabbits treated with the serum of fowls immunized to lens tissue.[34] The malformations in later generations include not only opacity of the lens but also microphthalmia with defects of other parts of the eyes. In the progeny of chickens treated with naphthalene or alcohol, cataract and coloboma have been described.[35]

The effect of overripeness of the egg cell on development should be mentioned at this point, even though there is no conclusive evidence that it is due to changes in the genetic structure. However, the effect is transmitted to the embryo by one of the germ cells, just as were the radiation effects in some of the aforementioned experiments on germ cells. Overripeness of the egg at the time of fertilization has been studied in several classes of vertebrates and found to be associated

30. Hertwig, P.: Jahresk. f. ärztl. Fortbild. **26**:50, 1935.

31. Landauer, W.: Arch. f. Gewerbepath. u. Gewerbehyg. **1**:791, 1931.

32. Landauer, W.: Personal communication to the author.

33. Weller, C. V.: J. M. Research **28**:271, 1915.

34. Guyer, M. F., and Smith, E. A.: J. Exper. Zool. **31**:171, 1920. Davis, F. A.: Tr. Ophth. Soc. U. Kingdom (pt. 2) **45**:555, 1925.

35. Kusagawa, S.: Arch. f. Ophth. **118**:401, 1927.

with a reduction in the number of offspring and a variety of developmental disturbances. In the trout various malformations, including double monsters, were found, as well as a change of the sex ratio in favor of males.[36] The latter has been explained by transformation of some genetic females, due to a somatic cause. Similar sex changes in frogs developing from overripe eggs were studied in detail by Witschi.[37] Various malformations, including duplicitas, were also found in them.[38] As a severe form of this disturbance, entirely unorganized growth was observed which, when transplanted to normal tadpoles, grew in the manner of a cancer.[39] Other investigators do not assume a true cancerous nature, pointing out that only in weak hosts aggressive growth occurs.[40] In the guinea pig and the rat a reduced number of embryos, frequent death in utero, and malformations follow delayed insemination.[41] No normal development occurs in the guinea pig if fertilization is delayed more than twenty hours after ovulation, and no development at all if the interval is longer than thirty-two hours. In the rat the limit for normal development of at least part of the embryos is fertilization ten hours after ovulation. In most mammals overripeness of the egg is normally prevented, as the female admits the male only during estrus, i. e., at the optimal time. Nothing is known to suggest the occurrence of malformations due to overripeness in man, in whom no such protective mechanism exists.

Another probable instance of genetically controlled malformations has been reported but not sufficiently worked out. Loeb [42] and Newman [43] found that eggs of the fish Fundulus when fertilized with spermia of other fish species yield large numbers of various malformations. These malformations resemble those Werber obtained by chemical treatment of normally fertilized eggs (page 417) and include, among others, double monsters, cyclopia and various other defects of large parts of the body, leaving in some instances only isolated eyes or hearts. Loeb is inclined to assume that in these cases the eggs develop parthenogenetically as far as their sets of chromosomes are concerned—in other words, that the chromosomes of the spermia are

36. Mršić, W.: Arch. f. mikr. Anat. u. Entwcklngsmechn. **98**:129, 1923.

37. Witschi, E.: Arch. f. Entwcklngsmechn. d. Organ. **102**:168, 1924

38. Witschi, E.: Proc. Soc. Exper. Biol. & Med. **31**:419, 1934. Zimmerman, L., and Rugh, R.: J. Morphol. **68**:329, 1941.

39. Witschi, E.: Proc. Soc. Exper. Biol. & Med. **27**:475, 1930.

40. Briggs, R. W.: Anat. Rec. **81**:121, 1941. Briggs, R. W., and Berrill, N. J.: Growth **5**:273, 1941.

41. Blandau, R., and Young, W.: Am. J. Anat. **64**:303, 1939. Blandau, R. J., and Jordan, E. S.: ibid. **68**:275, 1941.

42. Loeb, J.: J. Morphol. **23**:1, 1912; Biol. Bull. **29**:50, 1915.

43. Newman, H. H.: Biol. Bull. **32**:306, 1917.

lost. In support of this he shows that similar malformations can be obtained from normally fertilized eggs by various external influences. However, it was mentioned in a preceding paragraph that if spermia damaged by various agents [16] or derived from a different species [44] merely induce parthenogenesis, the incidence of malformations is not unusually high. Further investigation of this problem should be of great interest.

Numerous hereditary malformations of unknown cause have been found and extensively studied in the breeding of laboratory and domestic animals. Several of these have been investigated embryologically, with highly gratifying results, as will be reported in part II.

The details of the genetic mechanisms involved will not be discussed here, as such an excursion would lead far into the field of genetics. Several reviews of various aspects of the subject are available.[45] Many of the well studied hereditary malformations are transmitted by single factors, the abnormal trait being either dominant or recessive, or having a different expression in homozygous and heterozygous form. A large number of hereditary traits are known which in the homozygous condition interfere with life beyond embryonic or early postnatal periods, and the genes producing these severe malformations in homozygous individuals are called lethal factors. The embryos carrying this condition and destined to die before reaching maturity should, according to Cairns,[46] be called prothanic rather than lethal. The best studied example is the Creeper chick which will be discussed later in more detail.

The expression of single genetic factors may be modified by other genetic [47] or by environmental [48] factors. Some of these modifications increase the severity of the defect. There is, for examples a cumulation of severity of malformations if Creeper chick embryos develop under the influence of Selenium intoxication.[49] Other instances are known in which the embryo may benefit from the action of additional influences. Remarkable among these are: changed uterine environment in an early lethal malformation of the mouse,[50] temporary lowering of

44. Rugh, R., and Exner, F.: Proc. Am. Philos. Soc. **83**:607, 1940.

45. (*a*) Mohr.[1e] (*b*) Snyder, L. H.: Medical Genetics, Durham, N. C., Duke University Press, 1941; (*c*) Am. Naturalist **76**:129, 1942. (*d*) Baur, E.; Fischer, E., and Lenz, F.: Human Heredity, New York, The Macmillan Company, 1931.

46. Cairns, J. M.: J. Exper. Zool. **88**:481, 1941.

47. (*a*) Reed, S. C., and Snell, G. D.: Anat. Rec. **51**:43, 1931. (*b*) Reed, S. C.: Genetics **21**:361, 1936. (*c*) Dunn.[1n] (*d*) Grüneberg.[1r] (*e*) Dunn, L. C., and Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **31**:82, 1945.

48. Dunn, L. C.; Gluecksohn-Schoenheimer, S.; Curtis, M. R., and Dunning, W. F.: J. Hered. **33**:65, 1942. Dunn.[1n] Grüneberg.[1r]

49. Landauer, W.: J. Exper. Zool. **83**:431, 1940.

50. Robertson, G. G.: Genetics **27**:166, 1942.

the incubation temperature [51] and possibly also increased oxygen supply [46] in the case of the homozygous Creeper chick. Similarly, the development of hereditary polydactyly in chickens may be suppressed by lowering the temperature at incubation.[52] However, no influence of lowered temperature was found in several other hereditary malformations of the chick.[53]

Another noteworthy fact is that identical or very similar abnormalities are produced by mutations occurring at different points of the pattern of genes.[54] Finally one should mention the peculiar effect which results if an abnormal genetic factor is located in the sex chromosome. The possibilities for the expression of this factor will then differ according to sex, and this has been termed sex-linked inheritance. Many examples have been described in human and animal pathology—for example, hemophilia and certain forms of color blindness. For the details of this hereditary mechanism textbooks of genetics should be consulted.

The mechanism of gene action as observed during the development of an individual and throughout life is not well understood. In some instances it appears that the presence of enzymes is governed by genes, and genetically controlled enzyme deficiencies are followed by accumulation or excretion of intermediary products which cannot be metabolized. This has been studied in detail in fungi, and similar conditions seem to be present in human alkaptonuria and phenylketonuria,[12] lipid storage diseases [55] and glycogen storage disease.[56] Structural abnormalities are not readily explained by this mechanism. Only in the case of gargoylism (lipochondrodystrophy) it has been suggested that the disturbances in skeletal development are caused by accumulations of an as yet unidentified substance in the cartilage cells.[57] In many malformations the gene action is supposed to affect the rate of metabolism and of growth at a definite stage of development,[4] interfering primarily with those parts which grow most actively at that moment.[1a] The problem of multiple ("pleiotropic") gene action will be discussed in part II of this review.

In the preceding pages only those malformations have been considered which are caused by the action of abnormal genes. There

51. Landauer, W.: Science **100**:553, 1944.

52. Sturkie, P. D.: Genetics **27**:172, 1942; J. Exper. Zool. **93**:325, 1943.

53. Sturkie, P. D.: Am. Naturalist **79**:286, 1945.

54. Dunn, L. C., and Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **30**:173, 1944.

55. Sobotka, H.; Glick, D.; Reiner, M., and Tuchman, L.: Biochem. J. **27**:203, 1933. Sobotka, H.: J. Mt. Sinai Hosp. **9**:795, 1942.

56. Bridge, E. M., and Holt, L. E.: J. Pediat. **27**:299, 1945.

57. Schmidt, M. B.: Centralbl. f. allg. Path. u. path. Anat. **79**:113, 1942.

are, however, a few conditions in which abnormalities develop through unfavorable constellations of genes which are normal in themselves. This is the case in certain instances of genetic intersexuality.[58] While the intersexuality which occurs in some breeds can be accounted for by gene mutations,[59] that in others has been produced by abnormal combinations of normal genes. There are not only polyploid individuals (in lower species of animals) in which abnormal numbers and proportions of normal male and female determining factors can produce abnormal sex development by their interaction, but also diploid individuals in which intersexuality has been found to be due to a faulty quantitative relationship of sex determining factors.[60] This has been studied extensively by Goldschmidt [61] in crosses of various local races of the gypsy moth, Lymantria dispar. It was found that some of these races differ in the quantitative effect of their male and female determining factors. Within each race the strengths of these factors are so matched that a sufficient preponderance of one sex is present to assure normal sex development. In crosses, however, this preponderance may be insufficient if male and female factors come from races with factors of different strength. Thus, in an individual which, according to the number of its sex chromosomes, should be female, the male factors may be relatively too strong, or vice versa, and intersexuality will result. The two opposing views on the manner in which intersexual traits develop in these individuals during embryonic life will be discussed in part II. It must be emphasized that in most species the strengths of the sex factors do not vary among races and, consequently, the overwhelming majority of interracial crosses will not result in intersexuality. There are few examples of intersexuality appearing in interracial crosses in vertebrates, and these have not been fully analyzed from the genetic angle. If this analysis could be undertaken, these instances might well turn out to be due to mutations rather than to an abnormal combination of normal factors as was just discussed.

In another instance of developmental disturbances caused by normal genes the mechanism is entirely different. It is not an interaction of ill matched genes during the development of the traits which they

58. The term "intersexuality" was used by Goldschmidt [61] to indicate those abnormal sexual conditions which result from sex reversal during development (see part II of this review). This theory is not generally accepted. The term is now frequently used to indicate sexual intergrades of various kinds.

59. (*a*) Bonnevie, K.: Arch. f. Entwcklngsmechn. d. Organ. **106**:611, 1925. (*b*) Riddle, O.; Dunham, H. H., and Schooley, J. P.: Genetics **27**:165, 1942. (*c*) Eaton, O. N.: ibid. **30**:51, 1945.

60. Bridges, C. B., in Allen, Danforth and Doisy,[64] p. 15.

61. Goldschmidt, R.: Die sexuellen Zwischenstufen, Berlin, Julius Springer, 1931.

determine, but an effect of incompatibility of these traits themselves after they have developed. This is the case when an embryo of an Rh-positive blood group sensitizes the mother [62] whose blood does not contain Rh factor. As a rule, the embryo develops undisturbed unless the mother has previously been sensitized by Rh-positive substance, usually in the course of an earlier pregnancy, and has developed antibodies. These, transmitted to the embryo through the placenta, produce deleterious effects, the best known of which is the so-called erythroblastosis fetalis. Details of the ill effects of this constellation on the embryo will be described later. Other blood group incompatibilities between mother and fetus account for a small percentage of cases of erythroblastosis.[63]

There is a controversial and possibly highly important genetic cause of localized developmental abnormalities, namely, somatic mutation. This means that during the development of the embryo a mutation arises in one cell and is transmitted only to the descendants of that cell, which are thus genetically different from all other cells of the body. Most striking are those cases in which this appears to have happened at the first division of the fertilized egg cell, and one half of the body differs from the other in some genetically determined character. Examples are gynandromorphism,[63] unilateral gigantism [65] and unilateral pigmentary anomalies.[66] In arthropods genetic mosaics of various kinds are well known.[67] Another field in which somatic mutations have been taken into consideration is that of cancer.[68] This will be referred to in part III of this review.

ENVIRONMENTAL CAUSES OF MALDEVELOPMENT

It is obvious that abnormal development can be induced by any number of physical or chemical agents which will damage but not kill

62. Levine, P.; Burnham, E.; Katzin, E. M., and Vogel, P.: Am. J. Obst. & Gynec. **42**:925, 1941. Levine, P.: J. Pediat. **23**:656, 1943. Davidsohn, I.: J. A. M. A. **127**:633, 1945.

63. Polayes, S. H.: Am. J. Dis. Child. **69**:99, 1945.

64. Allen, E.; Danforth, C. H., and Doisy, E. A.: Sex and Internal Secretions, Baltimore, Williams & Wilkins Company, 1939.

65. (*a*) Mohr.[1e] (*b*) Hollander, W. F.: Quart. Rev. Biol. **19**:285, 1944. (*c*) Warren, D. C.: J. Hered. **36**:227, 1945. (*d*) Wartenberg, R.: Arch. Neurol. & Psychiat. **54**:75, 1945. (*e*) Zondek, L. H.: Arch. Dis. Childhood **20**:35, 1945. (*f*) Rugel, S. J.: Am. J. Dis. Child. **71**:530, 1946.

66. Zlotnokoff, M.: J. Hered. **36**:163, 1945. Glass, B.: ibid. **36**:192, 1945.

67. Goldschmidt.[61] Mohr.[1e]

68. (*a*) Furth, J.; Boon, M. C., and Kaliss, N.: Cancer Research **4**:1, 1944. (*b*) Furth, J., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1944, vol. 6, p. 25. (*c*) Strong, L. C.: Arch. Path. **39**:232, 1945; Yale J. Biol. & Med. **18**:359, 1946.

the embryo. No attempt will be made here to review the large amount of older work in which various agents were applied to embryos at random and without regard to the mechanism of action. Great caution must be exerted in the evaluation of such work, because unknown accidental factors may be more potent than those intended to act. An example of this is the extensive work of Ferret,[69] who found that opening the shell of the hen's egg or manipulating the albumin of the egg has a profound influence on the embryo. Innumerable reports of experimental work with chick embryos have appeared since, and the necessary manipulations of the shell, of the albumin or even of the embryo itself have not produced the severe changes described by Ferret. On the other hand, workers in this field are often faced with the occurrence of numerous severe malformations in their material without an apparent cause. Shaking the eggs while they are being shipped to the laboratory has often been indicated and is certainly not without effect if it reaches a certain intensity. A few other causative agents have tentatively been identified—for example, fumes of a laboratory [70] or jarring.[71]

Mechanical Agents.—Displacement or destruction of parts of the embryo may be produced mechanically in accidental injuries or in surgical experiments. Comparable destructions are produced when parts of embryos are destroyed by chemical action or radiation, in contrast to true chemical or actinic action on development in which the affected tissue survives and shows the effect of the agent. Thus, electrolysis and roentgen rays have been used extensively in experimental embryology and teratology in order to eliminate certain tissues. The procedure is less hazardous than mechanical excision, and its effect on further development is essentially the same. Many of the common severe malformations, such as cyclopia or that of the sirenomelus, have been reproduced in chick embryos by localized roentgen ray destruction.[8c]

There are other kinds of mechanisms interfering with development in which the action is not so obvious. Numerous reports on invertebrates and lower vertebrates centrifuged early in their development will not be reviewed. A recent analysis of the literature and original work [72] relating to frogs revealed that at the early blastopore stage certrifugation is most effective in the production of malformations, and the optimum speed is about 2,500 revolutions per minute. Higher speeds cause early death. The malformations are varied and include, among

69. Ferret, P. E.: Arch. d'anat. micr. **7**:1, 1904.

70. Stockard, C. R.: Anat. Rec. **8**:33, 1914.

71. Stiles, K. A., and Watterson, R. L.: Anat. Rec. **70**:7, 1937.

72. Torrey, T. W., and Breneman, W. R.: Proc. Indiana Acad. Sc. **50**:213, 1941.

others, cyclopia and other defects of the head region, duplication of the caudal portion of the body and persistent open blastopore. The authors believe that dislocation of a portion of the organization center causes the defects of the head and the duplication of caudal parts.

An investigation of the effect of jarring on chick embryos [71] was precipitated by an unexplained increase in the occurrence of certain severe malformations in a laboratory in which other activities were also carried out. These malformations, including platyneuria (a peculiar form of nonclosure of the neural plate) and absence of yolk sac circulation, causing death, were reproduced by making a weight strike the table with the incubator many times in rapid succession during the early hours of incubation. In another series of experiments mechanical shaking of hen's eggs prior to incubation increased the incidence of all types of malformations, notably of "accidental" rumplessness.[78] In this case a combination of the mechanical factor and a genetic one was revealed, as the incidence of rumplessness was increased particularly in the offspring of those hens which were known to produce occasional rumpless chickens even without shaking.

In man and other mammals intrauterine development reduces the occurrence of mechanically caused malformations to a minimum. In the early phases of teratology, mechanical explanations were favored for almost all malformations and a narrow amnion or amniotic bands and adhesions were commonly indicated as the cause. Today investigators are more critical, and the incidence of these factors is found to be low when one relies on positive criteria, such as the demonstrable presence of amniotic bands and not just uncharacteristic furrows and irregular defects.[74] Moreover, some of the intrauterine "amputations" are known to be inherited in almost identical form,[75] which disproves an amniotic band causation. However, the occasional occurrence of malformations due to narrowing of the amniotic cavity (oligohydramnios) or to constriction of amniotic bands has been demonstrated beyond doubt.[74] That amniotic adhesions may be not accidental but sequelæ of defective conditions of the embryonic tissues, has been suggested by Streeter [76] and Seitz.[77] The histologic changes in the stumps of seemingly amputated legs of an infant have been described in detail by Taylor Gorostiaga and Lede.[78] The effect of constriction of em-

73. Landauer, W., and Baumann, L.: J. Exper. Zool. **93**:51, 1943.

74. Gruber, G. B., in Schwalbe, E., and Gruber, G. B.: Die Morphologie des Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1937, vol. 1, pt. 3, p. 278.

75. Koehler, O.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **19**:670, 1936.

76. Streeter, G. L.: Contrib. Embryol. **22**:1, 1930.

77. Seitz, L.: Monatschr. f. Geburtsh. u. Gynäk. **94**:236, 1933.

78. Taylor Gorostiaga, D., and Lede, R. E.: Prensa méd. argent. **31**:363, 1944.

bryonic extremities, with subsequent rapid autolysis, has been investigated experimentally in mammals.[79]

Gross mechanical injury rarely leads to malformations in man and other mammals. If the injurious force penetrates the protecting envelopes, abortion or, in some mammals, resorption is the most probable outcome. In the recorded cases in which human embryo obviously survived an injury, the brain as the most vulnerable part is the most commonly affected. Pertinent examples are (1) apparently hemorrhagic destruction of both hemispheres following an accident during pregnancy [80] and (2) atypical presence of encephaloceles not in the embryonic line of closure of the primordium of the brain, with embolism and growth of brain tissue in the lungs.[81]

Mechanical conditions unfavorable for normal development may prevail in twins. Omphalocephaly [82] and ourentery [83] (anomalies in which the cranial and the caudal end of the body, respectively, grow into the yolk sac) of twins are probably caused by pressure of one twin on the other, in contrast to similar malformations of single embryos, which are probably not caused by external pressure.[84] The development of an acardiac twin may have a hydromechanic cause if one of twins, by virtue of his stronger circulation, takes over the function of propelling blood through the common placenta and the other twin. The latter will then suffer complete degeneration of large parts of his body. This course of events is hypothetic, and it is quite probable that at least in part of the cases the acardius is primarily maldeveloped or has abnormal vascular relations to his co-twin and the placenta which secondarily lead to the changes of circulation just described.[83]

Radiations.—An embryo may be deformed by radiation in two ways. One, in which a defect is caused by destruction of the irradiated tissue, has been mentioned in the preceding section. The other possibility is the survival of damaged tissue. While complete elimination of some tissue can usually not be ruled out, the following reports deal, in all probability, mainly with effects of the latter type. Hinrichs and Gentner [85] produced twins and double monsters in fish eggs with ultraviolet radiation, and determined as the most effective period the time just before the onset of cleavage. Many other malformations were also found, and various organs appeared to be affected to different degrees. The

79. Hellner, H.: Monatschr. f. Geburtsh. u. Gynäk. **95**:70, 1933.

80. Seitz, L.: Arch. f. Gynäk. **83**:701, 1907.

81. Gruenwald, P.: Am. J. Path. **17**:879, 1941. Potter, E. L., and Young, R. L.: Arch. Path. **34**:1009, 1942.

82. Gruenwald, P.: Ztschr. f. Anat. u. Entwcklngsgesch. **107**:782, 1937.

83. Gruenwald, P.: Anat. Rec. **83**:267, 1942.

84. Gruenwald, P.: J. Morphol. **69**:83, 1941.

85. Hinrichs, M. A., and Gentner, I. T.: Physiol. Zoöl. **4**:461, 1931.

circulatory and the central nervous system are more severely affected than other parts. Solberg [86] has reported on fish embryos that were exposed to roentgen rays. He distinguishes the following stages of the effect: (1) a latent period; (2) retardation through interference with mitosis; (3) disintegration of some tissues; (4) reorganization; (5) subsequent differentiation, depending on the changes previously produced. Uniform malformations can be obtained by properly controlled irradiation. Several workers have done similar work with amphibians.[87]

Von Hippel [88] produced cataracts in rabbit embryos by roentgen irradiation of the pregnant mother. Pagenstecher [89] obtained rosette formation in the retina by a similar procedure. Comparable effects have been observed in human embryos.[90] Experimentation with pregnant rats has yielded the following observations [91]: Hydrocephalus resulted most frequently from irradiation on the ninth day; ocular abnormalities, from that on the tenth day, and malformations on the jaws, from that on the eleventh day. In mice, irradiation on the seventh day resulted in resorption of embryos; that on the eighth day in meningocele; that on the ninth to fourteenth day, in kinked or short tail; that on the twelfth to fourteenth day, in hydrocephalus; that on the fourteenth to seventeenth day, in sterility, and that on the eighteenth and nineteenth day, in cataract.[92] This differential action may well be due to an effect of radiation proportionate to the growth rate at a given time and place,[93] although many other factors are probably involved as well. That roentgen rays have their predominant effect on cells in mitosis is well established.[14] A combination of irradiation and administration of ether [94] or increase of temperature [16b] has been reported to increase the effect in experimental animals.

Much has been reported on human embryos that received therapeutic doses of roentgen rays. In contrast to irradiation of the germ cells, which has not been proved to affect the offspring, irradiation of the embryo is responsible for a large number of cases of maldevelopment, which have been thoroughly investigated, notably microcephaly with

86. Solberg, A. N.: J. Exper. Zool. **78**:441, 1938.

87. Bardeen,[16b] Hertwig,[16c] Baldwin, W. M.: Anat. Rec. **17**:135, 1919; Am. J. Physiol. **52**:296, 1920; Anat. Rec. **22**:305, 1921. Curtis, W. C.; Cameron, J. A., and Mills, K. O.: Science **83**:354, 1936.

88. von Hippel, E.: Verhandl. d. deutsch. path. Gesellsch. **9**:174, 1905.

89. Pagenstecher, H. E.: Ber. ü. d. Versamml d. ophth. Gesellsch., 1916, p. 447.

90. (*a*) Lindenfeld, B.: Klin. Monatsbl. f. Augenh. **51**:443, 1913. (*b*) Goldstein, I., and Wexler, D.: Arch. Ophth. **5**:591, 1931; (*c*) **7**:434, 1932.

91. Job, T. T.; Leibold, G. L., Jr., and Fitzmaurice, H. A.: Am. J. Anat. **56**:97, 1935.

92. Kaven, A.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:238, 1938.

93. Woskressensky, N. M.: Arch. f. Entwcklngsmechn. d. Organ. **113**:447, 1928.

94. Haecker, V., and Lebedinsky, N.: München. med. Wchnschr. **61**:7, 1914.

mental deficiency and less frequently hydrocephalus, microphthalmia, malformations of the extremities and other parts.[95] Retinal rosette formations comparable to those experimentally produced in the rabbit [80] have been studied in detail in embryos irradiated in order to terminate pregnancy.[90] It has been found that well over 50 per cent of irradiated embryos (not counting those irradiated for intentional termination of pregnancy) suffer severe damage.[95b, c] In view of this fact it has been suggested that pregnancy be interrupted if through unfortunate circumstances the embryo has received therapeutic doses of roentgen rays.[95a] Diagnostic curettage of the uterus before irradiation of the organ during the generative period has also been advocated.[95c] While in most of the reported cases the damage of the embryo was due to early irradiation, ill effects have been observed to follow treatment given during the latter half of intrauterine life.[96] According to Bagg [97] and Goldstein and Murphy,[98] the effect of radium resembles that of roentgen rays. It need not be elaborated in detail that these malformations are not hereditary, in contrast to those caused by mutation after irradiation of germ cells (see page 404).

Chemical Influences.—Countless investigations have dealt with chemical alterations of the embryo. Only a few will be mentioned for their biologic or medical significance. Much of the early work was done on the fish Fundulus heteroclitus, and striking modifications of development were obtained. Werber [99] produced double monsters by means of acetone. In addition, he [100] obtained, by administration of acetone or butyric acid, cyclopia and various malformations of the eyes, ears, olfactory pits, mouth, central nervous system, heart and vessels, fins, tail and body form. There are also most interesting cases in which isolated eyes or lenses were found on the blastoderm far from the embryo itself.[101] This led Werber [101b] to develop the concept of blastolysis, which means an action of chemicals resulting in destruction or in

95. (*a*) Murphy, D. P.; Shirlock, M. E., and Doll, E. A.: Am. J. Roentgenol. **48**:356, 1942. (*b*) Zappert, J.: Wien. klin. Wchnschr. **38**:669, 1925; Arch. f. Kinderh. **80**:34, 1926. (*c*) Murphy, D. P.: Am. J. Obst. & Gynec. **18**:179, 1929; (*d*) footnote 21. (e) Feldweg, P.: Strahlentherapie **26**:799, 1927. (*f*) Goldstein, L., and Murphy, D. P.: Surg., Gynec. & Obst. **50**:79, 1930. (*g*) Flaskamp, W., in Meyer, H.: Sonderbünde zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1930, vol. 12, p. 1. (*h*) Sternberg, H.: Chir. d. org. di movimento **24**:231, 1939. (*i*) Maxfield, F. N.: Am. J. Ment. Deficiency **45**:358, 1941. (*j*) Glass, S. J.: J. Clin. Endocrinol. **4**:47, 1944.

96. Goldstein, L., and Murphy, D. P.: Am. J. Roentgenol. **22**:322, 1929.

97. Bagg, H. J.: Am. J. Anat. **30**:133, 1922.

98. Goldstein, L., and Murphy, D. P.: Am. J. Obst. & Gynec. **18**:189, 1929.

99. Werber, E. I.: J. Exper. Zool. **24**:409, 1917.

100. Werber, E. I.: Anat. Rec. **9**:529, 1915; *footnote 6 e.*

101. Werber, E. I.: (*a*) J. Exper. Zool. **21**:347, 1916; (*b*) Anat. Rec. **10**:258, 1916.

splitting and dispersion of the germ. As this work was based on experimental use of acetone and butyric acid, which may be present in the human circulation under abnormal conditions, it was suggested [102] that human malformations may have the same cause. While this deduction cannot be subscribed to, for many obvious reasons, and the mechanism of Werber's blastolysis is not understood, the interesting facts remain and have been powerful stimuli for further work. Stockard used inorganic salts in experiments on the same species and found that magnesium salts produced a remarkable incidence of cyclopia. The most constant effect on various embryonic structures was obtained with lithium salts. These produced a retardation of development, malformations of the eyes, colorless (sic) blood and a slow heart rate.[103] McClendon [6d] examined the power of many inorganic and organic compounds to produce cyclopia, and along with the chemical properties of these agents he considered also their surface tension.

In regard to amphibians the work done with lithium salts overshadows all other experiments with chemicals in extent and importance. Lehmann [104] found the notochord absent and the myotomes (somites) fused across the midline in locations depending on the times at which the subjects were exposed to the salts. Similar results were obtained by Cohen,[105] and the original articles should be consulted for the somewhat different explanations given by the two workers. In the head region, not only faulty differentiation or arrangement of the primordia occurs, but severe defects up to cyclopia or complete anophthalmia with associated lesions of the brain. All these malformations are not specific for the action of lithium; they occur, though less regularly, after the use of other chemicals [106] and after an increase of temperature.[107] The action of trichlorbutyl-alcohol on lens formation in amphibians has also been investigated in great detail and with particular attention to the effect of various concentrations.[108]

An interesting and severe malformation in amphibians, which occasionally occurs spontaneously, has been produced with increased frequency by raising embryos in 0.35 per cent sodium chloride solution.[109] It is exogastrulation, an abnormality of the process of gastrulation in which the mesoderm, instead of moving into the interior at the blastopore, moves outward to form a separate mass. In extreme

102. Werber, E. I.: Anat. Rec. **9**:133, 1915; Bull. Johns Hopkins Hosp. **26**:226, 1915; footnote 6 *e*.

103. Stockard, C. R.: J. Exper. Zool. **3**:99, 1906; footnote 6 *a*.

104. Lehmann, F. E.: Arch. f. Entwcklngsmechn. d. Organ. **136**:112, 1937; **136**:106, 1938.

105. Cohen, A.: J. Exper. Zool. **79**:461, 1938.

106. Lehmann.[104] Cohen.[105]

107. Hoadley, L.: Growth **2**:25, 1938.

108. Lehmann, F. E.: Arch. f. Entwcklngsmechn. d. Organ. **131**:333, 1934.

109. Holtfreter, J.: Arch. f. Entwcklngsmechn. d. Organ. **129**:669, 1933.

cases it may constrict itself off from the ectoderm, and the embryo is then divided into two separate pieces. Just what the action of the salt is in provoking this process is not understood. Exogastrulation has also appeared occasionally after other experimental procedures.

In frog embryos 2,4-dinitrophenol produces a general retardation of development and any of the following malformations: persistent yolk plug, absence of external gills, papillary outgrowths of the epidermis and abnormalities of the eyes and the neural tube.[110]

There are many reports on the action of chemicals on the chick embryo. Various substances acting chemically or as foreign bodies were administered by Bauer [111] and Canat and Opie.[112] The former reports hypoplasia of the mesenchyme and inhibition of the outgrowth of peripheral nerves, supposedly consecutive to the disturbance of the mesenchyme. Ectodermal and entodermal structures are normal or retarded, or show foci of necrosis. Canat and Opie examined the local inflammatory reaction to the injection of india ink or turpentine. In embryos of 3 to 5 days the most prominent reaction is cell proliferation. Phagocytosis also occurs early. Granulocytes begin to appear at the end of the first week. Shortly before birth, inflammation assumes the well known postnatal forms. That scarlet red induces epithelial proliferations and irregularities of the neural tube has been claimed,[113] but not confirmed in a later investigation.[114] It is not known whether or not this discrepancy was due to differences in the chemical nature of the dye or to its impurities. Alcohol in suitable concentrations produces tachycardia in 48 hour embryos, but no malformations beyond disturbances of the curvature of the body.[115] Colchicine produces not only the well known abnormalities of mitosis ("colchicine figures") but also malformations of the neural tube [116] or, according to other authors,[117] strophosomus (an extreme dorsal flexion of the spine). When colchicine is applied to certain circumscribed parts of the embryo, dwarfed limbs may be obtained, as well as reduced numbers of digits.[118] Landauer [119] observed in chick embryos, after injecting Ringer solution into the eggs, an increased prenatal and a decreased postnatal mortality. This is

110: Dawson, A. B.: J. Exper. Zool. **78**:101, 1938.

111. Bauer, K.: Virchows Arch. f. path. Anat. **294**:477, 1935.

112. Canat, E. H., and Opie, E. L.: Am. J. Path. **19**:371, 1943.

113. Waelsch, L.: Arch. f. Entwcklngsmechn. d. Organ. **38**:509, 1914. I had an opportunity to see serial sections of Waelsch's specimens and to confirm the presence of the malformations described in this article.

114. Burnier, J., and Sauser-Hall, P.: Compt. rend. Soc. de biol. **116**:927, 1934.

115. Petry, E., and Ferrier, A.: Compt. rend. Soc. de biol. **116**:928, 1934.

116. Paff, G. H.: Am. J. Anat. **64**:331, 1939.

117. Lallemand, S.: Compt. rend. Acad. d. sc. **207**:1446, 1938. Ancel, P., and Lallemand, S.: ibid. **210**:710, 1940. Gabriel, M. L.: J. Exper. Zool. **101**:339, 1946.

118. Gabriel, M. L.: J. Exper. Zool. **101**:339, 1946.

119. Landauer, W.: Poultry Sc. **8**:301, 1929.

apparently an instance in which mostly the weaker ones, which would succumb to other influences after birth, are killed as embryos. As was mentioned in a foregoing paragraph, a similar explanation has been given for the action of alcohol on the germ cells.[31]. Landauer [119] also found that lithium salts and, to a less extent, magnesium salts cause a great increase of mortality shortly before hatching, but no malformations were seen. In another series of experiments, rumplessness was produced by insulin and several other organic compounds.[120] Catizone and Gray [121] have reported three types of distortion of the head following administration of lead compounds. The published views of whole embryos suggest that the malformations are all platyneuria, which occurs frequently in the laboratory without intentional interference. Other workers [122] found general retardation of growth and relatively greater retardation of somite formation. The development of head and eyes is inhibited. In both investigations the embryos were not sectioned. Gray and Worthing [123] injected tetanus toxin and observed a profound influence on the central nervous system and the head of the early embryo. These malformations, too, are of those types which frequently occur without apparent cause.

Of great economic importance is a series of investigations originally designed to discover the cause of so-called alkali disease of farm animals in the Middle West.[124] It was found that the disease is a poisoning due to consumption of selenium-containing plants. In connection with the present subject, only the findings in chick embryos are of interest. Mature chickens are not much affected by selenium in their diet, but embryos of selenium-poisoned hens show severe disturbances.[125] A high percentage of them fail to hatch and exhibit, among others, malformations of the brain, the eyes (including cyclopia), the beak and the extremities or an "edemic" (sic) condition. Similar malformations have been produced by injecting selenium compounds into eggs.[126] In the course of the same investigation arsenic, fluorine and lead compounds were also tested and failed to cause comparable disturbances. These substances caused a high embryonic death rate, also ectopia of the viscera.[126] Landauer [49] found that selenium-induced malformations share with certain others a predilection for one side of the body (eyes and wings of the left side; legs of the right side).

120. Landauer, W.: J. Exper. Zool. **98**:65, 1945. Landauer, W., and Lang, E. H.: ibid. **101**:41, 1946. Landauer, W., and Bliss, C. I.: ibid. **102**:1, 1946.

121. Catizone, O., and Gray, P.: J. Exper. Zool. **87**:71, 1941.

122. Hammett, F. S., and Wallace, V. L.: J. Exper. Med. **48**:659, 1928.

123. Gray, P., and Worthing, H.: J. Exper. Zool. **86**:423, 1941.

124. Moxon, A. L.: Bulletin 311, South Dakota Agricultural Experiment Station, 1937, p. 1.

125. Franke, K. W., and Tully, W. C.: Poultry Sc. **14**:273, 1935.

126. Franke, K. W.; Moxon, A. L.; Poley, W. E., and Tully, W. C.: Anat. Rec. **65**:15, 1936.

Few investigations have been undertaken to examine in mammals the influence on the embryo of substances injected into pregnant females. In recent years drugs have been tested in order to determine whether, if used during pregnancy, they might endanger the embryo. Sulfanilamide was tested in rats.[127] The concentration was equal in the maternal and the fetal blood and higher than that used in therapy. Prolonged administration increased the mortality before and after birth. The birth weight was diminished and postnatal growth retarded. Penicillin, on the other hand, had no detrimental effect on the embryo under the conditions of an independent investigation.[128] Thiourea was similarly examined.[129] The thyroid gland of the embryo showed the histologic changes characteristic of thiourea treatment. No malformations were recorded. Strontium compounds administered to pregnant rabbits replace calcium in fetal bones and produce a condition of "pseudorickets".[130] In rat embryos whose mothers receive a diet containing 25 per cent galactose cataracts develop. The changes resemble postnatal galactose cataract.[131] Alloxan has no direct effect on the rat embryo.[132] Hansmann and Perry [133] in a series of unselected cases in which there was no exposure to industrial hazards found lead in 62.5 per cent of human fetuses examined. In 25 per cent the amounts were considered dangerous. However, it is stated that the fetus is protected by the growing skeleton which binds the lead. The authors emphasize the possibility of abortion due to lead. No malformations were observed. Only one report has been found concerning the possibility that the fetus might be damaged by arsenicals used in antisyphilitic treatment during pregnancy.[134]

In the preceding pages the effects of foreign substances on embryonic development are reviewed. It is to be expected that striking effects would be produced in the embryo by those organic substances which even in the more stabile mature organism control structural changes, namely, hormones. It is unfortunate that Gley's [135] subdivision of these

127. Speert, H.: Bull. Johns Hopkins Hosp. **66**:139, 1940.

128. Greene, H. J., and Hobby, G. L.: Proc. Soc. Exper. Biol. & Med. **57**:282, 1944.

129. Goldsmith, E. D.; Gordon, A. S., and Charipper, H. A.: Am. J. Obst. & Gynec. **49**:197, 1945.

130. Lehnerdt, F.: Beitr. z. path. Anat. u. z. allg. Path. **46**:468, 1909.

131. Bannon, S. I.; Higginbottom, R. M.; McConnell, J. M., and Kaen, H. W.: Arch. Ophth. **33**:224, 1945.

132. Friedgood, C. E., and Miller, A. A.: Proc. Soc. Exper. Biol. & Med. **59**:61, 1945.

133. Hansmann, G. H., and Perry, M. C.: Arch. Path. **30**:226, 1940.

134. Arnold, W.: Virchows Arch. f. path. Anat. **311**:1, 1944.

135. Gley, E.: The Internal Secretions: Their Physiology and Application to Pathology, New York, Paul B. Hoeber, 1917.

active substances into hormones proper and harmozones, proposed in the early days of endocrinology, has not been generally accepted. According to Gley's definition, substances of the former group control functional activity, whereas harmozones control morphogenetic processes. Disturbances in the balance of harmozones should affect the embryo profoundly. The relationship of these agents to embryonic organizers has been discussed by Needham.[4]

The older literature on the endocrine glands of the fetus and their interrelation with one another, with other parts of the embryo and with agents in its environment has been reviewed by Thomas.[136] One important concept dating from that early period is that of synkainogenesis,[137] a term which designates all endocrine interactions between mother and embryo. Well known examples of this are the stimulation of the mammary glands of the newborn by maternal lactogenic hormone and the hyperplasia of the interstitial cells of the testes of the embryo, which is well marked in man [138] and reaches tremendous proportions, also those of the ovaries, in horse embryos.[139] Endocrine abnormalities of the mother may influence the embryo. Peculiarities of the children of diabetic mothers have recently been studied in great detail. They include increased birth weight, enlargement of the heart, extramedullary erythropoiesis, hyperplasia of the pancreatic islands and changes in other endocrine organs.[140] It was thought that the metabolic disturbance and the insulin deficiency of the mother were directly responsible for these changes. However, it has now been found that infants of mothers in whom diabetes develops only some time after termination of pregnancy have similar changes.[141] This has not been explained as yet. The fetal and neonatal mortality is increased during a five year period preceding the onset of maternal diabetes.[142]

Hereditary dwarfism of mice, apparent at birth, is probably mediated by an abnormality of the pituitary gland.[143] Mongoloid deficiency has been tentatively correlated with maternal and fetal pituitary dysfunc-

136. Thomas, E.: Innere Sekretion in der ersten Lebenszeit (vor und nach der Geburt), Jena, Gustav Fischer, 1926.

137. Kohn, A.: Arch. f. Entwcklngsmechn. d. Organ. **39**:112, 1914.

138. Mita, G.: Beitr. z. path. Anat. u. z. allg. Path. **58**:554, 1914. Neumann, H. O.: Ztschr. f. Geburtsh. u. Gynäk. **90**:100, 1930. Diaca, C.: Virchows Arch. f. path. Anat. **304**:171, 1939.

139. Kohn, A.: Ztschr. f. Anat. u. Entwcklngsgesch. **79**:366, 1926. Petten, J. L.: ibid. **99**:338, 1933.

140. (*a*) Potter, E. L.; Seckel, H. P. G., and Stryker, W. A.: Arch. Path. **31**:467, 1941. (*b*) Miller, H. C.; Johnson, R. D., and Durlacher, S. H.: J. Pediat. **24**:603, 1944.

141. Miller, H. C.: Am. J. M. Sc. **209**:447, 1945.

142. Miller, H. C.: J. Pediat. **29**:455, 1946.

143. Greene, H. S. N.: J. Exper. Med. **71**:839, 1940.

tion.[144] On the other hand, human subjects with pituitary dwarfism usually produce normal offspring.[145] Fetal thyroid or iodine deficiency produces congenital cretinism.[146] The mental development of persons with congenital hypothyroidism, particularly that of endemic cretins, often fails to respond adequately to administration of thyroid. It has therefore been suggested that damage of the brain may not be merely a consequence of thyroid deficiency but an associated lesion.[147]

A possible correlation of abnormalities of endocrine organs of the embryo is the repeatedly investigated severe atrophy of the adrenal cortex of the anencephalic monster.[148] It has not been definitely established whether or not this condition is caused by an abnormality of the hypophysis in the anencephalic monster, as was claimed by Kohn.[148b] I have seen a similar atrophy of the adrenal cortex in a stillborn infant with hydrocephalus and abnormalities at the base of the brain. No pituitary gland could be found (case unpublished).

By far the largest amount of work in embryonic endocrinology has been done with estrogenic and androgenic substances. It is of great interest because many of the experiments have resulted in abnormalities related to intersexuality. Work done up to 1939 has been reviewed by a group of outstanding experts.[64] Since then, experimental work has been done with the opossum,[149] the mouse,[150] the rat [151] and the monkey.[152] In all classes of vertebrates the genital organs and other sexually different characteristics may be transformed into forms more or less resembling those of the opposite sex by proper application of estrogen or androgen to embryos. A strong tendency of the genetic sex to assert itself and condition the effect of estrogen or androgen was noted in some of these experiments but until recently hormones were generally

144. (*a*) Benda, C. E.; Dayton, N. A., and Prouty, R. A.: Am. J. Psychiat. **99**:822, 1943. (*b*) Beidleman, B.: Am. J. Ment. Deficiency **50**:35, 1945.

145. Speck, G.: Am. J. Obst. & Gynec. **51**:217, 1946.

146. Benda, C. E.: Mongolism and Cretinism, New York, Grune & Stratton, Inc., 1946.

147. Bruch, H., and McCune, D. J.: Am. J. Dis. Child. **67**:205, 1944.

148. (*a*) Meyer, R.: Virchows Arch. f. path. Anat. **210**:158, 1912. Landau, M.: Verhandl. d. deutsch. path. Gesellsch. **16**:301, 1913. (*b*) Kohn, A.: Arch. f. Entwcklngsmechn. d. Organ. **102**:113, 1924. Angevine, D. M.: Arch. Path. **26**:507, 1938.

149. (*a*) Moore, C. R.: Physiol. Zoöl. **14**:1, 1941. (*b*) Burns, R. K., Jr.: Biol. Symposia **9**:125, 1942.

150. Raynaud, E.: Compt. rend. Acad. d. sc. **205**:1453, 1937; Compt. rend. Soc. de biol. **127**:503, 1938. Turner, C. D.: J. Morphol. **65**:353, 1939.

151. (*a*) Hamilton, J. B., and Wolfe, J. M.: Anat. Rec. **70**:433, 1938. (*b*) Greene, R. R.; Burrill, M. W., and Ivy, A. C.: Am. J. Anat. **65**:415, 1939; **67**:305, 1940. (*c*) Greene, R. R.: Biol. Symposia **9**:105, 1942.

152. van Wagenen, C., and Hamilton, J. B., in Essays in Biology in Honor of Herbert M. Evans, Berkeley, University of California Press, 1943, p. 581.

believed to play a dominating part in the morphogenesis of the genital organs (except the gonads themselves) under normal as well as under abnormal conditions. From experiments carried out during the last few years it has been concluded by some leading authorities that the gonads of the embryo do not produce hormones until after the genital tract is well established in male or female form. Even the gonadotropic hormone of the pituitary gland fails to stimulate the gonads in these stages to secrete hormones.[153] This is in accord with pathologic findings in human cases of congenital absence or severest hypoplasia of the gonads [154] in which the genital tract is definitely differentiated in the direction of one sex. In later periods sexual differentiation will, of course, suffer in gonadless individuals. The power of the genetic sex to assert itself, even in postnatal periods, is well illustrated in experiments in which male chick embryos were completely feminized by administration of estrogen. They had histologically normal ovaries, and yet were reconverted to the male sex after birth if estrogen treatment was not continued.[155] Thus the leading role of the genetic sex in determining not only the sexual form of the gonads but also that of the entire organism has come to be increasingly appreciated.

It is of great medical importance to realize the tenacity of the genetic sex. It explains the fundamental difference between genetic and hormonal intersexuality. In the former the sexual development is abnormal by the standards of the usual male or female organization but normal for the individual itself in that it conforms with that individual's genetic pattern. Hormonal intersexes, on the other hand, have a sex which is genetically normal by the usual standards but which is secondarily changed by hormones. These individuals will by themselves tend to revert to their genetic sex if the hormonal imbalance is eliminated. In addition, there is probably a condition of genetically determined hormonal intersexuality which may, if not properly analyzed, be confused with genetic intersexuality. It is produced by the action of genetically determined hormonal imbalance in individuals with a normal genetic sex. This occurs in genetically caused or conditioned growths with heterosexual hormonal activity. Familial intersexuality associated with hyperplasia or tumor of the adrenal cortex [156] probably belongs to this group.

153. Moore, C. R.: Am. Naturalist **78**: 97, 1944; J. Clin. Endocrinol. **4**:135, 1944.

154. Kermauner, F., in Halban, J., and Seitz, L.: Biologie und Pathologie des Weibes, Berlin, Urban & Schwarzenberg, 1924, vol. 3, p. 281. Rössle, R., and Wallart, J.: Beitr. z. path. Anat. u. z. allg. Path. **84**:401, 1930. Pich, G.: ibid. **98**:218, 1937. Erskine, C. A., and Rannie, I.: Arch. Path. **42**:381, 1946.

155. Wolff, E.: Arch d'anat., d'histol. et d'embryol. **23**:1, 1936. Dantchakoff, V.: Compt. rend. Acad. d. sc. **202**:1112, 1936.

156. Werthemann, A.: Schweiz. med. Wchnschr. **71**:1335, 1941.

While the domain of the sex hormones proper may not be as great in normal embryonic development as was formerly believed, it is quite possible that chemical correlations, perhaps more of the organizer type, are active in the early development of the genital organs. Here must be mentioned Witschi's [157] hypothetic substances which govern female development of the germ cells in the cortex, and male development in the medulla, of the gonad. Of undetermined normal significance is a hormone-like substance which produces a highly perplexing type of intersexuality in certain mammals: The freemartins among cattle are individuals with abnormal sex development, which are always twins of normal males. It was discovered simultaneously and independently by Keller and Tandler [158] and Lillie [159] that the abnormal twin is a genetic female modified by a substance transmitted from the male twin through anastomoses in their chorionic circulations. A considerable number of detailed investigations have since substantiated this theory and elucidated the development of the freemartin. Similar intersexual twins also occur among pigs and goats but not among heterosexual twins with vascular anastomoses in the cat, the peludo and the marmoset (see Witschi's review [160]). Moore [153] points out that conditions strictly comparable with those observed in the freemartin have not as yet been produced in experiments, and he concludes that the problem of the freemartin is just as mysterious now as ever. Marsman [161] compared freemartins with genetic intersexes and geldings as to morphologic aspects and excretion of hormones. His conclusion is that the freemartin is an individual deprived early of its sources of sex hormones and more thoroughly than the gelding.

It remains to mention the effect of certain tumors of the cortex of the adrenal gland on the genital tract. In a small proportion of cases of corticoadrenal tumors for which no specific histologic characteristics have yet been found, as well as in some instances of hyperplasia of the adrenal cortex, the female subjects may be masculinized at any period of prenatal or postnatal life (interrenalism [162]). This effect disappears promptly on removal of the excessive adrenal tissue unless sex transformation in early embryonic life has produced irreversible changes.[163] A much smaller number of cases in which men were

157. Witschi, E.: Biol. Rev. **9**:460, 1934.

158. Keller, K., and Tandler, J.: Wien. tierärztl. Wchnschr. **3**:513, 1916.

159. Lillie, F. R.: Science **43**:611, 1916.

160. Witschi, E., in Allen, Danforth and Doisy,[64] p. 145.

161. Marsman, W. S.: Acta neerland. morphol. **1**:115, 1937.

162. Mathias, E.: Zentralbl. f. Gynäk. **50**:2489, 1926. Berner, O., in Hirsch, M.: Handbuch der inneren Sekretion, Leipzig, Curt Kabitzsch, 1927, vol. 25, p. 1143.

163. McKenna, C. M.; Kiefer, J. H., and Bronstein, I. P.: Tr. Am. A. Genito-Urin. Surgeons **35**:41, 1943.

feminized by similar growths have been reported.[164]. Familial occurrence has already been mentioned.[156] Various theories have been proposed concerning the androgenic activity (neglecting the few cases in which feminization occurred) of one or another of the normal constituents of the adrenal cortex and he relationship of the just mentioned tumors to these. None of the theories is supported by convincing evidence, and they will therefore not be reviewed. Somewhat less problematic is the influence on sex of various tumors of organs which normally produce sex hormones, as the testis, the ovary and the placenta. This subject is adequately treated in many texts of pathology or of gynecology. A case of masculinization of a female fetus by an ovarian tumor of the mother, probably an arrhenoblastoma, is on record.[165]

Deficiencies.—If any of essential substances is lacking during embryonic life, this may produce severe developmental disturbances. Some of these disturbances have been investigated in chick embryos, partly because their occurrence in commercial hatching is of considerable economic importance. Micromelia was observed in chick embryos, caused by a deficient diet of the hen, by Byerly and co-workers,[166] and its morphologic aspects were studied by Landauer.[167] Lyons and Insko[168] and Caskey and Norris[169] have described the same manifestations in manganese deficiency and they, and later Landauer,[170] have assumed that the first-mentioned deficiency was also one of manganese. Gallup and Norris[171] found that manganese deficiency produces increased mortality of chick embryos. The embryos are fully developed but unable to hatch. The authors call this congenital debility in analogy to findings in the rat (see page 428). The influence of vitamin deficiencies has been examined in chick embryos. Low vitamin A content of the egg results in poor hatchability.[172] Riboflavin deficiency causes degeneration of the mesonephros, edema, anemia and abnormal down.[173] Another report mentions lethal changes in

164. Schiller, W.: Internat. Clin. **3**:87, 1940.

165. Brentnall, C. P.: J. Obst. & Gynaec. Brit. Emp. **52**:235, 1945.

166. Byerly, T. C.; Titus, H. W.; Ellis, N. R., and Landauer, W.: Proc. Soc. Exper. Biol. & Med. **32**:1542, 1935.

167. Landauer, W.: Anat. Rec. **64**: 267, 1936.

168. Lyons, M., and Insko, W. M., Jr.: Bulletin 371, Kentucky Agricultural Experiment Station, 1937, p. 61.

169. Caskey, C. D., and Norris, L. C.: Proc. Soc. Exper. Biol. & Med. **44**:332, 1940.

170. Landauer, W.: Sigma Xi Quart. **28**:171, 1940.

171. Gallup, W. D., and Norris, L. C.: Poultry Sc. **18**:83, 1939.

172. Bearse, G. E., and Miller, M. W.: Poultry Sc. **16**:39, 1937.

173. Lepovsky, S.; Taylor, L. W.; Jukes, T. H., and Almquist, H. L.: Hilgardia **11**:559, 1938.

the extraembryonic vessels ("lethal ring") and chondrodystrophy and other defects in those embryos which survive.[174] A third group [175] claims that the first changes to appear are those in the nervous system, including degeneration of myelin sheaths. These can be prevented by injecting riboflavin into the eggs. Vitamin D deficiency seems to occur in chick embryos as indicated by the high mortality in eggs of hens kept in closed rooms, which can be reduced by giving the hens cod liver oil.[176] Vitamin E deficiency causes retardation of development, and death through degeneration of vessels, rupture of the atrium of the heart or of large vessels, and impairment of growth of the allantois, which is essential for respiration.[177] However, the pertinence of these observations has been questioned,[178] because it is not certain that they are the effect of vitamin E deficiency alone. Lack of biotin is followed by increased embryonic mortality at certain stages, chondrodystrophy and syndactyly.[179]

Considerable experimental work has been reported on the effect of vitamin deficiencies on mammalian embryos. Many references have been gathered by Mason.[180] The same author has reported in detail on the results of vitamin A deficiency in pregnant rats[181]: Early death of many embryos, followed by resorption, results from inflammatory changes of the placenta. The surviving embryos are retarded in growth, gestation is often unduly prolonged, and many newborn young die soon. In the pig severe defects of the hindlegs follow maternal deficiency of a fat-soluble factor,[182] which Needham [4] states is vitamin A. In another investigation definite lack of this vitamin caused microphthalmia in every young one throughout several experiments, but no malformations of the legs.[183] In the rat, ocular malformations of a different kind are observed in association with maternal vitamin A deficiency.[184] A series of reports describes the development of cleft palate and malformations of the extremities of the embryos of rats

174. Romanoff, A. L.: Anat. Rec. (supp.) **78**:78, 1940.

175. Engel, R. W.; Phillips, P. H., and Halpin, J. G.: Poultry Sc. **19**:135, 1940.

176. Insko, W. M., and Lyons, M.: Bulletin 363, Kentucky Agricultural Experiment Station, 1936, p. 64.

177. Adamstone, F. B.: J. Morphol. **52**:47, 1931.

178. Mason, K. E.: Yale J. Biol. & Med. **14**:605, 1942.

179. Cravens, W. H.; McGibbon, W. H., and Sebesta, E. E.: Anat. Rec. **90**:55, 1944.

180. Mason, K. E., in Allen, Danforth and Doisy,[64] p. 1149.

181. Mason, K. E.: Am. J. Anat. **57**:303, 1935.

182. daZilva, S. S.; Golding, J.; Drummond, J. C., and Coward, K. H.: Biochem. J. **15**:427, 1921.

183. Hale, F.: Texas State J. Med. **33**:228, 1937.

184. Warkany, J., and Schraffenberger, E.: Proc. Soc. Exper. Biol. & Med. **57**:49, 1944; Arch. Ophth. **35**:150, 1946.

maintained on a certain deficient diet.[185] These disturbances can be prevented with dried pig liver. The same malformations result from riboflavin deficiency.[186] Similar, but not identical, defects were produced by feeding the mother a rachitogenic diet.[187] Avitaminosis C (deficiency of ascorbic acid) has been produced in guinea pig embryos.[188] Research on the effect of vitamin E deficiency started with the work of Evans, Burr and Althausen [189] and Urner.[190] Early abnormal development of the mesenchyme and general retardation were observed, as well as reduction of the entodermal villi of the placenta and impairment of the blood islands. The result was death of the fetus. Mason [191] described in hypovitaminotic embryos which survived to the sixteenth day a hemorrhagic state, with much blood accumulated in the vessels of the skin, and cerebral hemorrhages. He attributes the lack of cells in the blood-forming tissues to this escape of blood to the periphery.

Manganese deficiency produces in rats a state of "congenital debility" which renders them unable to live through the period of birth, even though they appear normal in utero shortly before term.[192]

Vitamin deficiencies of human embryos and newborn infants have repeatedly been reported.[193] In most cases neither the type of deficiency nor that of the structural changes has been clearly defined. The conditions found were called fetal rickets or osteomalacia, but no typical entities have as yet been demonstrated. The possible significance of vitamin deficiencies is indicated by the suggestion of Balfour and Talpade [194] that the high infant mortality of India may be caused by a deficiency, possibly of the vitamin B complex. Lack of iron in tuber-

185. Warkany, J., and Nelson, R. C.: Anat. Rec. **79**:83, 1941; Arch. Path. **34**:375, 1942. Warkany, J.; Nelson, R. C., and Schraffenberger, E.: Am. J. Dis. Child. **64**:860, 1942.

186. Warkany, J., and Schraffenberger, E.: Proc. Soc. Exper. Biol. & Med. **54**:92, 1943.

187. Warkany, J.: Am. J. Dis. Child. **66**:511, 1943.

188. (*a*) Ingier, A.: J. Exper. Med. **21**:525, 1915. (*b*) Reyher, P.; Walkhoff, E., and Walkhoff, O.: München. med. Wchnschr. **75**:2087, 1928.

189. Evans, H. M.; Burr, G. O., and Althausen, T. L.: The Antisterility Vitamine Fat Soluble E, Memoirs of the University of California, Berkeley, University of California Press, 1927, vol. 8, p. 1.

190. Urner, J. A.: Anat. Rec. **50**:175, 1931.

191. Mason, K. E., in Essays in Biology in Honor of Herbert M. Evans, Berkeley, University of California Press, 1943, p. 401.

192. Daniels, A. L., and Everson, J. G.: J. Nutrition **9**:191, 1935.

193. (*a*) Reyher, Walkhoff and Walkhoff.[188b] (*b*) Maxwell, J. P.; Hu, C. H., and Turnbull, H. M.: J. Path. & Bact. **35**:419, 1932. (*c*) Akamatu, K.: Okayama-Igakkai-Zasshi **52**:979, 1940.

194. Balfour, M. I.: Indian M. Gaz. **65**:630, 1930. Balfour, M. I., and Talpade, S. K.: ibid. **67**:601, 1932.

culous mothers and their fetuses has been suggested as the cause of anemia and decreased resistance of the newborn.[195] Iodine deficiency produces in mammals congenital goiter and hairlessness [196] and in man goiter and deaf-mutism.[197] Stott [198] attributes similar findings to an excess of calcium in the water rather than to iodine deficiency, or to a disproportion of calcium and iodine. Closely related conditions were mentioned in a foregoing section under thyroid deficiency.

Detrimental effects of an unspecified nutritional deficiency of the mother or the fetus are suggested by Burke, Beal, Kirkwood and

carbon dioxide presented platyneuria in all instances. The critical stage was found to last from the appearance of the primitive streak until the beginning formation of the notochord and neural plate. Byerly [202] described in somewhat older embryos as suffocation effect large blood vessels dilated into sinuses of excessive dimensions and death of the tissues in the deeper layers of the germ. I have seen the same abnormalities as "spontaneous" malformations in eggs which had not intentionally or knowingly been subjected to anoxia, which suggests anoxia as a natural cause of malformations. Anoxia due to failure of extraembryonic blood circulation to develop properly is thought to be responsible for some of the malformations and their peculiar lateral distribution in prothanic homozygous Creeper chick embryos.[46] The left eye, which is commonly more affected than the right, is turned away from the surface of the blastoderm by the normal rotation of the head and thus receives less oxygen by diffusion while the development of circulation lags. Transplantation experiments have shown that the primordia of the right and left eyes of these embryos are potentially equal.[203] Experimental obstruction of the extraembryonic circulation in genetically normal embryos leads to similar malformations with severer manifestations on the left side of the head, whereas an increase of the oxygen tension attenuates the defects of the left eye in prothanic Creeper embryos.[46] A subsequent examination of normal chick embryos incubated under favorable conditions showed that the left eye lags temporarily in the majority of them.[18] This asymmetry is present only during a short period beginning when the head turns to the side, and ending when an effective circulation is established. This is well in accord with the assumption of transitory anoxia of the left eye. A great preponderance of malformations of the left eye exists not only in Creeper embryos but also among sporadic [204] and selenium-induced malformations.[49] It may be assumed that temporary anoxia is a contributing factor which decreases the resistance of the left eye to other teratogenic agents.[18]

It is obvious that anoxia cannot be of great importance as a teratogenic factor in mammals. One can imagine that disturbances of oxygen supply occur before nidation in the uterus, or before the beginning of embryonic circulation, but this has not been observed. In later stages certain poisons may interfere with the metabolism of oxygen. A pertinent case of carbon monoxide poisoning of a pregnant woman has been described,[205] in which the mother recovered, while

202. Byerly, T. C.: Anat. Rec. **32**:249, 1926.

203. Gayer, K.: J. Exper. Zool. **89**:103, 1942.

204. (*a*) Gruenwald.[18] (*b*) Landauer, W.: Anat. Rec. **86**:365, 1943.

205. Maresch, R.: Wien. med. Wchnschr. **79**:454, 1929.

the child, which was born thirteen days after the poisoning and lived nine days, showed severest softening of the thalami and the corpora striata. In guinea pigs, anoxia preceding delivery produces severe cerebral damage.[206] In man, temporary anoxia of the older fetus stimulates respiratory movements, and may endanger the fetus by causing aspiration of amniotic fluid containing sebaceous material, cornified cells or meconium.[207] Damage of the brain similar to that observed in the aforementioned animal experiments may cause mental deficiency.[208]

Abnormal Temperature.—This environmental factor has no known teratologic importance in man and other mammals. Several authors have examined the influence of an abnormal incubation temperature in chick embryos and found various structural defects.[209] In the same species the expression of some hereditary malformations has been modified by a lowered temperature, as was mentioned.[210]

Petersen [211] has reported studies of the influence of various climatic factors on the incidence of malformations in man.

Infection and Inflammation.—The question whether maldevelopment may be caused by infection and subsequent inflammation leads to the frontiers of what is commonly considered to be maldevelopment as opposed to disease. That no strict borderline exists between these two divisions of the field of pathology, has been emphasized in the introduction.

The possible role of inflammation as a cause of abnormal development has been much discussed with respect to malformations of the heart. In the past it was believed that many of these malformations were due to embryonic endocarditis, but this explanation is no longer accepted for the great majority of cases. Gross [212] goes so far as to

206. (*a*) Windle, W. F.; Becker, R. F., and Weil, A.: J. Neuropath. & Exper. Neurol. **3**:224, 1944. (*b*) Windle, W. F., in Harvey Lectures, Lancaster, Pa., Science Press, 1945, vol. 40, p. 236.

207. (*a*) Farber, S., and Sweet, L. K.: Am. J. Dis. Child. **42**:1372, 1931. (*b*) Helwig, F. C.: Am. J. Obst. & Gynec. **26**:849, 1933. (*c*) Snyder, F. F., and Rosenfeld, M.: ibid. **36**:363, 1938. (*d*) Warwick, M.: New York State J. Med. **37**:2075, 1937.

208. Schreiber, F.: J. A. M. A. **111**:1263, 1938. Benda, C. E.: Am. J. Psychiat. **97**:1135, 1941. Clifford, S. H.: J. Pediat. **18**:567, 1941. Lamm, S. S.: Am. J. Ment. Deficiency **48**:131, 1943. Raymond, C. S.: ibid. **49**:8, 1944. Benda, C. E.: Medicine **24**:71, 1945.

209. Danforth, C. H.: Proc. Soc. Exper. Biol. & Med. **30**:143, 1932. Romanoff, A. L.: Poultry Sc. **15**:311, 1936. Smith, L. E. W.: Arch. Path. **28**: 422, 1939.

210. Sturkie.[52] Landauer.[51]

211. Petersen, W. F.: Am. J. Obst. & Gynec. **28**:443, 1934; Am. J. Orthodontics **27**:179, 1941.

212. Gross, P.: Arch. Path. **31**:163, 1941.

conclude from his own case and a review of the literature that there is no proved instance of fetal endocarditis, and others agree with him.[213] On the other hand, several authors[214] have described cases of acute endocarditis of undoubtedly prenatal origin. I have seen severe endocarditis of the tricuspid valve in an infant less than 1 day old.[215] These cases do not bear directly on the question of the inflammatory origin of cardiac malformations, as they concern late stages of intrauterine life. However, if one considers the relatively extremely small number of early embryos which are adequately examined (i.e. in serial sections), one must realize that failure to find acute endocarditis allows no conclusions. Fetal myocarditis has been found repeatedly.[216] Farber and Hubbard[216b] point out that of two groups into which congenital abnormalities of the heart may conveniently be divided, the one comprising gross departures from the normal developmental plan is not likely to be due to endocarditis. The other group includes hearts with normal septums and the typical relations of the large vessels and shows stenosis or atresia of valves, and these abnormalities are thought to be the effects of inflammation. I have found indications of a past inflammation, similar to those described by Farber and Hubbard, in the heart of a stillborn infant with atresia of the pulmonary ostium and in a newborn infant with stenosis of the mitral and aortic valves (cases unpublished).

The investigation of malformations of the heart and other organs occurring frequently in the infants of mothers who contracted rubella during the early months of pregnancy[217] may well contribute important information as soon as embryonic stages are examined. If the disease occurs during the first or second month of pregnancy, the inci-

213. Weintraub, T., and Himmelfarb, A. J.: Bull. Johns Hopkins Hosp. **72**: 299, 1943.

214. Plaut, A., and Sharnoff, G.: Arch. Path. **20**:582, 1935. Püschel, E.: Arch. f. Kinderh. **114**:1, 1938. Plaut, A.: Am. J. Path. **15**:649, 1939. MacGregor, R. R., and McKendry, R.: Canad. M. A. J. **50**:433, 1944.

215. I am indebted to Dr. A. J. Gitlitz for the opportunity to see this specimen.

216. (*a*) Stoloff, E. G.: Am. J. Dis. Child. **36**:1204, 1928. (*b*) Farber, S., and Hubbard, J.: Am. J. M. Sc. **186**:705, 1933.

217. Swan, C.; Tostevin, A. L.; Moore, B.; Mayo, H., and Black, G. H. B.: M. J. Australia **2**:201, 1943. Swan, C.; Tostevin, A. L.; Mayo, A., and Black, G. H. B.: ibid. **1**:409, 1944. Erickson, C. A.: J. Pediat. **25**:281, 1944. Reese, A. B.: Am. J. Ophth. **27**:483, 1944. Gregg, N. M.: M. J. Australia **1**:313, 1945. Welch, L. S. V.: ibid. **1**:574, 1945. Long, J. C., and Danielson, R. W.: Arch. Ophth. **34**:24, 1945. Greenthal, R. M.: Arch. Pediat. **62**:53, 1945. Albaugh, C. H.: J. A. M. A. **129**:719, 1945. Carruthers, D. G.: M. J. Australia **1**:315, 1945. Prendergast, J. J.: Arch. Ophth. **35**:39, 1946. Guerry, D.: Am. J. Ophth. **29**:190, 1946. Goar, E. L. and Potts, C. R.: ibid. **29**:566, 1946. Swan, C., and Tostevin, A. L.: M. J. Australia **1**:645, 1946. Aycock, W. L., and Ingalls, T. H.: Am. J. M. Sc. **212**:366, 1946.

dence of malformations is nearly 100 per cent. Most commonly affected are the eyes (cataract, microphthalmia), the brain (mental retardation, microcephaly) and the heart. Only a few cases have as yet been studied at autopsy.[218] In contrast to most other authors, Fox and Bortin [219] conclude from their investigation of epidemics of rubella in Milwaukee that many pregnancies are unaffected. Conte, McCammon and Christie [220] sent 120 questionnaires to the mothers of infants with malformations of those types which may be caused by rubella. Of 61 mothers who answered, 5 gave a history of having had rubella during pregnancy. In view of the large number of other possible causes of the malformations in question, this is a significantly high incidence.

The developmental changes in fetal syphilis are too well known to be discussed here. Numerous reports deal with less common agents infecting the embryo—with virus,[221] bacteria [222] and protozoa.[223] Particularly tubercle bacilli have received a great deal of attention.[224] Localization of infectious organisms in the placenta is important in the mechanism of spreading of some of the diseases due to them, par-

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218. Swan, C.: J. Path. & Bact. **56**:289, 1944; Tr. Ophth. Soc. Australia **4**: 132, 1944.

219. Fox, M. J., and Bortin, M. M.: J. A. M. A. **130**:568, 1946.

220. Conte, W. R.; McCammon, C. S., and Christie, A.: Am. J. Dis. Child. **70**:301, 1945.

221. (*a*) Reuss, A., in Halban, J., and Seitz, L.: Biologie und Pathologie des Weibes, Berlin, Urban & Schwarzenberg, 1927, vol. 8, p. 2. (*b*) Lynch, F. W.: Arch. Dermat. & Syph. **26**:997, 1932. (*c*) Brody, H.: New York State J. Med. **41**:1256, 1941. (*d*) Woolpert, O. C.: Am. J. Path. **12**:141, 1936. Gallagher, F. W., and Woolpert, O. C.: J. Exper. Med. **72**:99, 1940. Goodpasture, E. W.: Science **95**:391, 1942. Goodpasture, E. W., and Anderson, K.: Am. J. Path. **18**:563, 1942.

222. Reuss.[221a] Wohlwill, F., and Bock, H. E.: Arch. f. Gynäk. **135**:211, 1929; Beitr. z. path. Anat. u. z. allg. Path. **85**:469, 1930. Burn, C. G.: Am. J. Path. **12**:341, 1936. Kelley, R. W.: Arch. Path. **28**:248, 1939. Diddle, A. W., and Stephens, R. L.: Am. J. Obst. & Gynec. **38**:300, 1939. Roback, H. N., and Kahler, H. F.: J. Nerv. & Ment. Dis. **94**:669, 1941. Price, S., and Chang, T.: Arch. Path. **41**:450, 1946.

223. (*a*) das Gupta, B. M.: Indian M. Gaz. **74**:397, 1939. (*b*) Paige, B. H.; Cowen, D., and Wolf, A.: Am. J. Dis. Child. **63**:474, 1942. (*c*) Koch, F.; Wolf, A.; Cowen, D., and Paige, B. H.: Arch. Ophth. **29**:1, 1943. (*d*) Zuelzer, W. W.: Arch. Path. **38**:1, 1944. (*e*) Pratt-Thomas, H. R., and Cannon, W. M.: Am. J. Path. **22**:779, 1946.

224. Whitman, R. C., and Greene, L. W.: Arch. Int. Med. **29**:261, 1922. Schmorl, G., in Engel, S., and Pirquet, C.: Handbuch der Kindertuberkulose, Leipzig, George Thieme, 1930, vol. 1, p. 137. Siegel, M.: Am. Rev. Tuberc. **29**:297, 1934. Siegel, M., and Singer, B.: Am. J. Dis. Child. **50**:636, 1935. Conrad, C. A.: South. M. J. **32**:169, 1939. Schaefer, G.: Quart. Bull., Sea View Hosp. **4**:457, 1939. Schwartz, R., and Gerhart, O.: Compt. rend. Soc. de biol. **129**:1095, 1939. Schenck zu Schweinsberg, H. G.: Monatschr. f. Kinderh. **88**:145, 1941. Loewenstein, E.: Am. Rev. Tuberc. **51**:225, 1945.

ticularly tuberculosis. As far as the investigators know, developmental disorders are but rarely caused by these infections. However, the example of rubella shows how unsuspected correlations between embryonic infection and malformation may become apparent and their medical importance appreciated once they are brought to attention. Cellular inflammatory reaction to infection in embryonic tissues has been examined in experimental and clinical material.[225]

COMMENT

The knowledge of the causes of malformations reviewed in the preceding pages is based largely on experiments. Part of it, being based on experiments with lower vertebrates, does not apply directly to man and other mammals with their excellent protection of the embryo. It has been reviewed not only in order to illustrate general principles of teratology but also because the resulting malformations are often comparable in more or less detail with mammalian ones. The development of the malformation in an experimental animal shows how a similar condition in man may have arisen. This comparison cannot offer more than a suggestion, since it is known that different mechanisms may lead to similar final results. Incidental findings of a few early stages in man may then support or refute the comparison. Moreover, the time of action may be more important than the exact nature of a teratogenic agent, as will presently be discussed. Thus the action of agents which in themselves do not occur in man and other mammals may be duplicated by others which exert the same influence (e.g., inhibition of development) at a comparable time and place in the embryo.

It has repeatedly been postulated that the action of detrimental agents on development depends to a considerable degree on intrinsic circumstances in the embryo. As an example, the previously mentioned temporary anoxia of the left side of the head of the chick embryo may be recalled. It not only produces a transitory lag in the normal development of the left eye but is presumably the cause of the marked preponderance of sporadic malformations of the left eye of the chick embryos.[18] More often a rapid rate of growth and development has been regarded as a predisposing factor,[1a] and it is on this basis that investigators have explained how nonspecific detrimental factors acting on the entire embryo during limited periods may produce well defined

225. Wohlwill, F., and Bock, H. E.: Virchows Arch. f. path. Anat. **291**:864, 1933. Lillie, R. D.: Pub. Health Rep. **50**:1498, 1935. Goldsworthy, N. E., and Moppett, W.: J. Path. & Bact. **41**:529, 1935. Goodpasture, E. W., and Anderson, K.: Am. J. Path. **13**:149, 1937. Buddingh, G. J., and Polk, A. D.: J. Exper. Med. **70**:485 and 499, 1939. Canat, E. H., and Opie, E. L.: Am. J. Path. **19**:385, 1943.

malformations only in certain organs. Child [226] and his followers have developed the concept of gradients of vital activities in developing organisms and have shown how these gradients influence normal and abnormal development. Thus Hyman [227] has discussed the influence of high rates of growth at given points on the effects of injurious agents. The defect produced is more severe in a part whose rate of growth is higher than those of others exposed to the same agent. Up to a certain limit, this may be compensated by a great ability of the rapidly growing part to make restitution of itself. Beyond that limit, the permanent damage will be greater in a part with a high growth rate than elsewhere. Thus a pattern of sensitivity exists in the developing organism at a given stage which may be responsible for identical reactions to widely differing teratogenic agents, genetic and environmental. Not only can different abnormal genes produce the same malformation,[54] but exact phenocopies [228] of hereditary abnormalities may be produced by agents affecting the tissues of the individual itself and not its genetic constitution.[229] The concept has evolved that at least part of the teratogenic genes affect the embryo in a nonspecific manner at a constant stage of embryonic development. This agrees well with recent work suggesting that much if not all of gene action affects metabolic processes.[12] It has been pointed out that phenocopies have been produced only of those hereditary traits which are assumed to be caused by a change in the relative rates of developmental processes (which are of greater importance in the production of malformations). On the other hand, no phenocopies are on record of traits which interfere with definite steps of chemical synthesis.[230] Perhaps this is so because the former act but once, whereas the latter act throughout life and cannot very well be imitated by extrinsic agents. The assumption of an action of genes on metabolism is supported by several previously mentioned observations. Lowering the incubation temperature and thus the rate of growth of the embryo at a given time can reduce its reaction to gene action and diminish the expression of certain hereditary traits in the chick.[210] The cumulative effect of the hereditary Creeper factor and selenium poisoning in the chick embryo has been explained by the assumption that both interfere in a similar manner with metabolic activity.[49]

226. Child, C. M.: Am. Naturalist **58**:237, 1924; Patterns and Problems of Development, Chicago, University of Chicago Press, 1941.

227. Hyman, L. H.: Biol. Bull. **40**:32, 1921.

228. A phenocopy is an abnormality closely resembling a certain hereditary trait, but produced by an influence on the individual itself, and not hereditary.

229. (*a*) Cairns.[46] (*b*) Kaven, A.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:247, 1938; footnote 92.

230. Goldschmidt, R. B.: J. Exper. Zool. **100**:193, 1945.

It is apparent that malformations are the effect of a variety of intrinsic and extrinsic, synergistic or antagonistic agents. Many of these act in a nonspecific manner and may be replaced by others with similar action without changing the final result.

In the preceding pages one may find several instances of discrepancies in the results obtained by different workers with the same agent. This may be due to differences in the technic of the experiment or in the genetic constitution of the animals used. It is obvious from what has just been said that slight variations of the strength of the agent and particularly of the timing of its action may change the results fundamentally. The interaction of genetic and environmental factors in the production of malformations has been referred to on several occasions, and this may explain variations in the results of experiments with different strains of animals.

There is a method of approach to malformations and their causative agents which has not yet been mentioned in this review, namely, statistics. It is quite possible that in the future many questions of the action of hereditary or environmental influences on the developing organism will be answered by statistical evaluation of large groups of cases. Particularly concerning human maldevelopment, results might be obtained which would otherwise not be available, as experimental methods cannot be used. A detailed study of a large number of human cases correlated with information on various aspects of environment and other factors has been made by Murphy.[11] The only consistent result so far obtained in several independent statistical investigations is a striking difference between white persons and Negroes in the over-all incidence of malformations; the incidence is higher in white persons.[231]

231. Murphy,[11] Potter, E. L.: J. A. M. A. **115**:996, 1940. Gruenwald, P.: Illinois M. J. **79**:55, 1941.

MECHANISMS OF ABNORMAL DEVELOPMENT

II. Embryonic Development of Malformations

PETER GRUENWALD, M.D.
NEW YORK

MOST treatises on abnormal development classify their subject either according to morphologic aspects or according to hypothetic modes of origin deduced from the final structure as, for example, inhibition or excess of development and fusion or fission of primordia. Cyclopia, for instance, would be found in the category of malformations by fusion of normally separate bilateral primordia. The brief discussion of cyclopia given in the introduction to the first part of this review shows that this or any other classification of it under the system just mentioned must be incorrect and misleading. Many other malformations develop in a manner which cannot possibly be detected by the study of the final condition alone as, for instance, the defects of the extremities in a hereditary syndrome of malformations in mice ("myelencephalic blebs," see page 556). It thus appears that a system based on hypothetic mechanisms of development of malformations is of no value either for classification or as a guide for future investigations. Its inadequacy has been pointed out by Weiss.[3] The causes of malformations, on the other hand, do not constitute a suitable basis for classification for two reasons. One is that in cases of malformation not produced in the laboratory the cause is often unknown, and the other reason is that many different causes may produce similar effects, or the same cause different effects under but slightly differing conditions. This leaves, in the present state of knowledge, only a classification from the standpoint of morphology, and that will not be discussed here as this presentation is not concerned with purely morphologic aspects of the subject.

In the following pages some of the better studied mechanisms will be described by which a malformation develops into its final form after an initial lesion has been produced by a known or an unknown cause.

From the Department of Pathology, Long Island College of Medicine, Brooklyn.

This work was begun while the author was Fellow in Pathology at the Mount Sinai Hospital, New York.

The review of the literature was concluded in August 1946. However, many European journals of the past few years were not available at that time, on account of the interruption of communications during the war.

Grüneberg [1r] classifies the mechanisms by which mutations manifest themselves as structural abnormalities, as follows:

(*a*) A normal developmental process is suppressed or remains incomplete.

(*b*) A normal developmental process is exaggerated.

(*c*) A normal developmental process is deflected in the wrong direction.

(*d*) The changes are regressive (degenerations).

Grüneberg admits that there are no clearcut borderlines between these mechanisms and that some abnormalities could conceivably be classified under different headings. He also suggests that it may be necessary to reclassify a malformation if new information is obtained regarding its development. Many of the nonhereditary malformations can also be classified in this manner.

A more detailed classification, which also includes mechanisms occurring in malformations due to extrinsic factors, will now be given:

(*a*) Inhibition or excess of a developmental process.

(*b*) Abnormal developmental pattern (absence, excessive number, division, fusion or abnormal location of primordia).

(*c*) Qualitatively abnormal development.

(*d*) Degeneration of previously normal-appearing parts.

(*e*) Interference by nonspecific reaction to injury.

(*f*) Elimination of parts.

The basic abnormal process is usually limited to a short period of development, leaving a part of the body in an abnormal condition. The affected part will thereafter develop by normal mechanisms but will show the effect of the interference because these normal mechanisms occur in an abnormal substrate. In some cases the abnormality may be more or less eliminated by regenerative processes.

Syndromes of malformations occur when the primary abnormality affects more than one part, or when the primarily affected part influences other parts so that they, too, become abnormal. The correlations which link the various manifestations of teratologic syndromes may be of the following kinds:

(*a*) Genetic mechanisms.

(*b*) Equal susceptibility of parts.

(*c*) Developmental patterns governing several parts.

(*d*) Functional dependence.

(*e*) Mechanical correlations.

These mechanisms are by no means mutually exclusive. In fact, few instances will be found in which only one of them is in effect. They

can therefore not be used for a classification of fully developed syndromes but are used rather as guides for the study of the development of the syndromes. It must be remembered that some of these mechanisms normally safeguard the development of proper morphologic relations of parts, but they may greatly increase the extent of a malformation if they are shifted to wrong tracks, as it were, by an abnormality somewhere along the line of developmental steps. These chains of events explain the occurrence of syndromes of malformations and the often puzzling repetition of complex developmental aberrations in many persons. The following examples of mechanisms of developmental correlation are given to illustrate the aforementioned types.

Genetic Correlations.—When several malformations which are independent of each other in their development are caused by one gene they may be said to be genetically correlated. Grüneberg [232] holds that this so-called pleiotropic gene effect does not exist and that an adequate understanding of the developmental mechanism would in all instances reveal a single primary developmental disturbance responsible for all manifestations. It is true that in several instances complex hereditary syndromes of malformations have been traced back to a single initial disturbance. Gluecksohn-Schoenheimer [233] has recently criticized Grüneberg's view and holds that final judgment should be reserved until more is known about gene action. On purely speculative grounds one may say that since it is assumed that each gene is uniform, its primary action is likely to be uniform as well. Whether or not one accepts the concept of pleiotropic gene action on development depends on the arbitrary decision whether or not the primary action is considered a developmental process. Examples of apparently pleiotropic gene effect are instances of hereditary intersexuality in various mammals associated with a peculiar color of coat [59a] or with skeletal malformations.[234]

Another possibility of genetic correlation is linkage of genes which are located at nearby points in the same chromosome so that it is improbable that they will be separated during the maturation of germ cells. The results of numerous linkage tests in the mouse have been reviewed by Snell [1p] and Grüneberg,[1r] and recently in the form of a chromosome map.[235] If a gene is located in the sex chromosome, its transmission is modified by the peculiarities of that chromosome: sex-linked inheritance. In the present discussion of the embryogenesis of malformations, genetic correlations will not be considered, since their action is not on

232. (*a*) Grüneberg, H.: J. Genetics **45**:1, 1943; (*b*) footnote 1r.

233. Gluecksohn-Schoenheimer, S.: Genetics **30**:29, 1945.

234. Surrarrer, T. C.: J. Hered. **34**:175, 1943.

235. Staff of the Roscoe B. Jackson Memorial Laboratory: J. Hered. **36**:271, 1945.

a developmental basis, but precedes development. Only their results will occasionally be referred to.

Equal Susceptibility of Parts.—It must be recognized that equal susceptibility of primordia to the action of teratogenic factors of any kind is an important mechanism by which seemingly unrelated developmental processes may be affected by one agent. It was reported in part I that many workers in the field of teratology consider the majority of teratogenic stimuli as nonspecific retardations of metabolism and development, which have a seemingly specific effect on certain parts only because these parts are highly susceptible at the time of their action. This susceptibility is thought to be greatest while a given part is growing or differentiating rapidly, and if this is true, it is obvious that several parts may be in a highly susceptible phase at the same time. This has been emphasized in the explanation of multiple malformations in various parts of the body caused by the Px mutation in guinea pigs.[236]

Developmental Patterns Governing Several Parts.—Correlations determined by the developmental pattern exist when an abnormal pattern of a larger portion of the body determines the development of malformations in several parts, much in the same manner in which the normal pattern would determine normal development of the same area. In cyclopia, for instance, a change in the developmental pattern of the neural plate is responsible for malformations of brain and eyes.

Correlation by Independent Development.—This occurs in abnormal development just as in normal ontogenesis. If one part depends on another for its normal development, it will be abnormal if that other part is absent or abnormal. Thus absence of a wolffian duct in the embryo will result not only in absence of the ductus deferens, which arises from it, but in absence of the epididymis and the kidney because these organs depend in their development on stimulation by the wolffian duct and its branch, the ureteric bud, respectively.

Functional Dependence.—Correlation by functional dependence may occur in various forms. Defects of the respiratory organs of the embryo may produce malformations of various organs by anoxia before, or without, killing the embryo. This occurs in the homozygous Creeper chick embryo when the circulation of its yolk sac fails (see page 554). Abnormal function of endocrine organs may have profound effects on development as, for instance, excessive hormone production by adenomas of the adrenal cortex, which promotes sex reversal in the embryo as well as after birth. Correlations between the nervous system and the organs supplied by it are conspicuous in postnatal life and not entirely absent in the embryo.

236. Scott, J. P.: (*a*) J. Exper. Zool. **77**:123, 1937; (*b*) J. Morphol. **62**:299, 1938.

Mechanical Correlations.—If an abnormal condition of one part makes it mechanically impossible for another part to pursue its normal course of development, even though its primordium is present and normal, there is observed a mechanical correlation affecting development. The just mentioned absence of a wolffian duct, for instance, has a mechanical effect in addition to the one already noted. The duct serves normally as a mechanical guide of the müllerian duct, which grows caudad within the wolffian duct's basal membrane. In the absence of this guide the müllerian duct fails to grow, and as a result the tube and one horn of the uterus are absent if the individual is of female sex. Another example is the absence of a choroid fissure in maldeveloped eyes, as it occurs in certain cases of microphthalmia or cyclopia (see page 518). It deprives the optic nerve fibers of their passageway to the brain, and results in absence of the optic nerve even though the optic stalk and the nerve fibers are all originally present.

The foregoing may not be a complete listing of all the methods by which abnormal traits, once appearing at one point, may express themselves throughout the organism, but it comprises the essential mechanisms found in the malformations to be described. It is obvious that these correlations may appear in various combinations, as may be gathered from the fact that several of the few syndromes of malformations given as examples appear under more than one heading.

Malformations appear in nature and in the laboratory with varying degrees of regularity, and this has been used to classify them as typical or atypical.[237] Malformations of the former type are found repeatedly either as identical forms or as various degrees of the same type of aberration. They appear to follow a plan almost or wholly as definite as that of normal development. Atypical malformations, on the other hand, are irregular and unpredictable. The typical malformations are supposedly due to genetic causes, the atypical ones to environmental influences which may attack the embryo at any time and place. The latter statement is not correct, as is illustrated by the regular production of malformations by influencing the embryo itself (see the first part of this review) and particularly by the existence of phenocopies in which a genetically caused malformation is copied by interference with ontogenesis alone. However, it is obvious that some of the aforementioned types of correlation of abnormal developments will tend to yield typical, and others atypical, defects. Genetic correlations of abnormal traits are as regular as those governing normal development, and they yield consistent results unless other, modifying genes or extrinsic agents interfere. Syndromes caused by abnormal developmental patterns or dependent

237. von Szily.[1c] Politzer, G., and Sternberg, H.: Frankfurt. Ztschr. f. Path. **37**:174, 1929.

development will be among the most regular ones. On the other hand, the most irregular and atypical abnormalities may be found among simple malformations produced by extrinsic agents.

Another factor which may influence the final appearance of a structural abnormality is restitution of defective parts. In embryonic malformations the normal mechanisms of regulation of development will often provide some restitution of a defective pattern before it is structurally differentiated, and this will then not be apparent as regeneration. There are extensive studies of regeneration of extremities in larvae of amphibia [238]; their results have contributed materially to the understanding of malformations of these parts. An interesting example of the complicated mechanisms of regeneration is that of replacement of an extirpated lens in amphibia, the so-called wolffian lens regeneration. The new lens is formed not from the superficial ectoderm (cornea) as was the normal lens but from the upper portion of the iris, which is normally not concerned with lens formation.[239] In malformations of the eyes, abortive or even well organized lenses may develop in the same manner.[18] Schotté [240] has presented a concise review of the general problems involved in regeneration.

In the following pages some of those malformations on the embryonic development of which information is available will be discussed. Then the widespread manifestations of two extensively studied hereditary syndromes of malformations (Creeper, myelencephalic blebs) will be summarized, as well as the effects of heterospecific pregnancy.

TWINS AND DOUBLE MONSTERS

Of the two principal types of twins and multiple births, the dizygotic or fraternal twins are of little interest in the present discussion since they are not essentially different from brothers and sisters born at different times except that they may, on rare occasions, interfere with each other's development. This occurs particularly if close relations develop between the chorions of the siblings, leading to fusion of the membranes and often to anastomoses of their circulatory systems. This fusion is known to occur in man [241] as well as in other mammals.[242] In certain species of mammals, anastomoses between the circulations of heterosexual dizygotic twins result in abnormal sexual development of the female, apparently caused by some substance transmitted from the male partner. In other species, including man, no such influence is ever exerted in the presence of vascular anastomoses (see part I).

238. Mangold, O.: Ergebn. d. Biol. **5**:290, 1929. Weiss.[3] Needham.[4]
239. Mangold.[1f] Weiss.[3]
240. Schotté, O. E.: Growth (supp.) **3**:59, 1939.
241. Arey, L. B.: Anat. Rec. **23**:253, 1922.
242. Witschi.[160] Wislocki, G. B.: Am. J. Anat. **64**:445, 1939.

Monozygotic or identical twins, arising from a single, normally fertilized egg cell, are of great interest from the point of view of teratology. While each partner of the pair may be perfectly normal, monozygotic twinning in man is too rare to be considered as a normal occurrence. In certain animals, on the other hand, it occurs invariably and must be regarded as normal. The close relationship of twins to double monsters will be discussed subsequently. In the laboratory, twins and double monsters have been produced by a variety of methods. A thoroughly investigated procedure is that of constricting amphibian eggs to a varying extent, which results in separate twins or monsters with varying degrees of duplication depending on the intensity of constriction.[2] Many other methods have yielded twins and double monsters by mechanisms which are not so well understood, such as hybridization, delayed fertilization,[36] irradiation with roentgen [168] and ultraviolet rays,[85] splitting,[243] centrifuging [72] or chemicals.[244] A hereditary influence in human twinning is assumed by numerous authors [245] and denied by others.[246] An example of definite hereditary twinning is the development of multiple embryos in germs homozygous for the lethal gene "kinky" which in heterozygous condition produces abnormalities of the vertebrae, the tail and the labyrinth. These germs die early.[247] Danforth [248] described hereditary duplication of the caudal part of the body in mice.

There has been much speculation concerning the cause of polyembryony (monozygotic multiple birth) as it occurs regularly in the armadillo, partly with a view to explaining human twinning. Newman [249] and Stockard [18] hold that a temporary slowing of development, which is known to occur in these species, is responsible for a dispersion of the organization center into several independent centers; these authors also suggest that cooling of birds' eggs before gastrulation (which normally occurs before laying) is responsible for twinning. Sturkie [250] found that if hens are chilled about the time of ovulation and cleavage, the incidence of double embryos rises to 8.2 per cent. Newman set up a hypothesis for polyembryony of the armadillo, which includes the following steps:

243. Morita, S.: Anat. Anz. **82**:81, 1936; **84**:81, 1937. Twiesselmann, F.: Arch. de biol., Paris **49**:285, 1938.

244. Werber.[99] Morita.[243]

245. (*a*) Danforth, C. H.: J. Hered. **7**:195, 1916. (*b*) Davenport, C. B.: Proc. Soc. Exper. Biol. & Med. **17**:75, 1920. (*c*) Wehefritz, E.: Ztschr. f. menschl. Vererb- u. Konstitutionslehre **11**:554, 1925. (*d*) Hamlett, G. W. D.: Anat. Rec. (supp. 2) **73**:26, 1939.

246. Greulich, W. W.: Am. J. Phys. Anthropol. **19**:391, 1934.

247. Gluecksohn-Schoenheimer, S.: Anat. Rec. **94**:462, 1946.

248. Danforth, C. H.: Am. J. Anat. **45**:275, 1930.

249. Newman, H. H.: The Physiology of Twinning, Chicago, University of Chicago Press, 1923.

250. Sturkie, P. D.: J. Exper. Zool. **101**:51, 1946.

slow formation of the corpus luteum; no response of the uterine mucosa to the early embryo; late placentation; cessation of development for three weeks; deaxiation of the embryo; isolation of four growing regions. Hamlett [251] opposes this view and assumes that only genetic control, and not differences in temperature or in metabolic rate, can account for a multiplicity of organization centers that occurs as regularly as that seen in the armadillo. In support of this he [252] quotes the familial occurrence of twinning in man and the fact that many mammals regularly show a standstill of early embryonic development without twins ever developing. These arguments do not completely disprove the first mentioned hypothesis, as hereditary factors in man may be thought to produce twinning by reducing the metabolic rate at a given point, and the slowing of development in species without twinning may not occur just at the time when the organization center is established (of which time investigators have no knowledge). It is known that minute differences in the timing of teratogenic action may completely change the result (see part I). The assumption of cooling as the cause of twinning in birds is opposed by Riddle [253] on experimental grounds. Needham [4] tends to agree with Hamlett's view. Arey [254] finds an increased incidence of monozygotic human twins in ectopic pregnancies and is therefore inclined to assume the existence of extrinsic factors. Here, as in many other teratologic arguments, it is often forgotten that many different agents, hereditary and environmental, may produce the same result and that the occurrence of one agent in some cases does not disprove the presence of others in other cases.

The mutual relations of monozygotic twins and their membranes depend on the stage at which twinning occurs. According to Greulich,[246] all three theoretically possible forms may occur: a fertilized egg cell may divide and the daughter cells separate themselves from one another and develop into twins with completely separated membranes and placentas. If twinning manifests itself later in development, a single blastocyst may develop but may contain two embryoblasts resulting in a common chorion and separate amnions. Finally, from a single embryoblast in a single blastocyst two embryos may develop in one embryonic disk, resulting in twins with a common amnion and chorion, as well as a common yolk sac.[254] If all these possibilities should concur, monozygotic twins could have all possible relations of their membranes, including separate chorions, which are often assumed to be proof of dizygotic origin. This, together with the fact that the chorions of dizy-

251. Hamlett, G. W. D.: Quart. Rev. Biol. **8**:348, 1933.

252. Hamlett, G. W. D.: Quart. Rev. Biol. **10**:432, 1935.

253. Riddle, O.: Am. J. Anat. **32**:199, 1923.

254. Arey, L. B.: Anat. Rec. **23**:245, 1922.

gotic twins may fuse secondarily into one, shows that the membranes cannot be relied on in the determination of the type of twinning in a given case. More and more emphasis is therefore being placed on various hereditary somatic characters in the twins which should be significantly more similar in monozygotic than in dizygotic twins. However, work with these somatic traits revealed intermediate conditions, which led Danforth [245a] to postulate a third type of twinning. In this type only one gamete, which can only be the egg cell, is common to both twins, whereas their paternal component is derived from two different spermatozoons. No definite explanation of the mechanism is given, but the following possibility is suggested on the basis of work on lower animals. Immediately after an egg cell has been penetrated by a spermatozoon it divides in such a manner that only one of the daughter cells copulates with the entire chromatin of the spermatozoon. The other daughter cell is subsequently fertilized by another spermatozoon. This hypothesis has been taken over by Greulich [246] in a somewhat distorted form, according to which each of the daughter cells would finally be triploid, with chromosomes from two spermatozoons. This is an untenable as the old idea that twins derive from an egg cell fertilized by two spermatozoons. Boveri [255] showed long ago that fertilization of an egg cell by two spermatozoons leads not to twins but to early death, owing to unequal distribution of the chromatin of three gametes among the poles of a tetrapolar mitosis during the first cleavage.

The close topographic relations of monozygotic twins may lead to interference with the normal development of one or both partners. If extensive vascular anastomoses exist between the twins, particularly in a common placenta, one twin may become an acardius if its circulation is inferior to that of the other sibling and is finally taken over by the stronger one. The heart of the weaker twin will stop functioning, and the heart of the stronger one will drive the blood through the common placenta and the other twin. The acardius is invariably and severely maldeveloped in the late stage in which it is usually examined; large parts of the body are entirely absent. Two opposing views of the possible development have been advanced,[256] one holding that both twins are normal at first and differ only in vigor, and the other one assuming that the acardius is primarily maldeveloped, and its circulation taken over by the other twin as a consequence of this. Examination of a pertinent case of twins in a very early stage has recently [83] produced a third suggestion, namely, that the future acardius may in itself be normal, but have abnormal vascular conections with the other twin and the placenta. In the case in question, as well as in one briefly described

255. Boveri, T.: Zellenstudien, Jena, Gustav Fischer, 1907, vol. 6.

256. Schwalbe, E.: Die Morphologie der Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1907, pt. 2.

by Heaney and Bartelmez,[257] one of the twins has no direct vascular conections with the placenta. It is possible that any one of these three mechanisms may occur, depending on the conditions in each case.

Mechanical interference of twins with each other is occasionally observed. The history of the fetus papyraceus is not well understood. In early stages, pressure by a twin may cause invagination of the cranial end of the body into the yolk sac (omphalocephaly); this has been found in several birds.[82] Invagination of the caudal end into the yolk sac (ourentery), probably caused by the other twin, has been seen in a human ovum.[83]

Double monsters are structurally closely related to monozygotic twins. All transitions exist from low degrees of duplicity, which may be almost unnoticeable externally, through the commonly illustrated types of double monsters (conjoined twins) to identical twins which have separated bodies but which have in common one of their most important fetal organs, the placenta. The fact that the last-named twins are really also conjoined has been emphasized by designating them as choriopagi because of their common chorion, in analogy with the term "thoracopagi" for twins fused at their chests. Hamlett[245d] claims that monozygotic twins and double monsters are unrelated, basing his contention on the observation that double monsters do not occur in families with a hereditary tendency toward twining and are not related to inherited tendencies at all. Against this stand not only morphologic considerations but also the findings of Danforth[248] of hereditary posterior duplication in mice. It is doubtful whether statistically significant genetic results can be obtained concerning human double monsters in view of the rarity of the condition and the possibility of inheritance by more than one gene, perhaps in interaction with genetic or environmental modifiers. In Spemann's constriction experiments (see page 501) a variation in the intensity of the same agent produced all transitions from double monsters to twins.

The question has often been raised whether double monsters originate by splitting of one embryonic anlage or by fusion of two.[258] To me this appears immaterial, since it is obvious that at some early stage, probably before the beginning of visible differentiation, a division of the germ into two parts with their own organization centers must have occurred. Whether or not they fuse later on depends largely on topographic relations which, in turn, are partly determined by the time at which the duplicity is determined.

A considerable proportion of double monsters are asymmetric; that is, one of the partners is defective, depends for support on the other and

257. Heaney, N. S., and Bartelmez, G. W.: Anat. Rec. (supp.) **48**:47, 1931.

258. Newman, H. H.: J. Hered. **31**:371, 1940. Waddington, C. H.: ibid. **32**:268, 1941.

is referred to as a parasite. A comparable condition in separate twins is the acardius (see page 503). All grades of intermediate conditions have been described, leading from the relatively well formed parasitic twin to inclusions consisting of irregular mixtures of tissues or organs, namely, teratomas. Various theories of teratomas and their relationship to neoplasms will be briefly discussed later in this review. The occurrence of twins and teratomas in the same families has been observed,[239] and interpreted as indicating a related pathogenesis.

MEDIAN DEFECTS

An indication of the importance of median defects and related problems in the study of teratology was given in the brief review of cyclopia in the introduction to part I of this review. In addition to symmetric median defects of the head, including cyclopia, otocephaly and essentially similar malformations of minor degrees, defects of the caudal portion of the body have been investigated in great detail. Best known among these are sirens with fused legs, but there are many other varieties, mostly of less extent. Some of these are of medical importance, since they are viable or may be so with the help of plastic surgery. Finally, there is a small and not so well investigated group of similar defects of the trunk which do not affect either end of the body and will here be referred to as interstitial defects.

The median, symmetric defects of the head are often referred to under the common name of cyclopia (in the wider sense), and this will also be done here unless otherwise indicated. In the strict sense, "cyclopia" means a malformation in which the eyes are fused into one median organ. Several other defects are known to be regularly or frequently associated with this condition, such as a single median nasal cavity in a trunklike nose (proboscis) above the eye, defects of the oral cavity and of the derivatives of the branchial arches, and brain defects. Median defects of greater extent may lead to complete absence of the eyes. There may be ventral approximation or fusion of the ears, otocephaly. On the other hand, less degrees of median defects may leave the ears in their normal locations and the eyes approximated but separate (synophthalmia), or affect only the brain (arhinencephaly).

It is superfluous to describe in detail the development of knowledge of cyclopia as briefly outlined in the introduction, since all work up to 1936 has been reviewed by Adelmann.[260] His view is briefly as follows: In the normal embryo the pattern for the prospective eyes and nearby parts of the brain is determined in the neural plate stage in a labile manner. In the production and maintenance of this pattern the underlying tissue of the roof of the archenteron (the so-called substratum),

259. Edmonds, H. W., and Hawkins, J. W.: Cancer Research **1**:896, 1941.
260. Adelmann, H. B.: Quart. Rev. Biol. **11**:161 and 284, 1936.

and particularly the prechordal mesoderm, plays a leading role. The regular association of the malformation of the eyes and brain with defects of mesodermal parts in spontaneous and experimental cases indicates that an abnormality of the substratum (which develops into the defects of mesodermal parts) determines an abnormal pattern in the neural plate which in itself may have been normally formed. This was confirmed when cyclopic changes were induced in normal neural plates of amphibian embryos by experimental defects of the substratum alone.[261] It is not believed to be the rule that the primary defect comprises parts of mesoderm and ectoderm alike, although experimental defects of this kind have led to cyclopia.[262] Only minor contributions to the problem of cyclopia have been made since Adelmann's review. Lehmann [263] assumes that among the parts of the substratum the entoderm is more important for the determination of ocular development than the mesoderm. Marburg and Mettler [264] add to older studies of the morphologic aspects of cyclopia a detailed investigation of the nuclei of the cranial nerves. Gruenwald [265] gives an explanation of the frequent absence of the optic nerve in cyclopia, which puzzled previous workers because it could not readily be accounted for by changes in the pattern of the developing eye on the assumption of a primary absence of the optic stalk. In conformity with observations of microphthalmia of chick embryos it is assumed that an optic stalk is present in early stages but degenerates when nerve fibers fail to grow into it because of absence of a choroid fissure. A very recent interpretation of cyclopia [266] discards all previous hypotheses, however well founded, and proposes, on the basis of speculation, a different mechanism. The first aortic arches are assumed to fuse with one another and thus mechanically alter the direction in which the primordia of the eye grow out from the brain, causing them to fuse. Apart from all other difficulties of this explanation, it is obvious that cyclopia cannot develop by fusion of two eyes which are pushed toward each other. It must be determined in the pattern of the formation of eyes previous to the actual outgrowth, and fused aortic arches can have no part in this. It may be of interest to compile the various causes of cyclopia and related defects as observed in the laboratory (see also part I): elimination of tissue by excision or radiation,[8] centrifuging of fertilized eggs,[72] chemical treatment,[267] mutations [11] or hybridization.[43]

261. Adelmann.[9] Mangold.[1f] Sperling, F.: Anat. Rec. **85**:413, 1943.

262. Lewis.[7] Wolff.[8]

263. Lehmann, F. E.: Rev. Suisse de zool. **45**:413, 1938.

264. Marburg, O., and Mettler, F. A.: J. Neuropath. & Exper. Neurol. **2**:54, 1943.

265. Gruenwald, P.: Anat. Rec. **91**:13, 1945.

266. Krafka, J.: Arch. Ophth. **33**:128, 1945.

267. (*a*) Stockard.[6b] (*b*) McClendon.[6d] (*c*) Werber.[100] (*d*) Adelmann, H. B.: J. Exper. Zool. **67**:217, 1934. (*e*) Franke and Tully.[125]

The median symmetric defects of the caudal part of the body have not been investigated as thoroughly as those of the head. This is due largely to the peculiarities of the normal development of that region. There are not, as in the head, several distinct embryonic structures which, in order to develop properly, interact with each other in many ways. There is, on the contrary, a morphologically homogeneous mass of mesenchyme, the trunk-tail node. From this the respective parts of those organ systems arise which develop from distinct germ layers in the head region. This is a formidable obstacle to the experimental investigation of normal and abnormal correlations during early development.

The best known and most conspicuous representatives of caudal defects are sirens which have their legs fused into one median extremity. The skeleton shows parts of both extremities more or less incomplete or fused as, for instance, two femurs, two tibias and one median fibula. The normally lateral (fibular) sides of the extremities are turned medially, owing to changes in the pelvis, and are therefore the first to fuse. The knee points backward. For a review of the morphologic aspects of sirens, see Gruber.[268] Sirenoid malformations, as all caudal median defects have been called, also include minor degrees, such as sacral [269] and lumbosacral [270] defects, anchypodia (approximation and rotation of lower extremities without fusion) and other forms to be mentioned in the following pages. Examination of nonviable early embryos, homozygous for lethal genes, has revealed an instance of median defect severer than siren. In homozygous *T* mice the entire caudal portion of the body is absent.[271] These embryos die early. The reason why these forms are not usually seen is probably that they lack the allantois and its vessels, which are indispensable for the nutrition and the respiration of the embryo, and therefore die before they can be detected.

Feller and Sternberg [272] first devised a method by which all sirenoid malformations can be uniformly and systematically interpreted and classified. It is basically similar to that of Fischel in that it outlines the malformations in hypothetic early stages as wedge-shaped defects varying in length, width and position. This concept has been widely accepted, and Feller and Sternberg [273] extended it to certain defects of pelvic organs

268. Gruber, G. B., in Schwalbe,[256] 1937, pt. 3, p. 557.

269. Berman, W.: Am. J. Obst. & Gynec. **50**:447, 1945.

270. Zeligs, I. M.: Arch. Surg. **41**:1220, 1940. Sinclair, J. G.; Duren, N., and Rude, J. C.: ibid. **43**:473, 1941.

271. Chesley, P.: J. Exper. Zool. **70**:429, 1935. Gluecksohn-Schoenheimer, S.: Proc. Nat. Acad. Sc. **30**:134, 1944.

272. Feller, A., and Sternberg, H.: Virchows Arch. f. path. Anat. **280**:649, 1931.

273. Feller, A., and Sternberg, H.: Ztschr. f. Anat. u. Entwcklngsgesch. **108**: 283, 1938.

without abnormalities of the extremities, such as some forms of atresia ani and absence of the external genitalia. Klaften and Politzer [274] applied similar considerations to certain malformations with a persistent cloaca (a common terminal portion of the digestive and urogenital tracts). It is understood in all these considerations that, as in the corresponding concept of cyclopia, the defect is never present as a gap resulting from elimination of tissue but rather is present as a change which causes these median portions to be absent and more lateral ones to develop in contact with each other. While this concept can be used to classify sirenoid malformations, its validity as an explanation of the development of the malformations is open to the same criticism as the corresponding theory of cyclopia. It is implicitly based on the assumption of mosaic development, that is, development without correlation of the various parts concerned. That this cannot be taken for granted has been amply demonstrated in cyclopia. The possibility of a primary defect in only one organ in a key position, extending secondarily to other parts by developmental correlations, exists in sirenoid defects as well. Furthermore, the stage used by Feller and Sternberg in which to outline the defects, namely, a 6 mm. human embryo, is too old to use in pointing out the primary lesion, particularly since these authors themselves justly state that in a siren the malformation is determined in the earliest known stages of human development, since the allantois and its vessels are affected.

Very early stages of spontaneous sirens are not known. The youngest human siren on record [275] was a 19 mm. embryo, and its malformation is probably not a typical representative of the group. There is, however, ample information on the genetics, the structure and the embryonic development of related hereditary conditions, namely, taillessness and allied defects in mice [276] and rumplessness in fowl.[277] In all these cases the caudal end of the body is normal until, at a stage characteristic of each form, degeneration sets in and previously normal-appearing parts disintegrate and disappear. In some cases there are associated malformations of the cloaca and the urogenital organs, even though no

274. Klaften, E., and Politzer, G.: Beitr. z. path. Anat. u. z. allg. Path. **99**:70, 1937.

275. Gruenwald, P.: Beitr. z. path. Anat. u. z. allg. Path. **97**:417, 1936.

276. (*a*) Chesley, P., and Dunn, L. C.: Genetics **21**:525, 1936. (*b*) Steiniger, F.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **22**:583, 1938. (*c*) Dunn, L. C.; Gluecksohn-Schoenheimer, S., and Bryson, V.: J. Hered. **31**:343, 1940. (*d*) Dunn, Gluecksohn-Schoenheimer, Curtis and Dunning.[48] (*e*) Gluecksohn-Schoenheimer, S.: Genetics **28**:341, 1943. (*f*) Dunn and Gluecksohn-Schoenheimer.[54] (*g*) Gluecksohn-Schoenheimer, S., and Dunn, L. C.: Anat. Rec. **92**:201, 1945.

277. Landauer, W., and Dunn, L. C.: J. Hered. **16**:153, 1925. Landauer, W.: ibid. **19**:453, 1928. Dunn, L. C., and Landauer, W.: J. Genetics **29**:217, 1935; **33**:401, 1936. Zwilling, E.: Genetics **27**:641, 1942. Landauer, W.: ibid. **30**:403, 1945. Zwilling, E.: J. Exper. Zool. **99**:79, 1945.

degeneration has been found in these parts. This illustrates once more the inadequacy of an over-all explanation based solely on the extent of the defect in its final form. In chick embryos another mechanism occurs which leads to defects of the caudal median portions and sometimes to approximation of the lower extremities. This is a ventral displacement of all axial organs (ourentery) or of the notochord alone (chordentery) with subsequent partial or complete disappearance of the displaced parts.[278] Severe degrees are probably not viable, and mild degrees may well be the hitherto missing early stages of sporadic rumplessness. In these embryos the deviation of axial organs creates conditions equivalent to absence of their caudal parts. The youngest known human siren [275] shows conditions closely resembling these ourenteric chick embryos. Similar defects have been produced experimentally by destroying certain parts of the primitive streak by means of roentgen rays at definite stages.[8c] According to the current concept of the action of teratogenic factors (see part I) it is easy to understand why the primitive streak with its great morphogenetic activity should be particularly susceptible to damaging influences, such as temporary retardations of metabolism.

In reviewing the subject of sirenoid malformations, Gruenwald [278] comes to the conclusion that some, among them perhaps most of the human sirens, may well develop by a defect of just one leading part with subsequent abnormalities in other parts due to developmental correlations with the primary defect. This is assumed in analogy with cyclopia. However, in contrast to cyclopia, degeneration of previously normal parts, due to a hereditary deficiency, or dislocation of median organs plays an important role in many other instances.

The common term "interstitial defects" will be used here for all those in which nondefective (though perhaps not entirely normal) regions bound cranially and caudally on a defect of the median organs. Human malformations with defects of the vertebral column and the spinal cord in the lumbar region but not in the sacral portion have been described by Lücke [279] and by Feller and Sternberg.[273] The latter authors also give references to similar reports in the veterinary literature. According to roentgenograms published by Dobrovolskaia-Zawadskaia and Kobozieff,[280] similar malformations occasionally occur in mice of short-tailed stocks; this indicates a close relationship of interstitial and sirenoid defects. Wolff [8c] used localized roentgen irradiation to produce in chick embryos interstitial defects with *symptérie,* a median fusion of the upper extremities resembling sirenoid limbs, the more caudal regions not being affected. Treatment of amphibian embryos with lithium salts may, under properly controlled conditions, result in interstitial defects of

278. Gruenwald, P.: J. Morphol. **81**:97, 1947.

279. Lücke, H. H.: Frankfurt. Ztschr. f. Path. **50**:492, 1937.

280. Dobrovolskaia-Zawadskaia, N., and Kobozieff, N.: Compt. rend. Soc. de biol. **109**:420, 1932.

the notochord. However, these are not followed by defects of the vertebral column and the spinal cord even though these organs may be retarded or otherwise abnormal.

Complete median defects of the entire length of the body have, to the best of my knowledge, not been reported and probably do not occur. One malformation of a chick embryo closely approaches this condition.[278] It shows cyclopia and, throughout the body, a defect of the notochord resembling that seen in cases of chordentery of caudal regions. The spinal cord is reduced in width throughout, the spinal ganglions are partly median and ventral to the spinal cord, the upper and the lower extremities are abnormally near the midline dorsally, the tail is reduced and the mesonephrons as well as the permanent kidneys are fused in the midline.

SKIN

Much work has been reported on the gross and minute morphologic aspects of prenatally developing abnormalities of the skin and its appendages. This has been reviewed by Landauer,[281] David,[282] Steiner,[283] Lynch,[221b] Cockayne,[284] Grüneberg [1r] and others. However, very little is known about the developmental stages of these conditions. Many of the hereditary malformations of the skin, such as hairlessness in certain instances, develop only after birth.

The following conditions have been well studied, though not with regard to their developmental mechanisms: ectodermal dysplasia, affecting in various combinations skin, hair, sweat glands, teeth, eyes and brain [285]; epidermolysis bullosa of hereditary [286] or infectious origin (syphilis, pemphigus,[286a] variola and vaccinia [221b]); hairlessness (hereditary [282] or resulting from maternal dietary deficiency [195]); the Frizzle character of plumage of fowl [287]; congenital tumors of the skin, often multiple [288]; hereditary reduction in the number of mammary glands in guinea pigs [236b] and mice.[289] Several studies are on record of the postnatal

281. Landauer, W.: Ztschr. f. indukt. Abstammungs.- u. Vererbungsl. **42**:113, 1926; **50**:356, 1929.

282. David, L. T.: Ztschr. f. Zellforsch. u. mikr. Anat. **14**:616, 1932.

283. Steiner, K., in Jadassohn, J.: Handbuch der Haut- und Geschlechtskrankheiten, Berlin, Julius Springer, 1932, vol. 4, pt. 1, p. 1.

284. Cockayne, E. A.: Inherited Abnormalities of the Skin and Its Appendages, London, Oxford University Press, 1933.

285. Gordon, W. H., and Jamieson, R. C.: Ann. Int. Med. **5**:358, 1931. Christ, J.: Zentralbl. f. Haut- u. Geschlechtskr. **40**:1,1932. Cole, H. N.; Simmons, J. T., and Stroud, G. M.: J. A. M. A. **129**:723, 1945. Wilkey, W. D., and Stevenson, G. H.: Canad. M. A. J. **53**:226, 1945.

286. (*a*) Herlitz, G.: Acta pædiat. **17**:315, 1935. (*b*) Davidson, L. T.: Am. J. Dis. Child. **59**:371, 1940.

287. Landauer, W., and Dunn. L. C.: J. Hered. **21**:291, 1930.

288. Wilcox, J. C.: Am. J. Dis. Child. **57**:391, 1939. Vero, F.; Machacek, G. F., and Bartlett, F. H.: J. A. M. A. **129**:728, 1945.

289. Little, C. C., and McDonald, H.: J. Hered. **36**:285, 1945.

influence of defective heat regulation on the entire organism in Frizzle fowl.[290] Snyder and Doan [291] published an interesting observation on a family with telangiectasia. The paternal grandmother, the maternal grandfather and both parents of a child had the usual mild type, and the child itself died with a severe, generalized form. The authors suggest that the gene for telangiectasia may be lethal, being incompatible with life in homozygous form.

Of considerable importance are fetal defects of skin and the ensuing scars, because of their clinical significance as well as their relationship to alleged amniotic adhesions (see the section on extremities). From case reports, the majority of which do not include histologic observations, it is impossible to judge whether necroses and ulcers have the same nature and cause in all cases. Liegner [292] describes necrotic lesions of both forearms and indicates that oligohydramnios has caused the parts to press on each other at the sites of these lesions. Terruhn [293] favors the old amniogenic theory in his discussion of cutaneous defects and scars. Pantschenko [294], in a report of a case with necroses of both forearms, mentions as possible causes defective hereditary constitution, trophoneurosis and *Lutschflecken* (sucking spots). Ombrédanne and Lacassie [295] give an excellent discussion of what they call *maladie ulcéreuse intrautérine,* with the conclusion that if there are amniotic adhesions they are the result rather than the cause of ulcers. These ulcers may be sufficiently deep to cause defects of skeletal parts. Greig [296] describes median defects of the scalp, in which the normal layers are replaced by a thin membrane. He considers this membrane as a developmental substitute for the scalp, due to arrested development, rather than a scar. The median location and the familial occurrence speak against amniotic origin. Another report of a thin membrane at the vertex, combined with a cutaneous defect of the left leg, has recently appeared.[297] Ingalls [298] finds bleb formation under the epithelium in the development of these scalp lesions and assumes an inherent abnormality of the structures concerned. All these reports are concerned with human anomalies. Hadley [299] describes hereditary defects of the skin of the legs of calves,

290. Benedict, F. G.; Landauer, W., and Fox, E. L.: Bulletin 117, Storrs Agricultural Experiment Station, 1932, p. 130. Landauer, W.: Am. J. M. Sc. **194**:667, 1937; Biol. Symposia **6**:127, 1942.

291. Snyder, L. H., and Doan, C. A.: J. Lab. & Clin. Med. **29**:1211, 1944.

292. Liegner, B.: Monatschr. f. Geburtsh. u. Gynäk. **76**:278, 1927.

293. Terruhn, E.: Arch. f. Gynäk. **140**:428, 1930.

294. Pantschenko, N. A.: Zentralbl. f. Gynäk. **55**:3462, 1931.

295. Ombrédanne, L., and Lacassie: Arch. de méd. d. enf. **33**:199, 1930.

296. Greig, D. M.: Edinburgh M. J. **38**:341, 1931.

297. Callaway, J. L.; Noojin, R. O.; Riley, K. A., and Kuhn, B. H.: J. Pediat. **28**:214, 1946.

298. Ingalls, N. W.: Am. J. Obst. & Gynec. **25**:861, 1933.

299. Hadley, F. B.: J. Hered. **18**:487, 1927.

occurring in certain herds in the ratio of 1 diseased to 3 healthy calves. This clearcut case of a hereditary defect occurring in definite and symmetric locations speaks strongly against amniotic adhesions as the cause. In connection with these considerations Streeter's [76] arguments against amniotic causation of limb defects (the so-called amniotic amputations) should be remembered, as well as the familial and identical forms of these defects in man.[75]

CENTRAL NERVOUS SYSTEM

The commonest gross malformations of the central nervous system are dysraphia (clefts dorsally in the midline: encephaloschisis and myeloschisis) and hydrocephalus. It has been commonly assumed that defects of the former type are due to failure of the neural groove to close. This is doubtless true for the majority of cases. However, Bonnevie [300] showed that in two different hereditary malformations of mice the previously closed brain breaks open again, thus producing a condition which may later on be indistinguishable from primary failure to close. In one of these instances (the "shakershort" mouse) abnormal brain development causes abnormalities of the inner ear although the otic vesicles are normal in early stages. Kaven [229b] observed changes essentially like those described by Bonnevie, in mouse embryos irradiated with roentgen rays in utero. Perhaps a comparable mechanism of secondary rupture can explain the observation of Paff [116] that chick embryos may show dysraphia when treated with colchicine at the age of 48 hours, that is, after the neural tube should have closed completely. Another instance in which the simple explanation—failure of a normal neural plate to close—may not be applicable is spina bifida of the caudal portions of the body. There is in the embryo a region in which the neural tube forms not by closure of a groove but by hollowing out of a solid cord; this occurs in the trunk-tail node. Just how large the caudal part of the body is which develops in this manner is not known. A region where development almost certainly occurs from the trunk-tail node is the base of the tail, where typical myeloschisis has been observed in a sheep embryo.[301] The appearance of the abnormality is too regular to make one assume a secondary dehiscence, and the most probable explanation is that a neural plate was formed where it would not normally develop. This consideration may very well hold for the sacral region of the human embryo, where dysraphia is relatively common. This will not be definitely known until the exact extent of body formation from the trunk-tail node in man is determined. The clinical aspects of dysraphia have been discussed by several authors.[302]

300. Bonnevie, K.: Genetica **18**:105, 1936; Skr. Norske Vid. Ak. Oslo **9**:39, 1936.

301. Gruenwald, P.: Unpublished data.

302. Ingraham, F. D., and Swan, H.: New England J. Med. **228**:559, 1943.

A peculiar and teratologically interesting form of encephaloschisis and myeloschisis occurs frequently in chick embryos but has, to the best of my knowledge, not been seen in man and mammals. This is platyneuria, characterized by a flat, abnormally thick neural plate. If there is any attempt to form a neural groove, it is not by rising of the borders as this occurs in early stages of normal development or remains permanently in some cases of the usual dysraphia but by a narrow, steep infolding of the median portion alone while the lateral parts remain flat.[84] The differentiation in later stages is severely disturbed in all cases, a phenomenon which is not observed in the usual dysraphia. In the brain the usual divisions cannot be recognized, and the eyes are either absent or so severely deformed that one may not recognize them until in late stages by their pigmentation. Histogenesis is also abnormal, and numerous rosettes may be found in the brain substance.[303] Thus platyneuria is not only an inhibition of the normal folding and closure of the neural plate but a thorough disturbance of many phases of its organization as well. In many laboratories platyneuria occurs frequently, perhaps owing to unfavorable conditions of incubation, such as inadequate ventilation. Experimentally, platyneuria has been produced by a great increase of the concentration of the carbon dioxide in the air.[201] In this connection a recent report of Patten [304] is of great interest. It indicates that in human dysraphia there is also an abnormal growth tendency, producing an abnormally large neural plate. This is considered as a possible cause of the failure to close. I have confirmed this in part of the cases at my disposition. The thickening of the neural plate is not accompanied by the other severe disturbances found in platyneuria of the chick.

It seems that there are rare cases in which mechanical injury causes the brain of the human embryo to rupture at points distant from the normal line of closure. These cases have added interest because there may be nodes of nervous tissue growing within the lungs, owing probably to embolism at the time of the injury.[81]

Hydrocephalus is usually the result of obstruction of the route which the cerebrospinal fluid takes from the ventricles to the subarachnoid space. At least three different kinds of hereditary hydrocephalus of mice are known, and these have been studied in some detail. In one of them the aqueduct was obliterated.[305] In another one an abnormal configuration of the roof of the fourth ventricle is caused by a disturbance of the growth of the cartilaginous base of the skull.[232a] The third muta-

303. (*a*) Podmaniczky, T.: Frankfurt. Ztschr. f. Path. **5**:255, 1910. (*b*) Gruenwald, P.: Anat. Rec. **94**:518, 1946.

304. Patten, B. M.: Anat. Rec. **94**:487, 1946.

305. Clark, F. H.: Anat. Rec. **58**:225, 1934.

tion,[306] as well as hydrocephalus produced in embryos by roentgen rays,[307] has not been investigated embryologically.

Lichtenstein [308] has given a plausible explanation of those relatively numerous cases in which hydrocephalus of man is associated with spina bifida of the lower portion of the spinal cord. He found that relative shortening of the spinal cord, which normally results in a cranial movement of the lower segments, produces a caudad movement of the upper segments if the lower portion is held in place. This draws parts of the brain stem and cerebellum through the foramen magnum into the spinal canal (Arnold Chiari malformation) and thus interferes with the drainage of fluid from the ventricles. Ingraham and Scott [309] studied 20 cases of this malformation in detail and observed, in addition to other phenomena, that all had microgyria. I have made the same observation in a smaller series of cases. Certain forms of hydrocephalus have been treated surgically.[310]

Porencephaly is thought to be due either to degeneration of previously normal brain tissue—for example, by occlusion of blood vessels—or to rupture of the brain in hydrocephalus.[311] Yakovlev and Wadsworth [312] have shown that there are, in addition to porencephaly due to secondary changes, rare conditions in which a developmental anomaly must be assumed (schizencephaly). These defects are strictly symmetric and in typical locations.

An accidental discovery in mice of normal behavior was a hereditary defect of the corpus callosum.[313]

Several abnormalities of the minute structure of the central nervous system, often associated with gross malformations, are on record. Rosette formation has been suggested as a possible link between malformations and neoplasms of the nervous tissue, as it is observed in both conditions.[303] When they appear as a malformation, rosettes consist of minute cavities with lining cells in a radial arrangement, resembling ependyma. Numerous rosettes are often found in the brains of platyneuric chick embryos.[303] Occasionally rosettes occur associated with other malformations. Their development has not been adequately followed as yet. Their form and location, as well as their similarity to

306. Grüneberg, H.: J. Genetics **45**:22, 1943.

307. Job, Liebold and Fitzmaurice.[91] Kaven.[92]

308. Lichtenstein, B. W.: Arch. Neurol. & Psychiat. **47**:195, 1942.

309. Ingraham, F. D., and Scott, H. W.: New England J. Med. **229**:108, 1943.

310. Sachs, E.: J. Mt. Sinai Hosp. **9**:767, 1942. Michelsen, J. J.: Am. J. Ment. Deficiency **48**:15, 1943.

311. Marburg, O.; Rezek, P. R., and Marks, M. B.: J. Neuropath. & Exper. Neurol. **4**:43, 1945.

312. Yakovlev, P. I., and Wadsworth, R. C.: J. Neuropath. & Exper. Neurol. **5**:116 and 169, 1946.

313. King, L. S.: J. Comp. Neurol. **64**:337, 1936.

rosettes of the retina, which are known to develop independent of the ventricle,[314] suggest that they also develop in loco and are at no time continuous with the ventricles. Occasionally rosettes are found in the brains of embryos with hereditary microphthalmia and rosettes of the retina.[314] Groups of rosettes occur normally in the human brain—for example, in the medulla oblongata near the insertion of the tela choroidea.

Other kinds of cavities and rarefactions in the brain tissue occur normally in embryos of birds and mammals, including man, and disappear without leaving a trace.[315] The only indication of a teratologic significance of these formations is the observation that they also occur in association with gross malformations of the nervous system (brain, spinal cord, cranial and spinal ganglions) in regions where they are not normally found.[315]

There are several syndromes of multiple abnormal growths of the central nervous system and other organs, and perhaps the best studied of these is tuberous sclerosis. There is fair agreement that the condition is congenital, even though in the reported cases the patients were not very young persons. There are multiple tissue malformations involving the brain, the eyes, the heart, the kidneys and the skin; they show a tendency to form noncancerous and, less frequently, cancerous tumors. Feriz[316] suggests that the abnormal foci develop as overgrowth of atypical, functionally inferior tissue, at the points of defects due to inhibition of the genesis of the normal tissue; this explains the coexistence of defective and excessive formations. Yakovlev and Guthrie[317] classify tuberous sclerosis as one of several types of congenital ectodermoses, and they emphasize the existence of abortive forms which on superficial examination may pass for idiopathic epilepsy. Moolten[318] describes tuberous sclerosis as an example of "disseminated hamartiosis" and explains it in vague terms as due to "a defective mechanism of induction by embryonic organizers." It was shown in foregoing paragraphs that there are many mechanisms of development of simple and combined malformations which Moolten does not consider and rule out. Obviously, all theories based on the final structure of the malformation alone are at best working hypotheses until they are confirmed either by examination of early stages or by experimental reproduction of the condition.

Nonhereditary feeblemindedness is due in a large proportion of the cases to causes operating during embryonic development or at birth. Research has shown or suggested several possible causes during the

314. Gruenwald, P.: Anat. Rec. **88**:67, 1944.

315. Gruenwald, P.: J. Neuropath. & Exper. Neurol. **4**:178, 1945.

316. Feriz, H.: Virchows Arch. f. path. Anat. **278**:690, 1930.

317. Yakovlev, P. I., and Guthrie, R. H.: Arch. Neurol. & Psychiat. **26**:1145, 1931. Yakovlev, P. I.: ibid. **41**:119, 1939.

318. Moolten, S. E.: Arch. Int. Med. **69**:589, 1942.

past few years. Besides those causes which have been known for a long time, one has to consider: syphilis, rubella [217] or toxoplasma infection [319]; maternal endocrine deficiency [144] or abnormal conditions of the endometrium [320] in the case of mongolism; maternal dietary iodine deficiency [321]; injury caused by mechanical trauma or anoxia at birth [208]; heterospecific pregnancy (Rh blood group and less frequently others) [322]; roentgen irradiation of the fetus (see part I). Now that these causes of feeblemindedness begin to be appreciated, a wide field is opening up for preventive medicine.[323] In addition, some of the just mentioned causes also confine persons to institutions by producing deafmutism or blindness. In the cases of syphilis, toxoplasmosis, roentgen irradiation and birth injury the morphologic basis of the disturbance is established with relative ease; in the cases of anomaly associated with rubella occurring during pregnancy and of mongolism the mechanism is unknown. For the damage that occurs in heterospecific pregnancy several mechanisms have been suggested. Damage of the brain may result from damage of the liver [322d] or from a disturbance of development due to anemia and anoxia.[322c] Recently Wiener and Brody [324] observed thrombi in cerebral vessels. The well known condition of kernicterus (encephalomyopathy with icterus) indicates damage of the brain as it is not explained by jaundice alone.[322a,d] However, it is not yet established whether in all infants with kernicterus disturbances develop if they survive, nor do investigators know whether mental deficiency is limited to patients who had kernicterus, since the presence of the latter cannot be determined in the living patient. A more comprehensive discussion of the effects of heterospecific pregnancy will be given in a later section (page 557).

There are several types of hereditary defects associated with mental deficiency, which are only recently being identified and segregated from one another and from those without a genetic basis.[325]

319. Cowen, D.; Wolf, A., and Paige, B. H.: Arch. Neurol. & Psychiat. **48**:689, 1942. Zuelzer.[223d]

320. Mayerhofer, E.: Ann. pædiat. **154**:57, 1940. Engler, M.: Am. J. Ment. Deficiency **50**:27, 1945.

321. Stoot, H., and Gupta, S. P.: Indian J. M. Research **21**:655, 1934. Benda.[146]

322. (*a*) Zimmermann, H. M., and Yannet, H.: Am. J. Dis. Child. **49**:418, 1935. (*b*) Yannet, H., and Lieberman, R.: Am. J. Ment. Deficiency **49**:133, 1944; **50**:242, 1946; J. A. M. A. **130**:335, 1946. (*c*) Cook, R.: J. Hered. **35**:133, 1944. (*d*) Gilmour, J. R.: Arch. Dis. Childhood **19**:1, 1944. (*e*) Snyder, L. H.; Schonfeld, M. D., and Offerman, E. M.: J. Hered. **36**:9, 334, 1945. (*f*) Doctor, J. M.: J. Pediat. **27**:327, 1945.

323. Gruenwald, P.: Am. J. M. Sc., to be published.

324. Wiener, A. S., and Brody, M.: Science **103**:570, 1946.

325. Allan, W.; Herndorn, C. N., and Dudley, F. C.: Am. J. Ment. Deficiency **48**:325, 1944. Benda, C. E.: ibid. **49**:32, 1944. Halperin, S. L.: ibid. **50**:8, 1945

Correlations between various parts of the body and the region of the central nervous system which supplies them have been observed in malformations. Tsang [326] and Baumann and Landauer [327] found increased numbers of cells in the ventral horns of the spinal cord corresponding to polydactylous limbs in mice and chicks, respectively. In experimental chick embryos the differentiation of the spinal cord is influenced by extirpation or transplantation of the primordia of limbs.[328] Chase [329] describes in detail the defective development of certain cranial nerves and brain centers in mice with hereditary microphthalmia. Conversely, defective innervation may affect the development of various organs. The differentiation of the skeleton in nerveless transplanted limbs of chick embryos has been examined by Hamburger and Waugh.[330] The occurrence of clubfeet in newborn infants with lumbosacral spina bifida is well known.

EYES

Very large numbers of ocular malformations, hereditary as well as sporadic, are on record. Mann's book and Howe's [331] enumeration of hereditary malformations of the eye show the number and the variety of types. Stockard [1a] has expressed what is probably the generally accepted explanation of this great tendency of the eyes to abnormal development. He assumes that the rapid rate of growth and development in early stages accounts for the high susceptibility to various injurious agents which act by retarding development at a given moment. There is no known type of teratogenic agent which will not under certain conditions produce ocular malformations (see part I).

The present discussion will be limited to a few instructive examples of ocular malformations which have been thoroughly investigated as to their embryonic development.

Many malformations of the eye have been described as microphthalmia because they are associated with a reduction in the size of the eye. Often microphthalmia has erroneously been called anophthalmia if the small eye was not externally visible. True anophthalmia is very rare, and when it occurs it may be due to regression rather than to complete primary absence of the primordium of the eye.[332] Best studied are several hereditary forms of microphthalmia. While in each of these

326. Tsang, Y. C.: J. Comp. Neurol. **70**:1, 1939.

327. Baumann, L., and Landauer, W.: J. Comp. Neurol. **79**:153, 1943.

328. Bueker, E. D.: J. Exper. Zool. **93**:99, 1943.

329. Chase, H. B.: J. Comp. Neurol. **83**:121, 1945.

330. Hamburger, V., and Waugh, M.: Physiol. Zoöl. **13**:367, 1940.

331. Howe, L.: A Bibliography of Hereditary Eye Defects, Eugenics Record Office Bulletin 21, Cold Spring Harbor, Carnegie Institution of Washington, 1928.

332. Chase, H. B., and Chase, E. B.: J. Morphol. **68**:279, 1941.

the changes in embryos are fairly uniform and follow a definite pattern, no uniformity of structure or development is seen when the malformations caused by different mutations are compared. In the following discussion microphthalmia will be divided into two types comprising, respectively, severe general underdevelopment of all derivatives of the optic cup, and abnormalities restricted primarily to one part—for example, the choroid fissure or the retina.

Representative of hereditary microphthalmia with severe general inhibition of development are the mutations described by Chase and Chase [332] and by Browman and Ramsey.[333] The former authors examined the genetics and the embryologic aspects of a mutation causing anophthalmia or microphthalmia in the mouse. The optic vesicles, and often the cups, form as in normal embryos. However, the choroid fissure never closes, remaining as a coloboma. Then retardation sets in, and in severe cases degeneration seems to occur, so that at birth all remnants of the eye may have disappeared or may be too inconspicuous to be found. A lens vesicle forms only if the optic vesicle comes close to the epidermis.

Browman and Ramsey examined the embryonic development of microphthalmia in a strain of rats and found that an optic cup always exists in the embryo. In later stages it fails to grow and differentiate normally, and at birth the small rudiment of an eye may be degenerating. Lenses are formed in these eyes. The authors attribute much importance to failure of the hyaloid artery to develop, so that vessels of the rim of the optic cup are the only ones to supply the interior. Development goes on normally only as long as the tissues do not depend on the blood supply of the hyaloid artery. The microphthalmic eyes as well as many otherwise normal eyes in animals of this strain are devoid of an optic nerve. In both of the mutations which have thus far been described, not only growth but also histogenesis of the eye is severely impaired.

Sporadic microphthalmia is relatively common and often reaches degrees comparable with the just described hereditary forms. Here the embryologic aspects cannot be investigated systematically, and it is necessary to examine whatever forms and stages happen to present themselves, often without knowing what the earlier or later phases of the same malformation may be. This has been done in a fairly large number of chick embryos,[18] and the following conclusions resulted from the interpretation of the findings. Malformations grossly visible as microphthalmia are often not limited to the reduced eye. The adjacent parts of the forebrain, as well as the normal-sized other eye may be affected as well. The ocular malformations do not conform to any one type. Any part except the pigmented epithelium may be com-

333. Browman, L. G., and Ramsey, F.: Arch. Ophth. **30**:338, 1943.

pletely absent. The only manner in which these varied defects can be accounted for is that of an abnormal determination of the parts in the early primordium of the eye. An indication of the stage in which this determination can still be altered is given by the predominance of this kind of microphthalmia on the left side. This is most probably due to a temporary handicap of the left eye at the time when the head turns to the side, in embryos of about 2 days. The left eye is then far from the egg shell and does not receive as much oxygen by diffusion as the right eye, which is just under the shell. Circulatory oxygen supply sets in somewhat later, and during this interval a high percentage of normal embryos show a temporary lag in the development of the left eye. This is the only known factor which may selectively damage the left eye and thus increase its susceptibility to other injurious agents. At that time the optic vesicle begins to transform itself into the cup, and at this late stage the determination of the various parts of the eye is apparently not sufficiently rigid to prevent changes.

The interpretation of these malformations suggested that teratogenic agents of the following three kinds are all active: An agent within the eye itself is its high susceptibility due to rapid development (see opening paragraph of this section). An agent extrinsic to the eye but within the embryo is the rotation of the head which favors malformations of the left eye. That these two agents in themselves are not sufficient is obvious, since they are present in the normal embryo. However, they influence the intensity and the localization of the reaction to the third kind of factor, apparently extrinsic to the embryo. A hint of the possible nature of the latter is given by Stockard,[70] who found an increased incidence of sporadic microphthalmia in eggs incubated in an atmosphere contaminated by laboratory fumes. This may well hold for the cases used in the investigation just quoted. Landauer [11] emphasizes that a higher percentage of maldeveloped chicks are found in eggs incubated under unfavorable conditions. A higher incidence of microphthalmia of the left side of the chick embryo was also found elsewhere in sporadic cases,[204b] as well as in selenium-induced conditions [49] and in homozygous Creeper embryos.[46] The last-mentioned observation led Cairns to refer for the first time to the unequal oxygen supply of the eye when the head rotates; in that particular case the condition is aggravated by an inadequate circulation in later stages.

Detailed information is available on a type of microphthalmia of mice which is genetic so far as the causative agent develops on the basis of a hereditary abnormality; the immediate action on the eye is mechanical if that agent, a bleb of cerebrospinal fluid moving under the epidermis, happens to reach the region of the eye.[334] The

334. Bonnevie, K.: J. Exper. Zool. **67**:443, 1934.

hereditary condition is usually referred to as "myelencephalic blebs"; it will be described in a later section (page 556) in its various manifestations.

A number of mutations are known which produce milder forms of microphthalmia with little impairment of histogenesis and no secondary degeneration of the eyes, but with formation of a coloboma. All colobomas to be considered here are clefts of the eyeball produced by abnormalities of the choroid fissure. Diagrams of the form and the development of the three fundamental types of coloboma are given by Mann [11] and Gayer.[203] One type is the embryonic coloboma in which the fissure remains open. The boundary of the retina and the pigmented epithelium is at the lips of the fissure. The second type, embryonic coloboma with orbital cysts, also has an open fissure, but the retina extends beyond the lips and forms both layers of the cup in an area adjacent to the fissure. The outer layer is inverted, with the inner limiting membrane facing outward. These inverted portions of retina in the outer layer of the cup near the fissure tend to bulge outward in later stages, forming orbital cysts. The third type is the ectatic coloboma. This has in common with the second type the presence of inverted retina in the outer layer near the fissure, but the fissure closes later on. As in all areas where the outer layer consists of retina instead of pigmented epithelium, the connective tissue coats of the eyeball are deficient, and both layers of the cup bulge outward together.

Von Hippel [335] was the first to examine systematically the embryonic stages of hereditary coloboma in rabbits. Other authors [336] have since carried out similar investigations in apparently unrelated strains of rabbits with the same malformation. All agree that the choroid fissure remains open and that an increased amount of mesenchyme is found between its lips and in the interior of the cup. Hippel assumes that this mesenchyme causes coloboma by preventing closure of the fissure, whereas von Szily maintains that the fissure, that is, the ectodermal tissue, is primarily abnormal and that the mesenchyme enters because of this abnormality. All authors found inverted retina in the outer layer of the optic cup adjacent to the fissure, and all three of the aforementioned types of coloboma may result. Von Szily has published illustrations of numerous plastic reconstructions of these eyes. Warkany and Schraffenberger [184] have observed the development of coloboma in the embryos of rats with vitamin A deficiency.

Much experimental work has been done with chick embryos homozygous for the Creeper factor, in which coloboma develops if they

335. von Hippel, E.: Arch. f. Ophth. **55**:507, 1903.

336. Seefelder, R.: Verhandl. d. deutsch. ophth. Gesellsch. **42**:210, 1920. Koyanagi, Y.: Arch. f. Ophth. **104**:1, 1921. von Szily.[1c]

reach sufficiently late stages. If incubated without experimental interference, the majority of the homozygous Creeper embryos die on the fourth day, apparently because of a defective circulation of blood in the wall of the yolk sac.[46] Those which survive have phokomelia and coloboma and die shortly after hatching time. The various manifestations of the Creeper mutation will be summarized later in this review (page 554). Gayer [203] found that in eyes of homozygous Creeper embryos transplanted to the flanks of normal embryos colobomas develop similar to those of phokomelic embryos. However, eyes of normal control embryos transplanted in the same manner show colobomas of the same type. Thus the altered environment produces a phenocopy of the Creeper coloboma in genetically normal eyes. Orthotopic transplantation of a homozygous Creeper eye to the ocular region of a normal embryo produces an eye without coloboma.[337] The reverse experiment, namely, transplantation of a normal eye to the corresponding site in a homozygous Creeper host, was successful only in 1 case, owing to the high mortality of the Creeper hosts. The transplanted eye had a coloboma. All these experiments demonstrate the importance of an influence of the environment on the optic cup in the formation of coloboma. Gayer and Hamburger [337] conclude that in the homozygous Creeper embryo the optic cup is primarily normal and that only its environment is abnormal. Landauer [338] points out that this is only one of several possible interpretations of the experiments and that the normal properties of the cup are not proved, even though the influence of the environment on the cup is beyond doubt.

In addition to cases of microphthalmia with a fairly good differentiation of most parts and with coloboma, there are other small eyes in which the principal parts are also differentiated but the retina shows histologic abnormalities. Rosettes and folds of the retina have been found under various conditions. They occur in a strain of chickens with hereditary bilateral microphthalmia caused by a simple recessive gene.[339] The embryologic aspects of this condition have been studied,[314] and it has been shown that the rosettes arise in previously normal eyes without any folding and that their cavities are at all times separated from the cleft between the two layers of the cup. Later on folds develop in such a manner that the rosettes are on their crests, or else they are independent of rosettes. As soon as these abnormal differentiations appear, the eyes lag in growth.

Similar rosettes have repeatedly been found in the eyes of mammalian,[89] including human,[90] embryos exposed in utero to roentgen

337. Gayer, K., and Hamburger, V.: J. Exper. Zool. **93**:147, 1943.

338. Landauer, W.: Am. Naturalist **78**:280, 1944.

339. Jeffrey, F. P.: J. Hered. **32**:310, 1941.

rays. They also occur, though rarely, without apparent cause.[340] Goldstein and Wexler [90c] hold that rosettes may be formed from retinal folds as well as from dislocated cells, the latter apparently because the normal cells in the same layer do not normally multiply as do those of the rosettes. While it is true that the cells of the rosettes resemble those bordering on the cleft between the two walls of the optic cup, there is little to be gained by referring to them as dislocated, since all cells of the retina are derived from that one layer. In the rosettes the cells have apparently retained the ability to multiply, which is normally lost when cells move away from the outer border, and have at the same time failed to differentiate like the surrounding cells.

An entirely different abnormality of the retina, occurring in eyes of normal size, has been found as a mutation in mice. The formation of rod cells is partly or completely inhibited, depending on the presence of modifying genes.[341]

The optic nerve may be absent in cyclopic, in microphthalmic or in otherwise normal eyes. Fischel [5] assumes that cyclopic eyes are devoid of the nerve because the cyclopic defect eliminates the optic stalk, the eye subsequently constricting itself off from the brain. Adelmann [267d] describes this process in lithium-induced cyclopia. However, in part of the cases of cyclopia and in cases of sporadic microphthalmia of chickens absence of the optic nerve has a different cause.[265] All these malformed eyes have optic stalks in early stages. If no nerve fibers grow into the stalk either because there is no retina or because the usual pathway is obstructed by absence of the choroid fissure, the stalk degenerates at the end of the first week of incubation and soon disappears completely. In several cyclopic eyes without optic nerves indications of a primary absence of the choroid fissure were found.[265] In a strain of microphthalmic rats described by Bowman and Ramsey [333] (see page 518) the optic nerve is frequently absent. The authors consider absence of the hyaloid artery as the cause, without explaining the mechanism. Atrophy of the previously normal optic nerve has been found in cattle as a sequel of abnormal narrowness of the bony canal. This, as well as paralyses, appeared in the offspring of cows with a nutritional deficiency of an unidentified factor, perhaps vitamin A.[342]

The lens may be affected during embryonic development by a variety of factors. The action of chlorobutanol on the early phases of its formation in amphibians has been examined by Lehmann.[108] This compound produces undersized but otherwise normal lenses. In later stages of the

340. Jaensch, R. A.: Arch. f. Ophth. **116**:464, 1925.

341. Keeler, C. E.: J. Exper. Zool. **46**:355, 1927.

342. Moore, L. A.; Huffman, C. F., and Duncan, C. W.: J. Nutrition **9**:533, 1935.

development of the eye, cataract may develop on a hereditary basis [343] or through the action of chemicals (galactose,[131] naphthalene [35]) or roentgen rays.[344]

Numerous reports have recently described cataract in children of mothers who contracted rubella during the early months of pregnancy.[345] The mechanism is not known.

The development of accessory structures of the eye, such as extrinsic muscles, cornea, lids, conjunctiva and lacrimal glands, is apparently independent of the eyeball to a considerable degree, and, as a result, these structures are often better developed than the eye itself in cases of microphthalmia.[346]

DIGESTIVE AND RESPIRATORY TRACTS

Abnormalities of the teeth will be treated in part III of this review, since most of the experimental work has been done with continuously growing teeth of adult animals.

Major malformations of the digestive tract have gained clinical importance during the past few years since technics have been perfected for the surgical treatment of the most common forms.[347] Accounts of the development of malformations, such as atresia, are based largely on observations of the final conditions and on conjectures concerning the early stages. It has, for instance, been claimed that atresia of the duodenum is favored by the epithelial occlusion of the lumen which normally occurs in the embryo, the lumen in abnormal cases not being properly canalized but penetrated by connective tissue. This, however, does not explain the very similar atresia occurring elsewhere in the intestine, where there is no temporary epithelial occlusion. In some of these cases an intestinal loop may have been cut off when it failed to retract properly from the normal umbilical hernia.[348] Atresia of the esophagus combined with tracheoesophageal fistula in its most common form, in which the lower segment of the esophagus opens into the trachea, has received much consideration. The hypothesis that it is due to delay of the process whereby the respiratory anlage is separated from the digestive tube and to a consecutive process whereby part of the esophagus is incorporated into the posterior wall of the trachea is supported by the fact that esophageal tissue has been seen in the posterior wall of the trachea in newborn infants and, in an early stage, in a chick embryo.[349]

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343. Gregory, P. W.; Mead, S. W., and Regan, W. M.: J. Hered. **34**:124, 1943. Lutman, F. C., and Neel, J. V.: Arch. Ophth. **33**:341, 1945.
344. von Hippel.[88] Kaven.[92]
345. Footnotes 217 and 218.
346. Gruenwald.[18] Chase and Chase.[332]
347. Swenson, O., and Ladd, W. E.: New England J. Med. **233**:660, 1945.
348. Misgeld, G. C.: Virchows Arch. f. path. Anat. **310**:697, 1943.
349. Gruenwald, P.: Anat. Rec. **78**:293, 1940; **85** (supp.):23, 1943.

Malformations of the liver and the pancreas consist largely of occlusion of their ducts at various points and of cysts of the ducts. Polycystic malformations of the liver, the pancreas and the kidneys or of two of these organs are relatively frequently combined in one person.[350] This is intriguing since the development of the pancreatic and hepatic ducts differs considerably from that of the renal tubules.

Cysts, atresia and stenosis of bile ducts are multiple in a high proportion of cases.[351] No direct observations of developmental stages of these malformations exists. Numerous speculations concerning this subject will not be reviewed. Moolten [350d] has recently favored the hypothesis that polycystic disease of the liver and the kidneys is a type of hamartiosis, that is, a multiple tissue malformation, and that it is due to deficient organizer action. Atresia of ducts, on the other hand, is thought to be aplasia due to a deficiency of the tissue itself. However, it is obvious that atresia of the extrahepatic bile ducts, as in Moolten's own case, cannot be aplasia. The duct must have been present at one time, or no liver could have been formed. If atresia is due to a defect of tissue, this defect cannot be in the form of aplasia but must occur only as a secondary change.

Cystic fibrosis of the pancreas is of considerable clinical importance because its signs must be differentiated from those of other diseases not caused by malformations. At birth the changes in the pancreas are in their early phases. There is moderate distention of small ducts with inspissated secretion, as well as slight fibrosis. These changes progress to severe fibrosis with destruction of many acini and cystic dilatation of ducts. The deficiency of pancreatic secretion in the intestine causes either meconium ileus in the fetus or a severe nutritional deficiency and disturbance of intestinal function in the infant. Meconium ileus is due to inadequate digestion and subsequent hardening of the meconium.[352] This meconium can easily be digested by pancreatic enzymes in the test tube, but attempts to dissolve it by the same agents in vivo have not yet been successful.[353]

Four explanations have been offered for the cause and development of cystic fibrosis of the pancreas. In evaluating these, one must remember that any obstruction of the flow of secretion may produce cystic fibrosis and that it is not necessarily due in all cases to the same cause.

350. (*a*) Moschcowitz, E.: Am. J. M. Sc. **131**:674, 1906. (*b*) von Meyenburg, H.: Beitr. z. path. Anat. u. z. allg. Path. **64**:477, 1918. (*c*) Rumler, E.: Virchows Arch. f. path. Anat. **292**:151, 1934. (*d*) Moolten, S. E.: New York State J. Med. **43**:727, 1943.

351. Feyrter, F.: Virchows Arch. f. path. Anat. **271**:20, 1929.

352. Kornblith, B. A., and Otani, S.: Am. J. Path. **5**:249, 1929. Hurwitt, E. S., and Arnheim, E. E.: Am. J. Dis. Child. **64**:443, 1942.

353. Farber, S.: J. Pediat. **24**:387, 1944.

Several workers [354] have proved by an examination of serial sections of the pancreas that congenital stenosis or atresia of the main duct was present in their cases. In other instances of pancreatic fibrosis no anatomic malformation of this kind was found in serial sections.[355]

Andersen [356] found in a considerable percentage of cases of pancreatic fibrosis squamous metaplasia of the ducts, and she suggested that this may be the cause of stenosis and of retention of secretion. The interpretation of this metaplasia is difficult because it may also be the sequel of vitamin A deficiency secondary to pancreatic achylia. This is demonstrated by a case examined by Oppenheimer,[354a] in which there was squamous metaplasia of the bronchi and salivary ducts, while atresia of the pancreatic duct accounted for pancreatic fibrosis and vitamin A deficiency.

Brody [221c] found in cases of pancreatic fibrosis inclusion bodies suggesting a fetal infection. However, occlusion bodies occur not rarely in infants without pancreatic disease and, on the other hand, are absent in the majority with that disease. That they are related to the cause of pancreatic fibrosis is therefore doubtful.

Farber [357] assumes that an abnormal secretion is produced which is so viscid that it occludes the ducts ("mucoviscidosis"). This is thought to be caused by abnormal stimulation originating from the autonomic nervous system. Farber [358] produced in kittens with pilocarpine a condition similar in all important respects to pancreatic fibrosis of human infants. More recently Glanzmann [359] and Riniker [360] have also referred to an abnormally thick secretion, and Andersen and co-workers [361] have voiced an opinion similar to that of Farber. The associated pulmonary changes (bronchiectasis, emphysema) are explained by Farber [357] on the basis of viscid bronchial secretion, whereas Andersen and co-workers [362] assume a secondary vitamin A deficiency with squamous metaplasia of the bronchial epithelium.

The familial occurrence of pancreatic fibrosis has long been known. It has recently been discussed by Andersen and Hodges.[361b]

The subject of pancreatic fibrosis has recently been reviewed by Wiglesworth.[363]

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354. (*a*) Oppenheimer, E. H.: Arch Path. **29**:790, 1940. (*b*) Footnote 352.

355. Baggenstoss, A. H., and Kennedy, R. L., Jr.: Am. J. Clin. Path. **15**:64, 1945.

356. Andersen, D. H.: Am. J. Dis. Child. **56**:344, 1938; J. Pediat. **15**:763, 1939.

357. Farber, S.: Arch. Path. **37**:238, 1944; J. Michigan M. Soc. **44**:587, 1945.

358. Farber, S.: Am. J. Dis. Child. **64**:953, 1942.

359. Glanzmann, E.: Ann. pædiat. **166**:289, 1946.

360. Riniker, P.: Ann. pædiat. **166**:314, 1946.

361. (*a*) Di Sant'Agnese, P. E., and Andersen, D. H.: Am. J. Dis. Child. **72**:17, 1946. (*b*) Andersen, D. H., and Hodges, R. G.: ibid. **72**:62, 1946.

362. Footnotes 356 and 361.

363. Wiglesworth, F. W.: Am. J. M. Sc. **212**:351, 1946.

Large numbers of newborn infants die with poorly aerated lungs. In some cases this is doubtless due to extrapulmonary factors, such as disturbances of the respiratory center or mechanical obstruction of the air passages. In many others, however, the cause is probably in the lungs themselves.[364] This problem is closely linked with that of fetal respiratory movements. It has been claimed that the contents of the amniotic sac may be aspirated in utero and that this may cause an inflammatory reaction of the lung tissue which later on prevents expansion of the alveoli.[207b] Some authors hold that respiratory movements, by which amniotic fluid is necessarily aspirated, regularly occur in the fetus and are even instrumental in the normal development of the lungs.[365] The latter contention has been disproved by Potter and Bohlender,[366] who showed in a case of complete atresia of the larynx and in one of accessory lung that lung tissue which could not have expanded during respiratory movement nevertheless developed normally. The assumption that normal fetal lungs are partially expanded [207c] is based on inconclusive evidence. A slight experimental interference, such as anesthesia and laparotomy, even without opening the uterus, may cause anoxia of the fetus, particularly near term. If one sees respiratory movements under these conditions, this does not necessarily represent the normal condition.

It is true that almost all stillborn infants have expanded alveoli filled with amniotic fluid,[367] but this is not the normal state. Windle [206b] has shown that if the trachea of the animal fetus is clamped before it is in anoxia, the lungs are atelectatic. If a little more time elapses during the experiment, the fetus begins to gasp in utero and then shows histologically the same condition which prevails in the stillborn infant. It is common experience that large parts of the lungs of the young infant with poor respiration have collapsed alveoli. Therefore several authors [368] assume that there are normally no intrauterine respiratory movements. If such movements occur during temporary anoxia of the

364. Farber, S., and Wilson, J. L.: Am. J. Dis. Child. **46**:572, 1933. Wilson, J. L., and Farber, S.: ibid. **46**:590, 1933.

365. Snyder, F. F., and Rosenfeld, M.: Proc. Soc. Exper. Biol. & Med. **36**:45, 1937; J. A. M. A. **108**:1946, 1937. Bonar, B. E.; Blumenfeld, C. M., and Fenning, C.: Am. J. Dis. Child. **55**:1, 1938. Patterson, J. C., and Farr, J. T.: Canad. M. A. J. **41**:31, 1939. Snyder, F. F.: Am. J. Obst. & Gynec. **41**:224, 1941. Davis, M. E., and Potter, E. L.: J. A. M. A. **131**:1194, 1946.

366. Potter, E. L., and Bohlender, G. P.: Am. J. Obst. & Gynec. **42**:14, 1941.

367. (*a*) Windle.[206] Potter, E. L., in discussion on Zettelman.[368d] (*b*) Gruenwald, P.: Am. J. Obst. & Gynec. **53**:996, 1947.

368. (*a*) Farber and Sweet.[207a] (*b*) Windle, W. F.; Becker, R. F.; Barth, E. E., and Shulz, M. D.: Surg., Gynec. & Obst. **69**:705, 1939. (*c*) Whitehead, W. H.; Windle, W. F., and Becker, R. F.: Anat. Rec. **83**:255, 1942. (*d*) Zettelman, H. J.: Am. J. Obst. & Gynec. **51**:241, 1946. (*e*) Gruenwald.[367b]

fetus and lead to aspiration of contents of the amniotic sac, future respiration may be impaired in one or both of two developments: One is mechanical, solid or fatty material filling or lining the air spaces [369]; the other is a fetal pneumonia which is caused in rare cases by the aspirated material. The manner in which the lung tissue reacts to these foreign substances varies greatly.[207b] In addition to the irritation caused by sterile material there may be a congenital bacterial pneumonia which also occurs only after aspiration of contents of the amniotic sac.[370] During periods of anoxia the fetus may discharge meconium, which will stain the vernix caseosa yellow.[371] Even the aspiration of meconium or of yellow vernix often fails to cause an inflammatory reaction of the alveoli.

SKELETON AND EXTREMITIES

Several reviews of the tremendous variety of skeletal malformations are available.[372] The developmental mechanism has been investigated in a relatively small number of these, and only this will be discussed here.

Duplication of entire extremities or large parts has repeatedly been produced in embryologic transplantation experiments in forms comparable with those occurring in man and other mammals, and the mechanisms of both kinds may well be similar in some respects. A single embryonic limb bud produces a double limb when transplanted in certain abnormal positions, and it has been concluded that the normal limb bud has an inherent tendency toward duplication which is normally suppressed.[373]

Excessive or reduced size of extremities may develop in several ways. It may be due to a somatic mutation, as has been assumed particularly when it is part of a general difference in size between the two sides of the body. A good example has recently been observed in a chicken.[65c] More references to similar lateral differences and other mosaics believed to be caused by somatic mutation may be found

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369. Farber and Sweet.[207a] Nelson, W. E., and Smith, L. W.: J. Pediat. **26**:36, 1945.

370. Johnson, W. C., and Meyer, J. R.: Am. J. Obst. & Gynec. **9**:151, 1925. Hook, H., and Katz, K.: Virchows Arch. f. path. Anat. **267**:571, 1928. Kaldor, J.: Am. J. Obst. & Gynec. **25**:113, 1933. Bufe, W.: Ztschr. f. Geburtsh. u. Gynäk. **113**:265, 1936. Benner, M. C.: Arch. Path. **29**:455, 1940.

371. Clifford, S. H.: Am. J. Dis. Child. **69**:327, 1945.

372. (*a*) Aschner, B., and Engelmann, G.: Konstitutionspathologie in der Orthopädie, Berlin, Julius Springer, 1928. (*b*) Brandt, W.: Die Entstehungsursachen der Gliedmassenmissbildungen und ihre Bedeutung für das Vererbungsproblem beim Menschen, Leipzig, Johann Ambrosius Barth, 1937. Gruber.[74]

373. Brandt, W.: Arch. f. Entwcklngsmechn. d. Organ. **106**:193, 1925. Swett, F. H.: J. Exper. Zool. **44**:419, 1926.

in textbooks of genetics and in Hollander's review.[65b] Another cause of abnormal size of extremities or of parts of them, with normal proportions within the part concerned, has been discussed by Politzer.[374] It is recalled that the embryonic limb buds develop as harmonious equipotential systems; that is, their cells have equal potencies, and the whole primordium develops into a harmonious limb, to a high degree independent of the amount of tissue present. Increased or decreased size of the whole will thus produce a limb of abnormal size but normal proportions. As a limb develops and its various parts are determined in its tissue, new harmonious equipotential systems develop for smaller parts, such as the forearm, the hand, the joints and the digits. At any of these stages an abnormal amount of tissue will produce parts that are of normal proportions within themselves but out of proportion with the rest of the body. In addition to obvious abnormalities of size, another malformation has been explained on this basis, which at the first glance does not seem to have anything in common with these, namely, congenital dislocation of the hip joint. Braus[375] made experiments in amphibians which indicate that at certain periods there exist independent harmonious equipotential systems for the two components of the joint. He showed that in congenital dislocation the fossa is in itself well proportioned in its structure but is too small for the head of the femur and that a reduction of the amount of tissue available for the fossa brings about a condition comparable with the human malformation. It is well known that in man dislocation of the hip joint is familial, and Faber[376] described a lesser degree occurring in affected families, which he called dysplasia. It produces no dislocation but can be detected by roentgenogram.

Many defects of the extremities, of varying extent and appearance, have long been regarded as the results of intrauterine amputation by amniotic bands and adhesions. Many authors still adhere to this concept even though strong arguments have been brought forward in favor of a different origin of all or most of these defects. Streeter[76] emphasizes the histologic abnormalities of the tissues at and near the site of the defect. He assumes that primary and intrinsic abnormalities of the tissues, and not mechanical action of amniotic adhesions, are responsible, and that adhesions are sequelae of the fundamental abnormalities. In the same year Ombrédanne and Lacassie[295] arrived at similar conclusions when studying clinical cases. They point to various associated lesions, such as syndactyly, scars of the skin and the tongue, skeletal defects of the nonamputated parts, pseudarthroses and the

374. Politzer, G.: Beitr. z. path. Anat. u. z. allg. Path. **100**:273, 1938.

375. Braus, H.: Arch. f. Entwcklngsmechn. d. Organ. **30**:459, 1910; München. med. Wchnschr. **57**:1742, 1910.

376. Faber, A.: Ztschr. f. Orthop. **66**:140, 1937.

frequent symmetric locations of lesions. These cannot be satisfactorily referred to amniotic adhesions. On the other hand, the authors emphasize the occurrence of ulcers, which may be superficial or deep. One case is presented in which at birth an ulcer involved a large part of the forearm, healing with a considerable defect. This is considered as an unusually late occurrence of the disease which is called intrauterine ulcerative disease *(maladie ulcéreuse intrautérine)*. Amniotic bands are assumed to be adhesions developing in the course of the disease. Other reports of intrauterine defects of the skin have been reviewed in an earlier section of this paper. Seitz [77] also assumes a primary abnormality affecting the fetal tissues to account for the development of amniotic bands. The abnormality may be either intrinsic or environmental. Gruber [74] has reviewed much of the older literature concerning amniotic amputations. He accepts amniotic bands as the cause of the defect in some cases. In support of the amputation theory, Hellner [79] put ligatures around fetal extremities in animal experiments. The defects resembled those observed in nature, and the amputated parts rapidly disappeared by autolysis. Movers [377] states that not only defects of the extremities but also severe distortions of large parts of the body are not satisfactorily explained by amniotic adhesions. Taylor Gorostiaga and Lede [78] recently supported Streeter's view with histologic observations made in a pertinent case. In connection with fetal ulcers and amputations, intrauterine fractures of long bones occur.[378] One may wonder whether these are not pathologic fractures of diseased bones.

In favor of an intrinsic cause of the defects in question is the occasional familial occurrence. A good example is a family in which several children had so-called amputations of forearms and legs of identical form, bilateral and symmetric. Koehler [75] has reviewed several conflicting accounts of this family. According to the version which he considers most reliable, the mother of 6 defective and 6 normal children was the father's niece or sister. In spite of this obvious familial background, one of the authors reporting on this family considers the malformations as amniotic band amputations.[379]

The morphologic aspects and the development of a condition possibly related to those just reviewed were described by Greene and Saxton.[380] Brachydactyly and other defects occur in rabbits as simple recessive mutations. In the embryos, dilatation of blood vessels, hemorrhage and eventually necrosis and sloughing are observed. Somewhat similar is a group of malformations which have been investigated in great

377. Movers, F.: Arch. f. Gynäk. **168**:22, 1939.
378. Granzow, J.: Zentralbl. f. Gynäk. **55**:3458, 1931.
379. Joesting, H.: Therap. Beitr. **10**:51, 1933.
380. Greene, H. S. N., and Saxton, J. A., Jr.: J. Exper. Med. **69**:301, 1939.

detail, namely, hereditary defects of limbs and other parts of the body in a strain of mice. Early investigations revealed blisters and hemorrhages in the regions where malformations occur.[381] The complete sequence of events has been described by Plagens[382] and Bonnevie.[334] The authors agree in the essential points, and in the following presentation the description of Bonnevie will be followed. As has been mentioned in the description of ocular malformations occurring in the same strain, an excess of cerebrospinal fluid escapes from the brain and moves under the epidermis in the form of blisters ("myelencephalic blebs") until it reaches points where it can apparently go no farther. This happens frequently in the limb buds, and malformations ranging from clubfeet and syndactyly to defects resembling the so-called amniotic amputations result from the mechanical interference of these blisters which later become hemorrhagic.

There are various forms of micromelia, or disproportionate dwarfism (as against proportionate dwarfism), in which the parts of the skeleton of the extremities are present but deformed. These abnormalities have been studied most extensively in fowl.[170] Various types of micromelia result in fowl from a deficiency of manganese[383] or riboflavin[174] or from selenium poisoning[125] during embryonic life. Typical chondrodystrophy occurs sporadically[384] or as a hereditary trait.[385] Several mutations produce similar but quantitatively different forms of chondrodystrophy in heterozygous embryos and more severe changes, namely, early death or phocomelia, in homozygous embryos. Of these mutations, the Creeper fowl has been studied by several groups of workers. The results are of particular interest, because it has been shown that the chondrodystrophy or the phocomelia of these specimens closely resembles the human malformation.[385c] In man and fowl, chondrodystrophy is characterized by short, thick, curved bones of the extremities, while the vertebral column usually shows minor changes, if any, and the skull is normal or has a shortened base. The details of the malformation of the long bones vary considerably, and

381. Bagg, H. J., and Little, C. C.: Am. J. Anat. **33**:119, 1924. Bagg, H. J.: ibid. **43**:167, 1929.

382. Plagens, J. M.: J. Morphol. **55**:151, 1933.

383. Byerly, Titus, Ellis and Landauer.[166] Landauer.[167] Lyons and Insko.[168] Caskey and Norris.[169]

384. (*a*) Landauer, W., and Dunn, L. C.: Proc. Soc. Exper. Biol. & Med. **23**:562, 1926. (*b*) Landauer, W.: Arch. f. Entwcklngsmechn. d. Organ. **110**:195, 1927.

385. (*a*) Landauer, W., and Dunn, L. C.: J. Genetics **23**:397, 1930. (*b*) Landauer, W.: Ztschr. f. mikr.-anat. Forsch. **25**:115, 1931; (*c*) **32**:359, 1933. (*d*) J. Genetics **31**:237, 1935; (*e*) Bulletin 233, Storrs Agricultural Experiment Station, 1939, p. 1. (*f*) Am. Naturalist **76**:308, 1942. (*g*) Lamoureux, F. W.: J. Hered. **33**:275, 1942.

various subtypes have been described. A good summary of findings in human chondrodystrophy and the theories concerning it has been given by Gruber.[74] The morphologic aspects and the development of hereditary chondrodystrophy of cattle have been described by Crew [386] and Mohr.[1d] Only the embryogenesis of the hereditary chondrodystrophy of fowl has been described.[387] In early stages the cartilaginous parts of the skeleton are of normal size and proportions. Later on, growth is retarded. The various processes involved in the growth of cartilage and its replacement by bone are not properly coordinated. Enchondral ossification starts at the usual time but soon lags severely behind the normal process in extent. Perichondral ossification at times exceeds the normal rate, and this probably causes the large size of the fibula in man and fowl, by preventing partial involution of the structure. Connective tissue is frequently observed to extend from the periosteum into the epiphysis *(Perioststreifen)* in man and, in sporadic cases, in fowl, and occasionally in Creeper chicks. Landauer [384b] found that this formation does not cause curvatures of long bones by unilateral inhibition of epiphysial growth as had been supposed, but is preceded by them.

Many old theories holding that chondrodystrophy is caused by extrinsic agents during embryonic life (Gruber [74]) have been discarded. In part of the cases it is caused by genetic factors; the cause of sporadic chondrodystrophy is unknown. Landauer,[388] in accordance with previous investigators, concluded that there are no abnormalities of endocrine organs of the embryo which could account for the malformation. Phocomelia of homozygous Creeper embryos and chondrodystrophy of heterozygous birds are degrees of the same basic type of disturbance.[385c] However, it is acknowledged that this does not necessarily hold for all cases of phocomelia.

Embryologic experiments with Creeper fowl have shown that in limb buds of homozygous or heterozygous Creeper embryos transplanted to genetically normal hosts previous to the development of their skeletons, abnormalities develop according to their own genotype and independent of the host.[389] This shows that the malformation is not caused by other parts of the embryo acting on the primordia of the limbs. Several lines of evidence, in addition to the general principles discussed in part I, suggest that the Creeper factor acts by reducing developmental activity during a critical stage. The action of this genetic factor and that of selenium poisoning are cumulative, and the latter is probably

386. Crew, F. A. E.: Proc. Roy. Soc., London, s.B **95**:228, 1923.

387. Landauer (footnotes 384 *b* and 385 *b* and *c*).

388. Landauer, W.: Virchows Arch. f. path. Anat. **271**:534, 1929.

389 (*a*) Hamburger, V.: Physiol. Zoöl. **14**:355, 1941. (*b*) Rudnick, D.: J. Exper. Zool. **100**:1, 1945.

represented by a retardation of metabolism.[49] When genetically normal limb buds develop in vitro in a growth-restricting medium they yield deformities resembling those of Creeper embryos.[390] A disturbance of the metabolism of older chondrodystrophic chick embryos has been reported by Patton,[391] who found that the tissues of these embryos contain in later stages significantly less aminoacetic acid than do those of normal embryos.

In embryonic manganese deficiency of fowl the long bones show, in addition to a reduction of length, histologic abnormalities of the cartilage and a matrix containing fragments of degenerated cartilage cells in place of periosteal bone. If the involved chickens receive an adequate diet, the abnormal tissue is replaced by normal bone.[167]

A mutation of fowl causing a short upper beak and short long bones is on record.[392] In all these forms, including chondrodystrophy, the legs are more severely deformed than the wings, and the distal bones more than the proximal ones.[392]

A nutritional deficiency, perhaps of riboflavin, during gestation produces various skeletal and other malformations in rat embryos.[393] The abnormal condition is determined in the cartilaginous stage of skeletal development, and addition of the missing substance to the mother's diet prevents malformations only if it is made before the fourteenth day of gestation.[185]

Micromelia not conforming to any established type and affecting the limbs of one individual to different degrees may result from roentgen irradiation of embryos,[394] though perhaps less frequently than malformations of the brain or the eyes.

Brandt[395] found it difficult to produce phocomelia experimentally in amphibians, because, after the excision of tissue, either the limb is absent or it regenerates and develops normally. Only by long-lasting interference such as occurs through implantation of other tissue into the limb bud could the desired effect be obtained. Gabriel[118] produced dwarf limbs in chick embryos by local application of colchicine.

In some of the so-called amniotic amputations the condition is really one of irregular micromelia rather than a terminal defect, as traces of digits are present.[396]

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390. Fell, H. B., and Landauer, W.: Proc. Roy. Soc., London, s.B **118**:133, 1935.

391. Patton, A. R.: J. Nutrition **13**:123, 1937.

392. Landauer, W.: Genetics **26**:426, 1941.

393. Footnote 185. Warkany and Schraffenberger.[186]

394. Murphy.[21] Feldweg.[95e] Flaskamp.[95g]

395. Brandt, W.: J. Exper. Biol. **20**:117, 1944.

396. Ombrédanne, L., in Ombrédanne, L., and Mathieu, P.: Traité de chirurgie orthopédique, Paris, Masson & Cie, 1937, vol. 1, p. 23.

Hereditary deformities of the legs of mice, produced by defects of the tibias, have been thoroughly examined by Hovelacque and Noel.[397] In the embryo the condensations of mesenchyme which initiate the formation of skeletal parts are present for the tibias as usual. However, instead of differentiating into cartilage, the greater part of the tibial condensation forms a fibrous ligament. Later on, the fibula is severely curved. However, it was observed that even in externally normal mice of the same strain, which had been considered as normal overlaps, the tibias might be defective, though the fibulas were without deformity. Defects of the tibias have also been found in polydactylous guinea pigs carrying the lethal gene *Px*.[236]

Hereditary polydactyly was the subject of one of the earliest systematic investigations of the embryonic development of a malformation. In 1908 Kaufmann-Wolf[398] and Braus[399] examined polydactylous chick embryos and observed that an enlargement of the foot represented a partial reduplication. The toes developing in such excessive tissue form part of a mirror image of the normal foot at its tibial border. Gabriel,[118] also, believes that polydactyly is an expression of reduplication of the limb. By treatment with colchicine he reduced the number of digits in normal and polydactylous chick embryos. Cole[400] investigated a lethal trait producing severe polydactyly of all extremities, ectopia viscerum and malformations of the face in fowl. At one hundred and ten hours of incubation the limb buds are deformed, and homozygous embryos die at six to eight days of incubation.

Wright[401] examined in guinea pigs the inheritance of a type of polydactyly which produces in heterozygous embryos five digits instead of the usual four on the forefeet and three on the hindfeet. Homozygous embryos have excessive polydactyly, with ten and more digits on a foot, and multiple other malformations, which cause death usually on the twenty-seventh day in utero or, rarely, immediately after birth. Scott[236b] found in newborn homozygotes, aside from approximately twice the normal number of digits, clubfoot, absence of the tibia, microphthalmia, malformation of the brain and, in part of the cases, harelip or absence of nipples. From the study of the embryogeny of these malformations[236a] it was concluded that the gene *Px* causes in homozygous form excessive growth and retarded morphogenesis about the age of 17½ days in utero, affecting rapidly growing parts more than others. The resulting abnormal correlation of developmental

397. Hovelacque, A.: Bull. biol., 1920, supp. 3, p. 1. Hovelacque, A., and Noel, R.: ibid. **57**:133, 1923.

398. Kaufmann-Wolf, M.: Morphol. Jahrb. **38**:471, 1908.

399. Braus, H.: München. med. Wchnschr. **55**:386, 1908.

400. Cole, R. K.: Poultry Sc. **18**:403, 1939.

401. Wright, S.: Genetics **20**:84, 1935.

processes, further augmented by a normal onset of histogenesis in the morphogenetically retarded parts, accounts for most of the disturbances. The development of polydactyly in this mutation may well be related in its mechanism to that found in various other mammals, including man.[236b]

It remains to mention several abnormalities concerning mainly the histogenesis of the skeleton. It was just mentioned that faulty differentiation in an early phase, namely, formation of connective tissue instead of cartilage, accounts for hereditary absence of the tibia in mice. Grüneberg [1r] points out that a disturbance in a comparable stage, though in the opposite sense, produces flexed tails and similar malformations of vertebrae in the trunk region in a mutation of mice: The intervertebral disks are partly transformed into cartilage instead of fibrous connective tissue.

The ossification of the cartilaginous parts of the skeleton is subject to a variety of abnormalities. These are too complex to be described here in detail. One example is chondrodystrophy, which has already been discussed. A hereditary failure of bone resorption in mice (the gray-lethal mutation) leads to deformities of bones and prevents eruption of teeth.[402] Transplantation experiments showed that in normal hosts gray-lethal bones develop nearly normally; in gray-lethal hosts they retain their abnormal features. Bones of normal mice transplanted to gray-lethal hosts sometimes show deformities resembling the gray-lethal condition; transplanted to normal hosts, they remain normal. This suggests an abnormal action of the tissue fluids of the gray-lethal host.[403] Administration of parathyroid extract to gray-lethal mice causes much resorption of bone. These mice tolerate larger quantities of parathyroid extract than would normal mice. However, in spite of the improvement of the condition of their bones, the mice are not cured by the treatment. Since they show no other evidence of deficiency of the parathyroid glands, it has been concluded that either their osteoclasts require an abnormally high concentration of the hormone or the hormone is excreted too rapidly, or both.[404]

With regard to chickens, a lethal factor *"sticky"* has been briefly described,[405] which was so named because the amniotic and allantoid fluids are viscid. The embryos have edema and soft bones, and do not hatch. The bone changes are described as similar to those associated with experimental vitamin D deficiency,[406] but more study is necessary to elucidate the relationship of the two disturbances.

402. Grüneberg, H.: Proc. Roy. Soc., London, s.B **118**:321, 1935.
403. Barnicot, N. A.: Am. J. Anat. **68**:497, 1941.
404. Barnicot, N. A.: J. Anat. **79**:83, 1945.
405. Byerly, T. C., and Jull, M. A.: J. Exper. Zool. **62**:489, 1932.
406. Byerly, T. C.: Poultry Sc. **10**:404, 1931.

A great deal of confusion exists in the literature concerning experimental and human vitamin deficiencies of the embryo and their effects on the skeleton. Only a few reports will be mentioned here, and in all cases the nomenclature of the respective author will be used. Ingier [188a] published a study of Barlow's disease (scurvy) produced in guinea pig embryos by feeding the mother a diet of oats and water. In the report, scurvy, rickets, osteomalacia and osteogenesis imperfecta are mentioned, and no clearcut identification of the changes is made. Reyher, Walkhoff and Walkhoff [188b] describe vitamin C deficiency of guinea pigs, resulting in scurvy of the mothers and the embryos. The changes are compared with those noted in a newborn baby of their own observation. Maxwell, Hu and Turnbull [193b] observed a mother with osteomalacia and her baby with fetal rickets, and in their reports, stress the close relationship of the two diseases. Warkany [187] studied the effect of a maternal rachitogenic diet on the skeletons of rat embryos and observed changes similar to, but not identical with, rickets. He calls attention to the fact that the "diet" of the fetus is not the same as that of the mother.

Strontium treatment of pregnant rabbits produces in the fetal skeleton "pseudorickets" with increased apposition and decreased resorption of bone.[130]

The skeletal manifestations of fetal syphilis are too well known to be reviewed; they are described in textbooks of pathology. Postnatal developmental disturbances of the skeleton will be briefly referred to in part III of this review.

UROGENITAL TRACT

The explanation of malformations of the urogenital tract is facilitated by knowledge of several phases of its developmental physiology. This holds particularly for the kidney. Long before experimental evidence was at hand, Fischel [407] suggested that nephrons differentiate in the metanephric blastema only after their differentiation has been properly induced by the ureteric bud which, by its own differentiation, forms the collecting tubules, the renal pelvis and the ureter. This was assumed because neither of these two components of the kidney was ever found alone, or separated from the other (except perhaps small rudiments of ureters without a pelvis). Experimental elimination of the ureteric bud of the chick embryo has since furnished ample confirmation of Fischel's hypothesis.[408] These investigations, as well as

407. Fischel, A.: Die Bedeutung der entwicklungsmechanischen Forschung für die Embryologie und Pathologie des Menschen, in Vorträge und Aufsätze über Entwicklungsmechanik der Organismen, Leipzig, W. Engelmann, 1912, no. 16.

408. Boyden, E. A.: J. Exper. Zool. **40**:437, 1924. Proc. Soc. Exper. Biol. & Med. **24**:572, 1927. Gruenwald, P.: Arch. f. Entwcklngsmechn. d. Organ. **136**:786, 1937.

examinations of serial sections of suitable embryonic human specimens,[409] have made it clear that in man as well as in the chick aplasia of the ureteric bud results in absence of the entire organ, including also the derivatives of the metanephric blastema. The latter is present as usual but fails to differentiate in the absence of the inductor.

In mice, the gene which produces malformations of extremities, jaws and eyes, due to migrating blebs of fluid (see other sections of this review); is also responsible for renal malformations.[410] In the embryo the ureteric bud may be reduced or absent. If it is present, it does not branch and thus fails to induce differentiation of kidney tissue in the metanephric blastema.[411] In another mutation of the mouse [276e] it was found that complete absence of the ureteric bud or its failure to branch properly is followed by a complete lack of differentiation of nephrons even though the blastema is present.[283]

Various theories of the development of polycystic kidneys have been reviewed by Gruber.[412] According to one theory, the condition is caused by an abnormality of the union of the two components of the kidney, resulting in lack of drainage and subsequent distention of some nephrons. Actually it has been demonstrated that the interruption of the lumen does not usually occur at the point at which the nephron fuses with the collecting tubule. Roos's [413] own specimen, which its investigator thought to be proof of the nonunion theory, shows atresia at the junction of Bowman's capsule and proximal convoluted tubule, far from the point of union in the embryo. Moolten [350d] speaks of the nephron as migrating to join with the collecting tubule and suggests that this process may have failed in cystic kidneys; no such migration actually occurs. Berner's [414] detailed study of a large number of cystic kidneys demonstrated long ago that the interruption of the tubule may occur at any point. This indicates either that there are several mechanisms of origin affecting different parts of the tubules or that one mechanism may affect various points, the particular one depending perhaps on the timing of its action.

Occlusion of the lumen is not generally accepted as the principal lesion of polycystic kidneys. Some authors assume that active overgrowth rather than distention accounts for cyst formation.[412] Moolten [350d] advocates the interpretation of cystic liver and kidneys as a

409. Boyden, E. A.: Anat. Rec. **52**:325, 1932. Gruenwald, P.: ibid. **75**:237, 1939.

410. Bagg, H. J.: Am. J. Anat. **36**:275, 1926.

411. Brown, A. L.: Am. J. Anat. **47**:117, 1931.

412. Gruber, G. B., in Schwalbe,[256] 1927, pt. 3, sect. 3, p. 157.

413. Roos, A.: Am. J. Dis. Child. **61**:116, 1941.

414. Berner, O.: Die Cystenniere: Studien über ihre pathologische Anatomie, Jena, Gustav Fischer, 1913.

type of hamartiosis, or tissue malformation "based on a disturbance in organizer action" which manifests itself as cystlike gigantism of bile ducts or renal tubules. Actually there is no proof of this. Occlusion, as well as overgrowth of tubules, may be determined by interaction of adjacent tissues, but this is no more likely than an inherent deficiency of the tissue, perhaps determined by direct gene action. An old theory having in view obstruction of renal tubules on an inflammatory basis [412] has not been substantiated by histologic examination in the great majority of cases and has generally been abandoned.

Kampmeier [415] found that some of the earliest formed nephrons, located in the innermost portion of the embryonic renal cortex, degenerate before birth, and believes that their remnants may form cysts. This may explain cysts of the inner portions of the cortex, but it cannot account for the polycystic kidney in which the peripheral nephrons are also, or sometimes predominantly, affected, even with the assumption that the cysts of the inner portion of the cortex compress other tubules and that this leads to the formation of additional cysts.[416]

Dystopic kidneys are most commonly pelvic or fused kidneys. To understand their development it is necessary to know that the kidneys are at first located in the sacral region and that they move upward by a complicated process.[417] If this movement fails to occur, a pelvic kidney results. On the other hand, a slight medial deviation of the renal primordia is sufficient to bring them in contact with each other, producing a horseshoe kidney, particularly since the kidneys are normally close to each other at one point of their upward route.[418] Other displacements of the kidneys are less frequent, and their explanation is unknown. Among these is the rare one, in which the right kidney is displaced upward into the thorax, which occurs in combination with diaphragmatic hernia.[419] The arteries supplying dystopic kidneys arise from the aorta or its major branches at abnormal points corresponding to the locations of the kidneys. It was believed that the abnormal blood supply might be the cause of the dystopia of the kidneys, but investigators now know that this was erroneous, since the permanent renal vessels develop only after the kidneys have reached their final locations. The abnormal origin of the vessels is a consequence rather than the cause of dystopic kidneys.

Just as the permanent kidney depends in its development on the ureteric bud as an inductor of the metanephric blastema, the mesoneph-

415. Kampmeier, O. F.: Surg., Gynec. & Obst. **36**:208, 1923.

416. McKenna, C. M., and Kampmeier, O. F.: Tr. Am. A. Genito-Urin. Surgeons **26**:337, 1933.

417. Gruenwald, P.: Anat. Rec. **85**:163, 1943.

418. Lewis, F. T., and Papez, J. W.: Anat. Rec. **9**:105, 1915. Gruenwald.[417]

419. von Mikulicz-Radetzki, F.: Zentralbl. f. Gynäk. **46**:1718, 1922.

ros depends on the wolffian duct as an inductor of its differentiation.[408] In addition, the wolffian duct acts as a guide for the growing müllerian duct.[420] However, the gonads and the adrenal cortex do not depend on any of the nearby organs for proper differentiation.[408] The development of the urogenital tract of the chick embryo after experimental elimination of a wolffian duct shows a combination of defects, owing to the leading role of that duct. The combination is an exact duplication of the most common combination of malformations observed in man and other mammals of either sex.[421] In experimental embryos mesonephrons and müllerian ducts are absent when there is no wolffian duct. In addition, the ureteric bud is missing, since it is normally formed from the caudal part of the wolffian duct, and with it the entire kidney. In the male the result is absence of one kidney and part or all of the epididymis, as well as of the ductus deferens and the seminal vesicle on the same side. In the female the same disturbance results in absence of a kidney and the corresponding half of the uterus (in man: uterus unicornis of the other side) with part or all of the tube. Gonads and adrenal glands are not affected. This is an instructive example of the action of various types of correlation which normally insure proper development; a minute lesion at one point (the growing end of the wolffian duct) is followed by a syndrome of defects.

INTERSEXUALITY AND HERMAPHRODITISM

The investigation of sexual intergrades has in the past yielded innumerable suggestions for their explanation and nomenclature. However, it was not until modern genetic and endocrinologic animal experiments had produced much information that a satisfactory concept could be evolved. One of the most extensively used systems of classification distinguished among individuals with partly male and partly female structural characters true hermaphrodites and pseudohermaphrodites. The former have both testicular and ovarian tissue, either as ovotestes or as one testis and one ovary. Pseudohermaphrodites have uniform gonads, but part of the other sex organs are differentiated according to the opposite sex. All pseudohermaphrodites and the hermaphrodites with ovotestes are now often referred to as intersexes. Lateral hermaphrodites, and particularly those in which all genital organs show a striking lateral difference, are most probably due to a somatic mutation which results in a different genetic constitution of the two sides.

The existence of two kinds of intersexuality, genetic and hormonal, and the chromosomal mechanism of the former were discussed in part I. It remains to review briefly the theories of their development.

420. Gruenwald, P.: Anat. Rec. **81**:1, 1941.

421. Gruenwald, P.: Beitr. z. path. Anat. u. z. allg. Path. **100**:309, 1938.

All persons have in their genes male and female determining factors, with one kind outweighing the other. It is generally believed that genetic intersexes are those in which there is a low degree of preponderance of one sex, insufficient to assure normal development. There are two theories concerning the manner in which structural intersexuality develops in this instance. Goldschmidt [61] concludes from his genetic work with arthropods, and assumes for vertebrates, that intersexes begin their development in a normal fashion in the direction of the predominant sex but that they reach a turning point if the predominance of one sex is below a certain value. If the predominance is but slightly below the limit of normal, the turning point is at a late stage; the smaller (and the farther from normal) the predominance, the earlier is the turning point. After that point development proceeds entirely in the direction of the opposite sex. The result depends on the amount of irrevocable differentiation which has occurred prior to the turning. What has definitely developed in the direction of one sex at this time mixes with the remaining traits which subsequently differentiate according to the other sex. Goldschmidt presents several not quite convincing arguments to show that male intersexes (i.e., those beginning with a male phase before the turning point) do not exist in man and other mammals. One of the principal arguments is the inability of the testis to form an ovarian cortex in the event of transformation; this has since been shown to be incorrect.[422] Severinghaus found in a human intersex chromosomes characteristic of a male (X and Y), and Witschi [160] concludes from statistical data that the intersexes in his material develop at the expense of the males. Moszkowicz,[423] who is otherwise an ardent follower of Goldschmidt, assumes on morphologic grounds that male intersexes exist.

Apart from the question of male intersexes, the theory of Goldschmidt has been severely criticized by Bridges,[60] who developed the theory of genic balance. This is also based on the assumption of an abnormal quantitative relation of male and female sex factors, leaving the margin of either male or female factors insufficient to determine normal development. However, it is assumed that the individual is intersexual and develops as such at all times, since the genic constitution does not change. It is pointed out that Goldschmidt's results in arthropods can also be interpreted according to this concept.

Goldschmidt's theory was a potent stimulus for the examination and interpretation of the structure of human and mammalian intersexes, as is evident from the review of Moszkowicz.[423] However, it has not been a successful working hypothesis. If it were correct, all

422. Gruenwald, P.: Am. J. Anat. **70**:359, 1942.

423. Moszkowicz, L.: Ergebn. d. allg. Path. u. path. Anat. **31**:236, 1936.

forms should fall into one linear succession of degrees according to the times of turning from female to male development. According to Moszkowicz, two such series of forms should exist, including also those resulting from transformation of primarily male individuals. However, the observed forms cannot be readily classified in this manner. Moreover, in many attempts to determine the turning point by examination of the final condition, the same mistake has been made which occurred in many attempts of older teratologists to determine in a similar manner the latest time at which a given malformation could have started *(teratogenctische Terminationsperiode)*. The morphologic differentiation of an organ at various stages of its normal development was taken as a criterion of its ability to deviate from the normal course and produce the malformation in question. Experimental embryology has definitely established the fact that in some cases a given step of differentiation may be determined before it is visible and that in others a fully developed differentiation may change under certain conditions. As illustrations from the field of sex differentiation which show that it is impossible to determine a turning point with the aid of descriptive embryology, the following examples may be given: In rats ovarian follicles have been transformed into structures resembling seminiferous tubules long after birth by administration of testosterone propionate.[424] Genetically male chick embryos which had been completely feminized by estrogenic treatment in early embryonic stages changed completely back to their genetic sex after hatching, their gonads, which were ovaries at hatching, becoming testes with spermatogenesis.[155] These considerations invalidate all attempts to support Goldschmidt's theory by reconstructing the turning point in human cases of intersexuality.

Hormonal intersexuality occurs occasionally through the action of hormone-secreting tumors, and it has been produced repeatedly in the laboratory by the administration of androgenic or estrogenic substances to embryos. In both types the result depends, as is to be expected, on the kind, the concentration and the time of action of the substance administered. Another factor of great importance is the genetic background. Not only do corresponding embryonic primordia of genetic males and females react differently to hormonal stimulation, but various traits are fixed and resistant to changes to different degrees in different species.

A condition related to hormonal intersexuality, in which most of these factors are of importance, is that of the freemartin; in this instance one of heterozygous twins is obviously influenced by substances transmitted through anastomoses of the circulatory system from the other twin. This has been discussed in part I, where it was mentioned that

424. Marx, L.: J. Exper. Zool. **91**:365, 1942.

the nature of the active substance and its relationship to sex hormones is not known.

When an androgenic or an estrogenic substance is introduced into an individual, it acts by inhibiting some traits which would normally develop and by stimulating others which would otherwise regress or fail to develop. Burns [425] has emphasized that sex characters fall into two groups, one in which there are two different structures, one of which develops in the male and the other in the female ("alternative characters"), and another in which the same primordium follows a course of progressive development in the male which is different from that followed in the female ("nonalternative characters"). It is obvious that if a primordium of the former group has disappeared in accordance with sexual differentiation (e.g., the wolffian duct in the female), it cannot be brought back into existence by hormonal reversal of sex. In regard to other primordia of organs which develop in both sexes but in different ways, it is extremely difficult to predict the extent to which transformation is possible at a given stage. Morphology alone cannot answer this question, as was discussed in a foregoing paragraph.

Witschi [160] assumes, on the basis of experiments with amphibians, that the transformation of sex organs by action of hormones occurs by inhibition of structures of the sex opposite to that to which the newly introduced hormone pertains and subsequent compensatory growth of those of the other sex. The latter is not caused by direct stimulation by the substance administered. In agreement with this is the experience that if the left ovary of the chicken is removed, the right gonad (which never has an ovarian cortex) hypertrophies and develops into a testis, with concomitant masculinization.[426] Witschi extends his view to all vertebrates. It remains to be seen whether all findings in mammals are in accord with this.

Embryologic experiments have not confirmed the expected division of sex hormones into strictly male and female ones. Stimulation of structures of the opposite sex has repeatedly been found. However, Burns [149b] holds that this occurs only when excessive doses are used. The extent of stimulation of the same organ (e. g., the prostate) differs in genetic males and females, apparently under the influence of the genetic constitution.[149] The morphologic results of the administration of androgenic and estrogenic substances to embryos have repeatedly been reviewed.[427] They are too complex to be described here.

425. Burns, R. K., Jr.: Am. Naturalist **72**:207, 1938.

426. Domm, L. V.: Proc. Soc. Exper. Biol. & Med. **26**:338, 1929.

427. (*a*) Domm, L. V., in Allen, Danforth and Doisy,[64] p. 227. (*b*) Burns.[149b] (*c*) Burns, R. K., Jr., in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1942, vol. 10, p. 27. (*d*) Greene and others.[151b] Moore.[153]

Information on the interaction of genetic and hormonal factors has changed modern views on sex development. It was assumed until a few years ago that the genetic mechanism determines the sex of the gonad and with it the type of hormone to be secreted. The other, so-called secondary sex characters were believed to be determined by those hormones, and not by the genes directly. This concept evolved from the examination of relatively late, namely, postnatal, changes in which the influence of the genetic structure is overshadowed by conspicuous hormone actions. Experiments with embryos, as well as continued work with older stages, have demonstrated the fundamental importance of the genotype in the development, the maintenance and the reaction of sexually differentiated traits, including also the so-called secondary characters. This has been summarized by Moore,[153] who comes to the conclusion that in the embryo there is a long period of active sexual differentiation during which the gonads do not produce hormones and are not able to do so even when stimulated by the administration of gonadotropic substances. During this period the principal features of the sex organs differentiate apparently by direct gene action. However, during this same period the organs are susceptible to the action of artificially introduced estrogen or androgen. Even later on the action of hormones of the opposite sex is counteracted to some extent by the genotype. This is illustrated by the development of genetically male chick embryos which are feminized by estrogen injected into the egg to the extent that they have normal ovaries and female genitalia. In spite of this morphologically complete transformation, they revert to their genetic sex after hatching if treatment is discontinued, and are found later on to have functional testes.[155]

Danforth [428] has shown in an instructive table the varying extent to which genes and hormonal influences affect the plumage of several species and breeds of birds.

With these factors of genetic and hormonal determination of sex development in mind, one may attempt to interpret intersexuality as it is found in man and to formulate a basis for its management. It is clear from what has been said that a genetic intersex has no normal male or female sex. What some authors call the genetic sex of the individual, namely, the sex indicated by the number of sex chromosomes (either XX or XY), is of little value in these considerations, as it does not indicate the actual relation of male and female factors in the set of chromosomes. Imbalance of sex factors may go so far as to produce an individual of normal sex development and instincts opposite to the

428. Danforth, C. H., in Harvey Lectures, Baltimore, Williams & Wilkins Company, 1939, vol. 34, p. 246.

sex indicated by the mere number of sex chromosomes. This is an extreme which may not occur in man, but all intergrades between it and the normal may be expected.

The apparent combination of male and female bodily traits in the intersex had led many to assume an antagonism between male and female tendencies. In reality the only antagonism is that of the male and female genic sex factors, and this exists in normal individuals as well. In physical development a genetic intersex is as true to its own genotype as is any normal individual. It is futile and misleading to seek the "real" male or female sex in intersexes. Since one knows that the genic control which guides embryonic sex development also exists later on and that hormone action is but superimposed on it, it is unreasonable to attempt to produce a normal sex by giving hormones or by removing them (gonadectomy). Each intersex produces its appropriate hormones, no matter whether its gonads are testes or ovaries. To remove testes because they seem to be in opposition to a predominantly female external development means to castrate the person, and shows a fundamental lack of understanding.[164] It is also clear that attempts to determine the sex (in terms of male or female) of an intersexual child is futile, since no correlation has as yet been found which would permit one to determine the future psychic sex from somatic traits. For the same reason biopsy of the gonads is of no avail. It is therefore dangerous to do a plastic surgical operation on the genitalia of the infant with genetic intersexuality, since one cannot know at that time what the psychic sex will be.[429] It is necessary to declare an intersex as a male or a female, but this is arbitrary and has to be changed at puberty in many cases. The relationship between intersexuality and homosexuality is not clear.

Hormonal intersexuality offers to the physician an entirely different problem. When it develops in the embryo, it is usually due to hyperplasia or adenoma of the adrenal cortex,[163] which apparently produces an unidentified hormone. In the majority of cases females are affected, as is also true of postnatal "interrenalism." Elimination of the source of abnormal hormonal activity will allow the normal genetic sex to assert itself, and the structural changes will follow as far as is physically possible.

In the medical literature the principles outlined in the foregoing paragraphs have been increasingly appreciated during recent years. Novak [430] emphasizes the important role of genes in the early phases of development of the genital organs. Moszkowicz,[423] Schiller [164] and Greenhill and Schmitz [429] discuss the nature of the sex determination of genetic intersexes and warn against corrective procedures until the

429. Greenhill, J. P., and Schmitz, H. E.: West. J. Surg. **48**:36, 1940.
430. Novak, E.: J. A. M. A. **105**:413, 1935.

psychic sex manifests itself. Schiller stresses the grave consequences of gonadectomy, even in cases in which the sex of the gonads is seemingly opposite to that of the individual intersex. McCahey's [431] position is not so well founded on biologic principles. He divides intersexes into genetic males and females. This leads him to the statement that 'erections of the phallus in genetic females are pathological" and that these must be prevented by excision of all testicular structures. It should be clear that erections of the phallus are normal in any individual, and if an intersex with female feelings resents them, it is because of the excessive size of the organ. That might be dealt with surgically after puberty, when there can be no doubt of the psychic sex, but under no circumstances by gonadectomy. McCahey uses as a criterion of genetic femaleness the presence of derivatives of the müllerian ducts, a criterion which is entirely unfounded.

The only genetic sex abnormality in which there can conceivably be antagonism between male and female parts is true lateral hermaphroditism. According to the only satisfactory explanation, this is a genetic mosaic, apparently due to a somatic mutation (see part I) which leaves the two halves of the body with different genotypes. The assumption that the entire body and not just the genital tract is affected is borne out by a corresponding lateral difference of the plumage of birds with a similar constitution.[427a] The fact that the halves of the body develop independent of each other points to the leading role of the genotype of the tissues, since the hormonal environment is the same. Gynandromorphism has been reviewed by Moszkowicz,[423] Witschi [160] and others. A recent report of a human case is that of Lattimer, Engle and Yeaw.[432]

CARDIOVASCULAR SYSTEM AND BLOOD

In spite of the large number and the intriguing features of cardiac malformations, no direct information concerning their developmental mechanisms is available. Several theories of the abnormalities of septation and of the relations of the large vessels to each other and the heart are on record.[433] The subject is too complex to be reviewed in brief, and the original articles should be consulted by those interested in this field. As a cause of cardiac malformations, maternal rubella occurring in the early months of pregnancy has been recognized,[217] but here, too, the mechanism is unknown. Fetal endocarditis as a possible cause has been suggested by some and rejected by others.[434] Farber and Hub-

431. McCahey, J. F.: Surg., Gynec. & Obst. **67**:646, 1938.

432. Lattimer, J. K.; Engle, E. T., and Yeaw, R. C.: J. Urol. **50**:481, 1945.

433. Spitzer, A.: Virchows Arch. f. path. Anat. **243**:81, 1923; **271**:226, 1929. Pernkopf, E., and Wirtinger, W.: Ztschr. f. Anat. u. Entwcklngsgesch. **100**:563, 1933. Lev, M., and Saphir, O.: J. Tech. Methods **17**:126, 1937.

434. Gross.[212] Weintraub and Himmelfarb.[213] Footnote 214.

bard [216b] point out that malformations of the heart may be divided into two groups, of which one, comprising severe disturbances of the structure, is caused by primarily abnormal development, whereas the other, consisting of stenoses or atresias of ostiums in otherwise normal hearts, may be due to endomyocarditis of the embryo. In some cases of the latter group, microscopic study reveals indications of a previous inflammation, such as myocardial fibrosis or areas of calcification. Cardiac malformations occur in more than 20 per cent of all cases of mongolism.[144b] Their relationship to the suggested causes of this deficiency is unknown.

Remarkable success has recently been reported with the surgical treatment of certain malformations of the heart and large vessels.[435] This emphasizes the need for understanding and exact diagnosis of these conditions. Several workers have given directions for making the diagnosis.[436]

Abnormalities of the blood vessels are also relatively frequent and poorly understood. The inferior vena cava and other retroperitoneal veins are subject to much variation, as is to be expected in view of the complex pattern of the embryonic veins from which they arise.[437] Hereditary anomalies of these veins are on record.[438] The relationship of vascular abnormalities to malformations of the organs supplied has led to much speculation, particularly in the case of dystopic kidneys and the anomalous origin of their vessels. The old theory that abnormal renal vessels cause dystopia of the kidney is now generally abandoned.[439] It is known that the permanent renal vessels develop only after the kidneys have reached their final location and in accordance with that location.

In early chick embryos abnormalities of the extraembryonic vessels may be essential parts of complex syndromes of disturbances. Cairns [46] has described in detail the inadequate development of the yolk sac vessels in homozygous Creeper embryos and the deleterious effect on the embryo. Another abnormality, the so-called lethal ring, occurs in the wall of the yolk sac in riboflavin deficiency.[141] Excessive dilatation of intraembryonic vessels is found as a sequel of anoxia in the chick.[202]

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435. Blalock, A.: Bull. New York Acad. Med. **22**:57, 1946.

436. Sussman, M. L.; Grishman, A., and Steinberg, M. F.: Am. J. Dis. Child. **65**:922, 1943. White, P. D.: Heart Disease, New York, The Macmillan Company, 1944.

437. McClure, C. F. W., and Huntington, G. S.: Mammalian Vena Cava Posterior, American Anatomical Memoir 15, Philadelphia, Wistar Institute of Anatomy and Biology, 1929, p. 1. Gruenwald, P.: Beitr. z. path. Anat. u. z. allg. Path. **101**:439, 1938.

438. McNutt, C. W., and Sawin, P. B.: Am. J. Anat. **72**:2595, 1943.

439. Gruber.[412] Gruenwald, P.: Virchows Arch. f. path. Anat. **303**:47, 1938.

Abnormalities of the blood and the blood-forming tissues may be primary, on a genetic basis, or secondary to other defects. Well studied examples of the former kind are described by Grüneberg.[1r] The latter type consists of anemia, more or less compensated by persistent extramedullary blood formation. It occurs if there is insufficient space for marrow in the skeleton, as in chondrodystrophy,[440] or if fetal blood is destroyed by specific antibodies of the mother in so-called erythroblastosis fetalis, which will be described in more detail in a later section.

CLEFTS OF THE FACE

In older accounts the development of clefts of the face is usually attributed to the persistence of an early embryonic condition in which parts of the future face (medial and lateral nasal processes and maxillary processes) are separated from one another by fissures. Several investigators have recognized the fallacy of this explanation; particularly, Fleischmann [441] has emphasized that the so-called clefts occurring in the face of the normal early embryo are mostly grooves with a solid bottom, which disappear later on, not by fusion of adjacent parts but by filling in of mesenchyme under the intact epidermis. In the case of the groove between the medial nasal and the maxillary process the so-called cleft is filled from the beginning with epithelium of the thickened lower portion of the nasal plate, which is then partly replaced by mesenchyme.[442]

Politzer [443] shows in a critical survey that various types of abnormal cleft of the face develop in entirely different manners. Harelip, with or without corresponding clefts of deep structures, develops at the site of the aforementioned epithelial mass separating the medial nasal from the maxillary process, when mesenchyme fails to penetrate it. Early human stages of this malformation are known.[444] An oblique cleft running from a lateral part of the mouth to the eye was formerly explained as a persistent separation of the lateral nasal from the maxillary process. Politzer [443] points out that this type of cleft represents an irregular laceration of the face by mechanical force (e. g., that of an amniotic band) connecting two weak points, namely, mouth and eye. This is supported by the relation of such clefts to various structures, particularly the nasolacrimal duct, a relation which could not occur in a persistent embryonic condition. A third group, the median defects of the upper jaw, is considered by Politzer to consist of low grade cyclopic defects.

In rats cleft palate has been produced by a nutritional deficiency of the pregnant mother, which also causes a variety of other skeletal

440. Landauer, W., and Thigpen, L. W.: Folia haemat. **38**:1, 1929.
441. Fleischmann, A.: Sitzungsb. d. phys.-med. Soz. zu Erlangen **69**:315, 1937.
442. Veau, V., and Politzer, G.: Ann. d'anat. path. **13**:275, 1936.
443. Politzer, G.: Monatschr. f. Ohrenh. **71**:63, 1937.
444. Veau, V.: Ztschr. f. Anat. u. Entwcklngsgesch. **108**:459, 1938.

malformations.[445] Hereditary cleft palate of laboratory animals has been studied in several instances.[446] One instance is that in which modifying genetic factors influence the expression of the defect and may completely inhibit its appearance.[47a] Reed [47b] assumes that the inheritance observed in his strain of mice may well be similar to that in man. The genetics of human harelip and cleft palate has been discussed by Mather and Philip.[447]

TISSUE MALFORMATIONS

In this section abnormal differentiations or locations of tissues will be discussed as far as they are not part of gross anatomic malformations, and exclusive of the problem of tumor growth, which will be referred to in part III of this review. Abnormal histologic differentiation is often discussed in connection with tumor growth, partly because abnormal growth seems to produce changes in differentiation and partly because abnormal tissues often remain unrecognized unless they give rise to tumors. The older literature contains several large reviews of tissue malformations.[448]

There is no essential difference between the embryo and the adult in the developmental mechanisms of tissue malformations, and in many cases in which these have been found in adults, the time of development is unknown. For these reasons embryonic and postnatal conditions will now be treated, and no attempt will be made to find essential differences between the two kinds.

The histologic identification of abnormal tissues must be discussed here. It is often difficult and in many cases is carried out inadequately. Identifications which should be not more than suggestions are often taken for well established ones. In most cases one can demonstrate little or no evidence of a specific cellular function which might aid in the identification, and often a tissue is classified only by its appearance in histologic sections stained routinely with hematoxylin and eosin or by a similar method. Size and texture of nucleus and cytoplasm as seen in these sections are compared with those seen in normal cells. It should be clear to every one that these are but superficial and

445. Warkany, J.; Nelson, R. C., and Schraffenberger, E.: Am. J. Dis. Child. **65**:882, 1943.

446. Reed and Snell.[47a] Reed, S. C.: Genetics **21**:339, 1936; footnote 47 *b*. Scott.[236b]

447. Mather, K., and Philip, U.: Ann. Eugenics **10**:403, 1940.

448. Meyer, R.: Ergebn. d. allg. Path. u. path. Anat. (pt. 2) **9**:518, 1905; **15**:430, 1911; Ztschr. f. Geburtsh. u. Gynäk. **71**:221, 1912. Herxheimer, G., in Schwalbe,[256] 1913, pt. 3. Fischer-Wasels, W., in Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: Handbuch der normalen und pathologischen Physiologie. Berlin, Julius Springer, 1927, vol. 14, pt. 2, p. 1211.

unreliable criteria. To give an example, tissues have been identified in many different instances as adrenal cortex on little more basis than the presence of vacuolated cytoplasm. This is justified if one finds in addition the characteristic structure of a glomerular and a fascicular zone as one sometimes does in the epididymis, or if the tissue in question is located where one would expect adrenal cortex to develop, as in close proximity of the main organ. On the other hand, a striking instance in which the diagnosis of adrenal cortex appears unwarranted to me is that of a node of large, foamy cells found in the orbit.[449] In the report of that case, neither the structure of adrenal cortex was described, nor were any special methods used which, by the demonstration of certain chemical compounds, would suggest (though not establish) the diagnosis. It is so improbable on embryologic grounds that adrenal cortex would be present in this particular location that the vacuolated appearance of the cytoplasm is insufficient for the suggested diagnosis. Similar difficulties are encountered in many other instances, particularly often in cases in which structures of the urogenital tract are involved, e.g., tumors of the gonads and the kidneys. This is probably due to the unusually wide variety of developmental potencies which the tissues of the urogenital tract possess.[450] Another field in which the identification of cells has aroused much argument is that of hemopoietic and lymphoid tissues; here, too, the cells have a great variety of potencies, at least in the early phases of their differentiation.

The range of developmental potencies plays an important role in many tissue malformations. In many instances there is no experimental proof of the multiplicity of potencies in later stages of development. However, descriptive histology and embryology have brought forward many instances in which several cell types develop from a common ancestor in late embryonic or postnatal stages. Since the possession of multiple potencies is not a peculiarity of embryonic cells, one is not justified in designating cells of the mature organism as embryonic because they appear to have latent potencies.[451] The visible differentiation of a cell type cannot serve as an indication of its range of latent potencies, and there is no reason to postulate "undifferentiated" cells when evidence of latent potencies is discovered, or to deny the possibility of activation of latent potencies because the tissue in question shows some differentiation. If one gives due consideration to the possibility of activation of normal latent potencies, the assumption of persistence of embryonic germs becomes superfluous for the explanation of most dystopic tissues.[451] There is little direct evidence of the existence of such germs.

449. Hughes, L. W., and Ambrose, A.: J. A. M. A. **126**:231, 1944.
450. Gruenwald, P.: J. Morphol. **70**:353, 1942.
451. Gruenwald, P.: Arch. Path. **36**:190, 1943.

Gruenwald [452] recognizes two principal types of development of dystopic tissues and describes examples in human embryos. One is true aberration of tissue germs; that is, they depart from the normal point of origin into abnormal locations. Examples are aberrant bile ducts and accessory adrenal medulla. The other type arises by abnormal differentiation of cells in loco; these cells do not migrate and are not displaced from the region where the tissue which they form is normally found. Examples are ectopic renal tubules, accessory adrenal cortex or bone developing in abnormal locations. These formations develop by activation of potencies which the cells in loco normally have. Induction may have a part in this activation; thus, ectopic renal tubules may under certain conditions be induced by nerve tissue [453] and bone by mucosa of urinary passages.[454]

To these mechanisms which produce tissues where they would not normally occur at any time, one might add inhibition of development, occurring either as a failure to differentiate fully or as a persistence of tissues which normally disappear. Finally, there may be devious differentiation not corresponding to any normal tissue at any stage. The occurrence of pure failure of differentiation (the undifferentiated tissue being present) is doubtful unless one wishes to regard as such the absence of a kidney in a case in which the nephrogenic tissue is present in its mesenchymal state but fails to differentiate into nephrons. However, incomplete differentiation occurs as part of a complex disturbance, such as inhibited myelination of nerve fibers in mongoloid deficiency.[144b] Failure of a tissue to disappear is responsible, for example, for ectopic thyroid tissue and epithelial ducts at the site of the embryonic thyroglossal duct or for persistence of mesenchyme in the vitreous body of the eye.[455] Devious differentiation offers great difficulties to interpretation, as the customary comparison with normal tissue fails. It occurs to some extent in many tumors, particularly if the mother tissue has an intricate structural pattern which the tumor cannot duplicate. A good example of devious differentiation is that of the grotesque cells found in the brain in tuberous sclerosis.

Of great theoretic interest are the multiple hamartioses [318] affecting, for instance, in the case of the tuberous sclerosis complex the brain, the eyes, the heart, the kidneys and the skin. Nothing is known of their cause except that they may be familial. To explain them all simply by defective organizer action, as Moolten [318] does, is not justi-

452. Gruenwald, P.: J. Urol. **48**:224, 1942.

453. Gruenwald, P.: Anat. Rec. **86**:321, 1943.

454. Huggins, C. B.: Arch. Surg. **22**:377, 1931. Huggins, C. B.; McCarroll, H. R., and Blocksom, B. H.: ibid. **32**:915, 1936.

455. Reese, A. B., and Payne, F.: Am. J. Ophth. **29**:1, 1946.

fied until many other possibilities are excluded and the organizers involved are better known.

The problem of dystopic adrenal cortex and related tumors has been studied extensively. They will be discussed here as examples of tissue malformation. Many writers have believed that accessory corticoadrenal nodules developed by aberration of some of the buds which grow into the retroperitoneal tissue from the mesothelium in the early phases of adrenal development. More recently Uotila [456] and Gruenwald [451] failed to find the ingrowth of distinct cell cords during normal development. The adrenal cortex is nonepithelial for a considerable period after its origin; it develops from mesenchymal cells of the celomic wall by differentiation in loco.[451] A similar mode of origin is postulated for accessory cortical tissue in any location. All definitely identified accessory nodules appear where the mesenchyma may reasonably be assumed to possess the necessary developmental potency, namely, in the retroperitoneal tissues and their derivatives. Investigators do not know what determines the activation of this potency in some cells and not in others of these tissues. Only in an isolated instance has a condition been observed in which cortical cells regularly developed outside the adrenal gland: After complete adrenalectomy adrenal cortex tissue differentiates in the ovaries of ground squirrels and mice and maintains life indefinitely.[457] This differentiation is apparently mediated by the hypophysis, as it does not occur in hypophysectomized animals.[457a]

It is not easy to determine the extent to which accessory cortical nodules should be considered as normal. They have been described as regular components of the epididymis and the epoophoron of the rabbit.[458] In the hedgehog and the cat the testis and the ovary commonly contain them.[459] In man, accessory nodules can be found in and near the adrenal capsule on careful search in almost every adrenal gland examined, particularly in those of young persons. They are encountered in the epididymis much more commonly than the occasional case reports [460] would make one believe. A genetic influence on the amount of accessory adrenal cortex that develops was found in rats when it became apparent that certain strains are unsuitable for experi-

456. Uotila, U. U.: Anat. Rec. **76**:183, 1940.

457. Groat, R. A.: (*a*) Endocrinology **32**:488, 1943; (*b*) Anat. Rec. **89**:33, 1944. (*c*) Hill, R. T.: ibid. **94**:470, 1946.

458. Lacassagne, A., and Nyka, W.: Compt. rend. Soc. de biol. **118**:1406, 1935; **121**:95, 1936.

459. Watzka, M.: Ztschr. f. mikr.-anat. Forsch. **43**:235, 1938.

460. Freeman, A.: Arch. Path. **39**:336, 1945.

mental adrenalectomy because of the frequent occurrence of accessory tissue.[461]

The origin of the so-called hypernephroma (in the widest sense) has been controversial because this renal tumor presents structural traits resembling those of adrenal cortex. The followers of the theory of adrenal origin of the hypernephroma have referred to the frequency with which adrenal nodules occur in the kidney, sometimes combined with hypernephroma.[462] Cases in which adrenal gland and kidney are enclosed in a common capsule have also been cited in this connection.[463] Furthermore, in the tumors in question the distribution of vitamin A resembles that in the adrenal cortex.[464] Actually, the adrenal tissue occurring in the kidney is of significance not so much to account for the point of origin of the tumor as to indicate that the tissue in which these cell groups have developed has adrenal cortical potencies. The presence of these potencies would make it possible for renal tissue to form an adrenal tumor or a growth of hybrid differentiation combining traits of kidney and adrenal tissue.[465] The fact that tubules are present in some of these tumors does not exclude a relationship to adrenal cortex, since lumens occur in corticoadrenal cell cords in mammals,[466] as well as in human embryos and infants.[467]

Dystopic endometrium can probably develop in several manners. When it occurs in the myometrium as adenomyosis, it has obviously appeared there by growing in from its normal location. On the other hand, endometriosis outside the uterus must be explained either by implantation of endometrial germs or by activation of latent potencies. It has been demonstrated that all known locations of endometriosis are such that the presence of the necessary potency is a reasonable assumption.[468]

The cutaneous nevus has received much attention during recent years and most observers now agree that it contains nervous elements

461. Gaunt, R.: Am. J. Physiol. **103**:494, 1933. Gaunt, R.; Gaunt, J. H., and Tobin, C. E.: Proc. Soc. Exper. Biol. & Med. **32**:888, 1935. Cleghorn, R. A.; Cleghorn, S. M. M.; Forster, M. G., and McVicar, G. A.: J. Physiol. **86**:299, 1936. Ingle, D. J., in The Rat in Laboratory Investigation, Philadelphia, J. B. Lippincott Company, 1942, p. 291.

462. Mitchell, N., and Angrist, A.: Arch. Path. **35**:46, 1943.

463. O'Clowley, C. R., and Martland, H. S.: J. Urol. **50**:756, 1943.

464. Popper, H., and Ragins, A. B.: Arch. Path. **32**:258, 1941.

465. Schiller, W.: Arch. Path. **33**:879, 1942. Gruenwald.[452]

466. da Costa, A. C.: Compt. rend. Assoc. anat. **23**:69, 1928.

467. Hett, J.: Ztschr. f. mikr.-anat. Forsch. **3**:179, 1925.

468. Gruenwald, P.: Am. J. Obst. & Gynec. **44**:470, 1942.

and distorted tactile corpuscles.[469] This conclusion has distracted attention from the old argument as to whether the nevus tissue originates from the epidermis or the connective tissue. Recent workers have not discussed the problem of the origin of the nevus cell. It seems to me that the relation to nerve tissue, the pigmentation and the presence in the skin and (as melanoma) in the meninges are satisfactorily accounted for by assuming an origin from neural crest cells. These cells are known to migrate in the vicinity of the central nervous system and also along the skin of the embryo. They form either nerve cells and their supporting cells (connective tissues)[470] or the pigment cells of the skin.[471] Foot[472] does not mention this obvious possibility in a chart of the normal tissues and tumors derived from the neural crest.

A special problem apart from others in this field is that of teratomas. It is obvious that teratomas containing a variety of tissues must originate from pluripotent, if not omnipotent, cells, which thus resemble either germ cells or blastomeres or the cells in a few areas of the embryo where germ layers are not distinct and one cell group gives rise to a great variety of tissues (e.g., the prechordal region or the primitive streak). All of these possibilities have been suggested as the germs of teratoma. The problem has been reviewed by Schwalbe.[256] More recently Holmdahl[473] has accepted all of the aforementioned possible sources but favors the origin from areas where no distinct germ layers differentiate. Schlumberger[474] assumes that teratomas of the gonads originate from primordial germ cells and that teratomas arising in other places are due to "dislocation of tissues during embryogenesis," particularly in his cases of teratoma of the anterior mediastinum. In support of the theory of origin from germ cells, reference has been made to the parthenogenesis-like development of egg cells observed in the ovaries of various mammals.[475] Michalowsky and

469. Masson, P.: Ann. d'anat. path. **3**:417, 657, 1926. Ewing, J.: Brit. M. J. **2**:852, 1930. Foot, N. C.: Am. J. Path. **8**:309 and 321, 1932. Laidlaw, G. F., and Murray, M. R.: ibid. **9**:827, 1933. Becker, S. W.: Arch. f. Dermat. u. Syph. **30**:779, 1934. Feyrter, F.: Virchows Arch. f. path. Anat. **301**:417, 1938. Montgomery, H., and Kernohan, J. W.: J. Invest. Dermat. **3**:465, 1940. Ramel, E.: Schweiz. med. Wchnschr. **71**:375, 1941. Roth, G.: Arch. f. Dermat. u. Syph. **183**:148, 1942.

470. Raven, C. P.: Arch. f. Entwcklngsmechn. d. Organ. **129**:179, 1933.

471. DuShane, G. P.: Quart. Rev. Biol. **18**:109, 1943; **19**:98, 1944.

472. Foot, N. C.: Pathology in Surgery, Philadelphia, J. B. Lippincott Company, 1945.

473. Holmdahl, D. E.: Acta path. et microbiol. Scandinav. **19**:603, 1942.

474. Schlumberger, G. H.: Arch. Path. **41**:398, 1946.

475. Loeb, L.: Arch. f. mikr. Anat. **65**:728, 1905; J. A. M. A. **56**:1327, 1911. Courrier and Oberling: Bull. Soc. anat. de Paris **93**:724, 1923. Kampmaier, O. F.: Am. J. Anat. **43**:45, 1929.

others [476] have produced teratomas in the testes of fowl by injecting zinc salts. It has recently been suggested that many of the common types of testicular tumors of man are teratomas or resemble teratomas in that they are "neoplastic expressions of the unlimited potencies of embryonic cells." [477] Many of these tumors contain trophoblastic tissue.

Budde [478] compares the origin of teratomas with that of double monsters. If the malformation originates early in the development of an embryo, it results in a double monster, and if a comparable disturbance occurs later, it produces a teratoma. This matches well with the observation of Edmonds and Hawkins [259] that twins and teratomas occur in the same families. The problem of the homology of teratoma with an embryo (i.e., a parasitic twin) has been discussed by many writers. Nicholson,[479] Willis [480] and Needham [4] deny any homology of the two. One of their principal arguments is based on the lack of organization of the whole structure as indicated by the absence of segmentation. However, Schauffler [481] reports a teratoma with a segmented vertebral column. Other cases of a fetiform inclusion with extremities and other fairly well organized parts of a body have recently been reviewed, and a new case added by Plaut.[482] He considers the (so far not observed) development of the highly organized malformation, with skull, vertebrae, extremities and other parts, as a distorted replica of the development of an embryo. It is true, as Needham [4] states, that many times "innominate" structures in teratomas have unjustifiedly been compared to complex organs; yet there are well authenticated instances of high differentiation (e.g., of liver and kidney [479] or cerebellum [483] in addition to the aforementioned fetiform inclusions.

In recent years interest has shifted from the mother tissue of teratoma to the mechanism of development. In this connection organizer phenomena have been widely mentioned as responsible for the structure of the teratoma. Krafka [484] assumes in his organizer theory the pro-

476. Michalowsky, I.: Centralbl. f. allg. Path. u. path. Anat. **38**:585, 1926. Bagg, H. J.: Am. J. Cancer **26**:69, 1936. Anissimova, V.: ibid. **36**:229, 1939. Falin, L. I., and Gromzewa, K. E.: ibid. **36**:223, 1939; Virchows Arch. f. path. Anat. **306**:300, 578, 1940.

477. Friedman, N. B., and Moore, R. A.: Mil. Surgeon **99**:573, 1946.

478. Budde, M.: Beitr. z. path. Anat. u. z. allg. Path. **75**:357, 1926.

479. Nicholson, J. W. D.: J. Path. & Bact. **32**:365, 1929.

480. Willis, R. A.: J. Path. & Bact. **40**:1, 1935; **45**:49, 1937.

481. Schauffler, G. C.: Pediatric Gynecology, Chicago, The Year Book Publishers, Inc., 1942.

482. Plaut, A.: J. Mt. Sinai Hosp. **12**:567, 1945.

483. Willis, R. A.: J. Path. & Bact. **49**:571, 1939.

484. Krafka, J., Jr.: Arch. Path. **21**:756, 1936.

duction of secondary embryonic axes by "interference with the normal effect of the organizer," in analogy with the secondary body axes induced in amphibians by implantation of additional organizers. In essence, this theory comes close to that of Budde [478] (see the foregoing paragraph) and to the theory of blastolysis of Werber [101b] (see part I). The presence of organizer action within organ primordia in teratomas, e.g., kidneys, is obvious.

Needham [4] distinguishes two phases of organizer action: evocation and individuation. Evocation stimulates differentiation but does not determine its exact pattern, whereas individuation includes the determination of complex and harmonious developmental patterns. In the ectoderm of experimental embryos the induction of nervous tissue produces under certain conditions irregular tubes or cell masses by evocation, and under other conditions a neural tube with brain vesicles at one end, which is individuation. If a piece of tissue which would have the latter effect when transplanted in the living state is boiled before implantation, it will still act as evocator but will no longer produce individuation. Needham therefore compares teratomas with their irregular arrangement of highly differentiated tissues to the effects of dead organizers (without implying that there was actually induction on the part of dead tissue). Since, however, some teratomas show a considerable measure of individuation (see a foregoing paragraph), it might be better to assume that there is loss of individuation in the development of teratomas to a varying, though usually high, degree. Needham further points out that evocators occur commonly in the organism, and it may therefore be more important to give consideration to the presence of cells which will react to these evocators by producing the variety of structures found in teratomas. This leads back to the older embryologic speculations about tissues which may be expected to have a sufficient range of developmental potencies (blastomeres, germ cells and others).

EXAMPLES OF SYNDROMES OF MALFORMATIONS

A few well studied syndromes will be described, which affect several parts of the body. Each of these has been referred to in the foregoing pages, and it remains to summarize and correlate the various abnormalities.

The Creeper Fowl.—The Creeper gene (Cp) is dominant over its normal allelomorph, or allele (cp), and produces in the heterozygous condition chondrodystrophy. The gene is lethal, and most CpCp embryos die on the fourth to sixth day of incubation. A few survive but fail to hatch; these have phocomelia and ocular malformations. In early stages Cpcp embryos show no abnormalities and cannot be

distinguished from normal (cpcp) ones. Among the CpCp embryos there is considerable variation of structure, and only recently satisfactory criteria have been established.[485] All of these embryos have a defective yolk sac circulation. If this is severe and the vitelline vessels do not develop as continuous channels at all, the embryos show severe general retardation, intraembryonic anastomoses between large arteries and veins, and asymmetry of the eyes and the otocysts.[46] This asymmetry exceeds the extent found in normal embryos and is explained by the fact that the oxygenation afforded the organs by the blood stream is deficient; the part near the egg shell (in normally rotated embryos, the right side of the head) gets a better supply of oxygen by diffusion than do the parts on the opposite side.[46] In those CpCp embryos in which a better vitelline circulation develops, the effects on the embryo are at first minimal or absent (these embryos have not been examined in serial sections). The vascular network of the yolk sac is abnormal in these as well; there is no terminal vein, and part of the vessels contain stagnant blood. Eventually all circulation in the vitelline vessels stops, while the heart keeps pulsating, and the embryo dies shortly thereafter.[485] Just what the early characteristics of those CpCp embryos are which live longer and become phocomelic is not known. Landauer [51] recently reported that a higher proportion of CpCp embryos survive to late periods of incubation if the temperature during the first day of incubation is kept lower than usual, at 96 F.

The developmental potencies of CpCp and Cpcp limb buds have been studied by explantation.[389a] In the limb bud stage the phocomelic and chondrodystrophic abnormality appears to be determined, since transplanted limbs develop as they would in the embryo from which they came (apart from changes which the altered conditions would produce in any limb). When the effect of the disturbed circulation in CpCp embryos was recognized, regions containing the limb material were transplanted from embryos prior to the development of blood vessels. These also developed into phocomelic or chondrodystrophic structures, depending on the genotype. Apparently abnormal circulation has no part in the determination of phocomelia.[389b]

Experimental transplantation of the eyes of CpCp embryos has been reviewed in a foregoing section. The experiments show that the coloboma of the phocomelic embryo is also independent of the abnormal circulation. In contrast to the skeletal changes, however, it is not inherent in the primordium of the eye at the time of transplantation. It seems that an influence of some adjacent part determines this malformation.[337] In a review of the manifestations of the homozygous Creeper condition which incorporates part of the work just quoted,

485. Cairns, J. M., and Gayer, K.: J. Exper. Zool. **92**:229, 1943.

Hamburger [19] finds four abnormal mechanisms which are not correlated by any developmental links known to investigators. (1) In the majority of CpCp embryos a deficiency of the vitelline circulation leads to various severe malformations and early death; a mild degree of this may exist in those embryos which survive to become phocomelic, and may exert a hitherto unknown influence. (2) A disturbance of chondrogenesis combined with (3) a reduction of growth of the limbs produces phocomelia. There is an insufficient amount of bone marrow in this condition, and this leads to anemia and enlargement of the heart and the spleen. (4) An abnormality of the head mesenchyme apparently induces ocular malformation in phocomelic emybryos. (The asymmetric malformations of the eyes of early prothanic CpCp embryos are due to the first-mentioned mechanism.)

As to the chondrodystrophy of Cpcp chicks, it will be remembered that Landauer [385c] holds that it differs from phocomelia only in degree. Ocular malformations do not occur in these chicks.

"Myelencephalic Blebs" in Mice.—Much of the interest in this hereditary trait has been directed toward the production of the mutation, which is supposed to have been caused by roentgen irradiation of an animal from which this stock was derived. Some investigators believe now that there was no causal relationship between the irradiation and the origin of the mutation (see part I). The genetics of this trait has been extensively studied.[19]

The malformations are partial defects of the jaws, the eyes, the extremities and possibly the kidneys.[381] There is considerable variability of expression, and only part of the defects are present in a single animal. Bonnevie [334] found that the initial abnormality consists in the discharging of an excessive amount of cerebrospinal fluid from the fourth ventricle through the normal foramen anterius into the overlying tissue. This fluid forms blebs between the epidermis and the cutis. Owing apparently to peculiarities of the shape of the body, these blebs move in the same layer until they are either resorbed or reach points from which they cannot escape, such as the tips of the jaws or the extremities or the eyes. The temporary presence of blebs causes no permanent changes. However, when they come to rest, hemorrhage occurs into their cavities, and eventually they produce a local disturbance of development in the regions mentioned.

When this trait was crossed out to different stocks of mice, the effects varied somewhat, so that the existence of genic modifiers had to be assumed. Bonnevie [334] suggests that these modifiers act by changing the curvatures of the embryonic body which, in turn, influences the direction in which the blebs move.

Brown [411] described in detail the development of the renal malformations of the same strain of mice. They consist in failure of the

ureteric bud to grow out to its full length or to branch properly and in associated failure of the metanephric blastema to differentiate into nephrons. These events are obviously not determined by the presence of blebs of cerebrospinal fluid. Bonnevie [334] failed to find any malformations of the kidneys in her mice and concluded that these abnormalities are probably not effects of the same gene which is responsible for the blebs. Another possible explanation for this discrepancy is the action of modifiers.

Bonnevie's account of the origin of the malformations of this strain has furnished what is perhaps the best known example of a mechanical correlation of multiple defects of various parts of the body. Unfortunately, this has stimulated unwarranted speculation and has led several writers to extend a theory of myelencephalic blebs to totally unrelated conditions. Ullrich [486] quotes as evidence of the validity of this theory in human teratology a description of a 4 month fetus with cystic dilatation of lymph spaces in the neck.[487] Both the stage of development and the nature and the location of the cysts in that case are entirely incompatible with myelencephalic blebs. Engel [488] explains by this bleb theory a dozen unrelated syndromes of malformations, among them mongoloid deficiency and gargoylism (lipochondrodystrophy). Fortunately, these theories have not received undue consideration by other authors.

Effects of Heterospecific Pregnancy.—Certain differences of blood groups between mother and fetus cause various, often fatal disturbances in the latter if antibodies against the group-specific substance of the fetus develop in the mother.[62] The best studied effect is the so-called erythroblastosis fetalis, caused by destruction of fetal erythrocytes by maternal antibodies. In about 90 per cent of the cases this is found in Rh-positive babies of Rh-negative mothers (i.e., the baby's erythrocytes are shown by test to contain an Rh factor, while the mother's are shown not to contain it); in the remaining instances there are other group differences, namely, of the A or B groups.[63] The complex genetics of the Rh-types has been studied by Wiener, Sonn and Polivka.[489] Newborn babies with this disease have enlargment of the liver and the spleen and often also of the adrenal glands, the heart and the pancreatic islets.[140b] Similar enlargements occur in children of diabetic mothers.[140b] and also occasionally without any known cause. The

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486. Ullrich, O.: Klin. Wchnschr. **17**:185, 1938.

487. Gruenwald, P., and Kornfeld, W.: Beitr. z. path. Anat. u. z. allg. Path. **96**:341, 1936.

488. Engel, D.: Am. J. Dis. Child. **60**:562, 1940.

489. Wiener, A. S.: Tr. & Stud., Coll. Physicians, Philadelphia. **13**:105, 1945. Wiener, A. S.; Sonn, E. B., and Polivka, H. R.: Proc. Soc. Exper. Biol. & Med. **61**:382, 1946.

mechanism of these changes is unknown. Histologically, extensive extramedullary blood formation is found in various organs in fetal erythroblastosis. However, this does not fully account for the gross changes just mentioned. Macklin [490] suggests that erythroblastosis be diagnosed only if iron deposits due to hemolysis are demonstrable in the liver by histochemical means.

Changes other than anemia and its immediate sequelae have been produced by heterospecific pregnancy. They are of great importance and perhaps not fully recognized at the present time. Universal hydrops of the fetus is a well known manifestation.[322] It occurs occasionally without apparent relation to heterospecific pregnancy.[491] A reaction between antigen and antibody occurring in the body cells has been suggested as the cause of the damage of the liver, since the jaundice in some cases is out of proportion to the hemolysis.[492] The damage of the brain may be due to the damage of the liver,[322d] to the anemia [322c] or to agglutination thrombi lodged in cerebral blood vessels.[493] If it occurs in jaundiced patients, it is often quite conspicuous as the so-called kernicterus (encephalomyopathy with icterus). The presumable sequelae of this condition have been reviewed by Docter.[322f] In a small number of cases kernicterus is not associated with erythroblastosis.[494] There are indications that damage of the brain due to heterospecific pregnancy may occur without other manifestations, remain unnoticed in the young infant and account for a considerable percentage of cases of undifferentiated feeblemindedness.[322] Levine [495] suggests that fetal or neonatal death without characteristic anatomic findings of hydrops or erythroblastosis may also be due to the same cause. Haldane [496] estimates that the effects of Rh-group differences between mother and fetus account for more human deaths than any other genic difference.

Wiener [497] has recently stated that a difference of action between two kinds of antibodies which the mother may form accounts for two different syndromes within the group of erythroblastosis (in the wider sense). Agglutinins are believed to cause fetal erythroblastosis (in the

490. Macklin, M. T.: J. Pediat. **25**:533, 1944.

491. Potter, E. L.: Am. J. Obst. & Gynec. **46**:130, 1943.

492. Gilmour,[322d] Leonard, M. F.: J. Pediat. **27**:249, 1945.

493. Wiener, A. S., and Brody, M.: Science **103**:570, 1946; Am. J. Ment. Deficiency **51**:1, 1946.

494. Forster, F. M., and McCormack, R. A.: J. Neuropath. & Exper. Neurol. **3**:379, 1944.

495. Levine, P.: J. Hered. **34**:71, 1943.

496. Haldane, J. B. S.: Ann. Eugenics **11**:332, 1942.

497. Wiener, A. S.: Am. J. Dis. Child. **71**:14, 1946; New York State J. Med. **46**:912, 1946; Proc. Soc. Exper. Biol. & Med. **61**:390, 1946.

stric+ sense), jaundice of the newborn (icterus gravis neonatorum) and encephalomyopathy with icterus (kernicterus). The agglutination thrombosis of blood vessels of various organs which Wiener holds responsible for damage of tissues, has not been found by other workers up to this time. If, on the other hand, blocking antibodies pass from the mother to the fetus, severe sequelae will include death of the fetus, which in many instances shows hydrops; in less severe involvement there will be congenital hemolytic anemia. The ability of the mother to produce one or the other kind of antibody against the fetal Rh antigen depends on a hereditary factor.

The knowledge of heterospecific pregnancy has already led to the establishment of diagnostic methods and to rational directives for transfusion therapy of the hemolytic anemia. However, improved postnatal treatment will prevent death from anemia only. Other, perhaps more serious damage, which occurred before birth, cannot be undone. Investigators have not as yet established the full range of abnormal conditions caused by heterospecific pregnancy, particularly those in the field of neuropsychiatry. It may turn out that many of the infants who are saved by better postnatal care have severe defects unless, as Wiener [497] has suggested, those infants who can be saved are not the ones with damage of the brain.

MECHANISMS OF ABNORMAL DEVELOPMENT

III. Postnatal Developmental Abnormalities

PETER GRUENWALD, M.D.
NEW YORK

IN the introduction to this review the fact was emphasized that no strict separation exists between developmental abnormalities of early life, on the one hand, and many of the pathologic conditions of the mature organism, on the other. One might contend that some measure of development is involved in all pathologic changes of the structure of the body, and thus bring morphologic pathology almost entirely into the scope of developmental pathology. However, the present part of this review will be confined to some of the more obvious deviations of postnatal development. It will not include those subjects which are customarily treated in a systematic manner in textbooks—for example, the structural changes resulting from inflammation or from the action of hormones. An attempt will be made to cite examples which are comparable to embryonic abnormalities mentioned in the preceding parts, in order to establish a link between the developmental pathology of the embryo and that of the adult, which is much better known. Usually they are considered from different points of view.

All those causes of structural changes which were discussed in part I with particular reference to the embryo are also effective during postnatal life. However, their relative importance is different in the two periods of life. After birth the action of intrinsic (genetic) causes, while still demonstrable in many instances, is overshadowed by that of extrinsic agents, such as mechanical forces, chemical substances, actinic rays or infectious organisms. Similarly, the mechanisms of correlation by which multiple effects are produced after one initial lesion of the

From the Department of Pathology, Long Island College of Medicine, Brooklyn.

This work was begun while the author was Fellow in Pathology at the Mount Sinai Hospital, New York.

The review of the literature was concluded in August 1946. However, many European Journals of the past few years were not available at that time, on account of the interruption of communications during the war.

embryo (see part II) are all still in existence after birth, though not with the same relative significance.

During normal development there is a gradual shift in the extent to which various correlations are active; this is reflected in a similar shift of the mechanisms of abnormal development. After birth, the basic developmental pattern of the organism is no longer subject to changes, and developmental correlations—for example, induction—are active only in the limited spheres of tissue differentiations. Genetic control of structure exists at any time, but it is not so obvious after birth. On the other hand, correlations based on organic functions, particularly those of the nervous systems and the endocrine glands, gain greatly in significance. The space allotted to the effects of various agents and correlations in the following pages will not reflect their relative significance, as those changes will be discussed in detail which are less known and which resemble the ones that are more prominent in the embryo.

HEREDITARY ABNORMALITIES

Many of the abnormal hereditary traits appear after birth, and with all methods at one's command it is impossible to detect in the newborn an abnormality which might have developed before birth. Examples to illustrate this will be cited here with the reservation that there may have been failures to discover prenatal changes. This consideration and the fact that the abnormal genotype is present in the organism at all times, no matter when it manifests itself structurally, show that there is no essential difference between hereditary changes appearing during various periods of life.

The purely descriptive literature on many of the hereditary conditions in man will not be reviewed. It may be found through references in texts of human genetics.[45]

Reference has been made previously in this review to the fact that even in the well protected embryo the manifestation of hereditary traits may be influenced by the environment. This holds, of course, to a much greater extent for postnatal life. The modifying effects of various extrinsic factors may be so great that the hereditary background is considered as merely a "disposition" toward a certain change—for example, diabetes mellitus.

Many hereditary abnormalities manifest themselves by degenerative changes which appear at a characteristic time, sometimes late in life. These have been termed heredodegenerative diseases. They are closely related to obvious malformations. It is known that such congenital defects as taillessness in mice develop in the early phase of the embryo by a very similar process of degeneration of tissues which were previously normal in appearance.

Skin.—The morphologic expression and the morphogenesis of hereditary hairlessness of man and other mammals have been examined and the literature reviewed by David.[282] The condition may appear before or after birth, depending on the genotype. Among mice with one form of dominant hairlessness, homozygous animals can be recognized at birth, whereas heterozygous animals do not appear abnormal at birth, their abnormality developing later on. According to the histologic changes, David distinguishes three forms of hypotrichosis, namely, *hypokeratotica, cystica* and *hypoplastica follicularis*. For details the original article should be consulted. Whether human baldness is comparable with these findings in animals is questionable. There seems to be a hereditary disposition, and, in addition, an influence of androgenic hormone.[498] Among the hereditary postnatal cutaneous diseases which show more or less clearly the character of developmental abnormalities are xeroderma pigmentosum, neurofibromatosis and psoriasis.

Nervous System and Sense Organs.—In the case of some abnormalities of the brain it is particularly difficult to determine the onset of abnormal changes in relation to birth, because many of the functions of that organ cannot be tested in the newborn.

Retardation or arrest of development occurs after birth in mongolism.[499] However, other manifestations of this condition date back to prenatal life.

Qualitatively abnormal development, producing a tissue structure different from any normal stage, occurs in tuberous sclerosis,[500] amaurotic familial idiocy [501] and other diseases. The exact time of onset is not known.

Numerous examples of heredodegenerative conditions of the nervous system are on record. Among these are paralysis agitans,[502] hereditary sclerosis, spinal form (hereditary ataxia, Friedreich's ataxia), hereditary chronic progressive chorea (Huntington's chorea), hepatolenticular degeneration (Wilson's disease),[45] loss of coordination in rabbits [503] and many others which may be clinically more important but which have not been thoroughly studied from the pathologic and the genetic point of view.

498. Hamilton, J. B.: Am. J. Anat. **71**:451, 1942.

499. Benda, C. E.: Am. J. Ment. Deficiency **45**:42, 1940.

500. Globus, J. H., in Penfield, W.: Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932. Yakovlev, P. I., in Blumer, G.: The Practitioners Library of Medicine and Surgery, New York, D. Appleton-Century Company, Inc., 1936, vol. 9, p. 745.

501. Globus, J. H.: J. Mt. Sinai Hosp. **9**:451, 1942.

502. Allan, W.: Arch. Int. Med. **60**:424, 1937.

503. Anders, M. V.: Am. J. Anat. **76**:183, 1945.

Several forms of hereditary metabolic deficiency affect the nervous system—for example, amaurotic familial idiocy and phenylketonuria. In the former the nerve cells accumulate the abnormal metabolic product; in the latter the mechanism by which the brain is affected is not understood.

The sense organs present excellent examples of hereditary postnatal maldevelopment. In the eyes of mice examined by Keeler [341] the differentiation of the retina was arrested shortly after birth, and this led to complete or partial absence of rod cells. A somewhat similar end result has been found in a strain of rats after degeneration of the previously well differentiated retina.[504] The latter strain, as well as a mutant of mice described by Grüneberg,[1r] shows a high incidence of cataract developing after birth.

In man, retinitis pigmentosa develops as a degeneration of the retina [505] at a varying age, depending on the mode of inheritance in the particular family.[506] Certain forms of atrophy of the optic nerves, cataract and glaucoma are also hereditary.[45]

Postnatal degenerative changes of the labyrinth account for deafness and other abnormalities of labyrinthine function in certain strains of mice. In shaker-1 mice the stria vascularis is abnormal, and as soon as the vas spirale atrophies in the usual manner, Corti's organ degenerates because it is not adequately supplied with endolymph from the stria vascularis.[507] In another mutant, the "waltzing" mouse, the inner ear is normal at birth. There is disagreement as to whether in this form there is also primary degeneration of the stria vascularis with consecutive damage of the labyrinth or primary degeneration of the ganglion spirale cochleae or of the acoustic tracts of the brain.[508]

In man a hereditary change which involves abnormal development is otosclerosis. The morphogenesis and the genetics of this condition have been reviewed in detail by Bauer and Stein.[509] Abnormal bone is formed and replaces normal bone; it forms in excessive amounts and causes fixation of the stapes. In addition there are degenerative changes in the labyrinth and its nerves. The relation of these changes to the abnormal growth of bone is controversial. Bauer and Stein conclude from investigations of the familial occurrence of diseases of the inner ear that there is a hereditary inferiority of the organ (*Organminderwer-*

504. Bourne, M. C., and Grüneberg, H.: J. Hered. **30**:130, 1939.

505. Friedenwald, J. S., and Chan, E.: Arch. Ophth. **8**:172, 1937.

506. Allan, W.: Arch. Ophth. **18**:938, 1937.

507. Grüneberg, H.; Hallpike, C. S., and Ledoux, A.: Proc. Roy. Soc., London, s.B **129**:154, 1940.

508. Grüneberg,[1r] p. 130.

509. Bauer, J., and Stein, C.: Konstitutionspathologie in den medizinischen Spezialwissenschaften, Berlin, Julius Springer, 1926, no. 2.

tigkeit), transmitted by a combination of two recessive genes. This inferiority may manifest itself either as otosclerosis or in the form of purely degenerative diseases. The histologic changes of otosclerosis have been found in cretins and deaf-mutes at a very early age.

Teeth.—The teeth present an excellent opportunity to study postnatal developmental disturbances because much of their complex development takes place after birth. Identical changes may originate before and after birth, and for this reason all malformations of teeth will be discussed jointly in various sections of this part of the review.

Weinmann [510] has classified hereditary abnormalities of enamel formation according to the developmental process involved. If the deposition of enamel matrix is inhibited, hypoplasia of enamel results; if the maturation is affected, hypocalcification is seen. Both abnormalities may also result from other than hereditary causes. Weinmann points out that the hereditary forms affect all teeth uniformly and regardless of the time of the formation of these, whereas systemic forms, caused by metabolic or endocrine disturbances, as well as those due to localized trauma, are limited to the portions of the enamel which develop at the time and the place affected.

In the mutation gray-lethal of mice, with skeletal changes which have been referred to in part II, severe abnormalities of tooth development and eruption are apparently due to a lack of bone resorption in the jaws which would normally provide the necessary space.[511] A somewhat similar hereditary malformation occurs in rats.[512] At birth the animals appear normal. Shortly after birth spicules of bone begin to encroach on the apical portions of the incisor and first molar teeth and cause severe deformity and ankylosis of the tooth germs. The incisor teeth develop into large, unerupted, tumor-like masses of dental tissues. The first molar teeth show a lesser degree of abnormality, and the second and third molar teeth, which develop later, are more nearly normal. This points to a selective action of the abnormal trait beginning shortly after birth. Only preliminary studies have been published as yet.

Skeleton, Muscles and Tendons.—Various skeletal malformations which begin to manifest themselves in the embryo (see part II) not only show after birth the effect of the prenatal abnormality but continue to follow a devious course of development. These include, among others, various forms of chondrodystrophy and the gray-lethal malformation of mice.

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510. Weinmann, J. P.: Bur **43**:20, 1943.

511. Grüneberg, H.: J. Anat. **71**:236, 1937.

512. Schour, I.; Massler, M., and Greep, R. O.: J. Dent. Research **23**:194, 1944.

A generalized malformation of cartilage which appears after birth is hereditary in the rat.[513] There is excessive growth of cartilage, and the resulting changes in the trachea and the ribs lead to emphysema and fatal pulmonary complications. Experimental transplantations have shown that the property is inherent in the tissue: grafts of cartilage from abnormal rats develop abnormally in normal hosts, and vice versa.

Postnatal hereditary abnormalities of the human skeleton have been reviewed by Aschner and Engelmann.[372a] Examples are: Legg-Calvé-Perthes disease (osteochondrosis of the head of the femur) and osteopsathyrosis (fragilitas ossium). In the muscle system there are such heredodegenerative changes as progressive muscular atrophy and peroneal atrophy (progressive neuropathic muscular atrophy). Concerning tendons, Dupuytren's contracture of the palmar fascia might be mentioned.

Other Organs.—Imperforate vagina occurs as a hereditary trait in mice.[1r] The abnormality manifests itself at puberty, when the normal vagina acquires a lumen. This process fails in the abnormal mice, and it cannot be initiated by estrogenic treatment. If the vagina is opened surgically, the female is fertile. However, the vagina retains a tendency to close, in contrast to the normal vagina, which tends to open again if it is closed surgically.

Another strain of mice shows, beginning at the age of 3 to 4 months, severe adenomatous thickening of the mucosa of the pyloric portion of the stomach.[514] This observation leads into a field which should be of considerable medical importance, namely, hereditary differences in the old age changes of various organs. The following examples are discussed more in detail in Grüneberg's book on genetics of the mouse.[1r] Gorer [515] found in each of three strains of mice which he examined a specific change in the kidneys in advanced age. One strain showed metaplasia of the parietal layer of Bowman's capsule more frequently than did other mice. In another strain necrotic lesions of the papillae appeared, with consecutive dilatation of tubules. The third strain showed hyaline degeneration of the connective tissue framework. It is highly significant that these changes were not the result of diseases caused by extrinsic agents but were rather the expression of the genetic constitution, either alone or, as a predisposing factor, in combination with extrinsic influences which would otherwise not have this effect. The changes just mentioned are obviously related to the so-called heredodegenerative diseases which have just been referred to and to prenatal malformations which develop by degeneration of previously normal-appearing parts.

513. Grüneberg, H.: Proc. Roy. Soc., London, s.B **125**:123, 1938. Engel, S., and Grüneberg, H.: J. Genet. **39**:343, 1940.

514. Andervont, H. B., and Stewart, H. L.: Science **86**:566, 1937. Stewart, H. L.: J. Nat. Cancer Inst. **1**:489, 1941.

515. Gorer, P. A.: J. Path. & Bact. **50**:25, 1940.

There are interstrain differences in the occurrence of the so-called brown degeneration of the adrenal glands and in the structure of the follicles of the thyroid gland and the incidence of goiter. Also in mice there are genetically determined differences in the time of appearance of old age changes of bones and joints.[516]

Progressive facial hemiatrophy of man has been considered by Wartenberg[65d] as a heredodegeneration, possibly mediated by unilateral changes in the nervous system.

MECHANICAL AGENTS

Mechanical injury in the widest sense accounts for many structural changes. In most cases the abnormality is just the result of an elimination or a disfiguration of parts plus the usual processes of wound healing; these cases offer nothing to be discussed here. There are, however, instances in which more complex developmental processes are modified by mechanical injury. Among these is the growth of tissue displaced by trauma.

Few noncancerous tissues of the mature organism grow for a long period when transplanted to abnormal locations, unless special precautions are taken as in surgical grafting. Several cases are on record in which multiple nodules of splenic tissue grew on the peritoneum after the spleen had suffered traumatic rupture.[517] Another example, namely, endometriosis, is controversial. Some authors assume that viable endometrial tissue is displaced either by being regurgitated through the tubal ostiums or in the course of surgical procedures[518] as suggested by the appearance of endometriosis in surgical scars. On the other hand, there are sites of endometriosis which are not explained by either of these theories. It has been demonstrated that all known locations of endometriosis can be accounted for embryologically without recourse to mechanical displacement (see part II). This, of course, does not disprove the claim that mechanical transportation of tissue has occurred in some cases.

It may appear strange at the first glance that bone, one of the tissues of the body which mechanically are most rigid, should react most actively to mechanical influences by developmental changes. Yet it is this very rigidity which makes developmental changes of the structure necessary in cases in which other tissues, such as muscles, tendons or ligaments, could adapt themselves by means of their flexibility. The arrangement of the trabeculae and the haversian systems of bone is adapted to mechanical

516. Silberberg, M., and Silberberg, R.: Am. J. Anat. **68**:69, 1941.

517. Buchbinder, J. H., and Lipkoff, C. J.: Surgery **6**:927, 1939. Jarcho, S., and Anderson, D. H.: Am. J. Path. **15**:527, 1939.

518. Sampson, J. A.: Arch. Surg. **3**:245, 1921. Wespi, H. H., and Kletzhändler, M.: Monatschr. f. Geburtsh. u. Gynäk. **111**:169, 1940.

strain, and any change occurring in the mechanical conditions of the environment is immediately followed by resorption and rebuilding of parts of the bone. This is well demonstrated by the minute structure of bones with abnormal curvatures.[519] Pressure causes resorption of bone not by crushing or destroying the tissue but by the action of osteoclasts. This explains, for example, why the bodies of vertebrae are less resistant to the pressure exerted by an aortic aneurysm than are the intervertebral disks. Mechanical pull, on the other hand, causes overgrowth of bone, as seen at the points of insertion of tendons.

Müller [520] has presented in book form the developmental physiology and pathology of bone, with particular reference to the differentiation of inherent and extrinsic factors of development. Iselin [521] has pointed out how the principles of developmental mechanics should be used as a basis for rational therapy in orthopedics.

Displaced tissues of the mature organism may in a few instances act on their new surroundings as inductors. The best studied example is that of bone formation inducted by transplants of mucosa of urinary passages.[454] It has been found that the connective tissue reacts by formation of bone only in certain locations (e. g., the abdominal wall). In other locations (e.g., the liver, the spleen, or the wall of the stomach) a connective tissue capsule develops around transplants of bladder mucosa, but no bone. This has been compared with similar observations in embryologic experiments demonstrating that only part of a morphologically uniform tissue is able to react to certain inductions.[522] The fact that alkaline phosphatase is present in the transplanted epithelium has led to the suggestion that phosphatase diffusing into the connective tissue might be the cause of ossification. This has been ruled out not only by the negative findings in certain areas, even though connective tissue was present and the epithelium contained phosphatase, but also by the negative results observed in all locations of other transplanted phosphatase-containing epitheliums. The phosphatase which is present in areas of ossification induced by bladder mucosa is produced by fibroblasts as the first known response to the induction, before routine staining methods show any change in these cells.[522]

THE INFLUENCE OF CHEMICAL AGENTS

Certain substances which may or may not be essential for normal development and function in certain amounts are poisonous when present

519. Landauer, W.: Arch. f. Entwcklngsmechn. d. Organ. **115**:911, 1929. Sternberg, H.: Ztschr. f. orthop. Chir. **63**:387, 1935. Murray, P. D. F.: Bones, London, Cambridge University Press, 1936.

520. Müller, W.: Die normale und pathologische Physiologie des Knochens, Leipzig, Johann Ambrosius Barth, 1924.

521. Iselin, H.: Schweiz. med. Wchnschr. **14**:465, 497 and 536, 1933.

522. Gomori, G.: Am. J. Path. **19**:197, 1943.

in excessive amounts. In many instances their action, though morphologically specific to some degree, is of little interest from the standpoint of this review. Among those substances which have a definite influence on development are carcinogens, which will be mentioned in a later section dealing with cancerous growth, as well as others which affect normal developmental processes. In addition, deficiencies of some inorganic compounds may disturb development.

Other groups of substances are specifically related to developmental processes. Vitamins are essential for normal development; their lack or, in rare cases, their excess causes disturbances. Hormones are elaborated in the body, and one of their principal functions is the control of certain phases of morphogenesis (see also parts I and II). It is obvious that excess or lack of these "messengers" will profoundly influence development and maintenance of structure. Most of the well studied morphologic sequelae of abnormal levels of hormones and vitamins have been treated systematically in many monographs and textbooks and will therefore not be dealt with here. Only a few examples will be given.

The continuously growing teeth of rodents are a good object for the study of the effect of all of the aforementioned types of chemically caused disturbances. The formation or the maturation of the enamel, the formation of dentin, the eruption of the tooth and the structure of the socket may be affected singly or in various combinations.

Deficiency of magnesium in the diet of rats reduces the rate of eruption. Dentin formation is also reduced, and ceases completely in focal areas.[523] An excess of fluorine compounds reduces the rate of eruption and causes defective calcification of dentin and enamel. If large doses are given, enamel formation suffers by a shortening of the appositional life span of the ameloblasts. Changes observed in human fluorosis are in some respects similar to these experimental results.[524] Strontium and manganese compounds cause hypoplasia of enamel but in somewhat different manners, as has been shown by Wessinger and Weinmann [525] in histologic studies and illustrative diagrams.

The effects of vitamin deficiencies on postnatal developmental processes in man and in experimental animals has been summarized by Wolbach and Bessey [526] in a review in which much information and a comprehensive list of references may be found. A smaller volume of data on hypervitaminoses is also contained in that review. At an earlier date, Wolbach [527] pointed out that studies of vitamin deficiencies "may be

523. Gagnon, J.; Schour, I., and Patras, M. C.: Proc. Soc. Exper. Biol. & Med. **49**:662, 1942.

524. Schour, I., and Smith, M. C.: Publication 19, American Association for the Advancement of Science, 1942, p. 32.

525. Wessinger, G. D., and Weinmann, J. P.: Am. J. Physiol. **139**:233, 1943.

526. Wolbach, S. B., and Bessey, O. A.: Physiol. Rev. **22**:233, 1942.

527. Wolbach, S. B.: Science **86**:569, 1937.

of value as premises in problems, hitherto approached only by the methods of experimental embryology . . . ". Wolbach and Bessey classify the morphologic manifestations of vitamin deficiencies as follows: (1) diffuse consequences expressive of inanition; (2) effects common to several deficiencies, especially degenerations of the nervous system and, with qualifications regarding fine details, lesions of the skin; (3) degenerative changes characteristic in kind and distribution, best illustrated by the cerebral lesions of thiamine deficiency and the degeneration of skeletal muscles and embryonal tissues observed in vitamin E deficiencies; (4) initial specific effects exhibited by striking changes of structural patterns, outstanding in relation to vitamins A, C (ascorbic acid) and D. As was found earlier in the present review, postnatal maldevelopment is particularly obvious in the skeleton and the teeth, and much of present information on vitamin deficiencies concerns these structures.

One of the most important effects of vitamin A deficiency is seen in the keratinizing stratified epithelium formed in many mucous membranes which normally have a different lining. In the continuously growing teeth of rodents there are atrophy and keratinizing metaplasia of the enamel epithelium with consecutive irregularity, and finally cessation of dentin formation. If the vitamin deficiency is not complete, irregular remnants form tumor-like aggregates of dental tissues.[528]

According to Johnson,[529] vitamin A deficiency produces in the eyes of rats degeneration of the retina and rosette formation. This is of particular interest because, as has been reviewed in part II, rosettes develop in the embryo as abnormalities during the differentiation of the retina. They can apparently also arise secondarily after normal differentiation has been completed. The mechanism is probably one of rearrangement of the remaining cells after extensive degeneration.

Skeletal growth and development are inhibited in avitaminotic young animals, and the ensuing limitation of space in the cranial cavity and the spinal canal causes herniations and other disturbances of the nervous system. An excess of vitamin A produces osteoporosis and decalcification of bone, leading to multiple fractures. Osteoporosis is most marked in areas in which bone is normally being remodeled at the time of the disease.[526]

Deficiencies of vitamins of the B complex are not followed by such characteristic and generalized changes as is exemplified by the epithelial metaplasia of vitamin A deficiency. Most of the pathologic aspects are those of degeneration, with which one is not concerned here. Examples of abnormal development that probably is a consequence of such degen-

528. Burn, C. G.; Orton, A. U., and Smith, A. H.: Yale J. Biol. & Med. **13**:817, 1941.

529. Johnson, M. L.: J. Exper. Zool. **81**:67, 1939.

erative changes are imperfect growth of hair, the hyalinization and vascularization of the tunica propria of the cornea seen in riboflavin deficiency and the cirrhosis following fatty changes of the liver in choline deficiency.[526]

Vitamin C produces a characteristic generalized change in the supporting tissues by a "failure of formation and maintenance of intercellular materials".[526] All the changes found in human scurvy and in deficiency experimentally produced in the guinea pig are explained on this basis. The viability of the cells of the affected tissues is not diminished. The formation of bone and dentin is abnormal and finally ceases. Osteoblasts and odontoblasts become indistinguishable from fibroblasts. Wound healing is poor. Wassermann [530] shows that in the teeth of guinea pigs enamel formation ceases in areas exactly corresponding to those of absence of dentin, and he explains this by stating that the ameloblasts depend on the presence of dentin for their normal function.

According to Wolbach and Bessey, vitamin D deficiency acts not directly on the bony structures which show the striking effects but on the absorption of calcium and phosphate, which becomes inadequate for the proper calcification of tissues. This affects the cartilage of the epiphyses of growing bones and thus prevents the normal changes preceding the destruction and bony replacement of that tissue. Consequently the cartilage accumulates in the epiphyses. Another feature of rickets is the deposition of much uncalcified osteoid tissue instead of bone and the resorption of some of the preexisting bone. Osteoid tissue is highly resistant to osteoclastic resorption. In older individuals, in whom epiphysial growth no longer occurs, bone tissue is gradually replaced by solid masses of soft osteoid tissue (osteomalacia). The changes in teeth have repeatedly been examined. Weinmann and Schour [531] report on rachitic changes of the continuously growing teeth and the alveolar bone of rats and on their modification by various agents. They found, in contrast to some earlier investigators, that the formation and the maturation of enamel were unaffected. The formation and the calcification of dentin were retarded. In addition there were qualitative abnormalities in the dentin. The alveolar bone showed the well known lack of calcification of newly formed bone tissue and failure of this osteoid tissue to be resorbed. This produced a distorted pattern of growth. The increased resistance of osteoid tissue to resorption remained in evidence after treatment with parathyroid extract. As in other instances, the rachitic changes were reversed by starvation or by the administration of sodium phosphate solution or viosterol.

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530. Wassermann, F.: J. Dent. Research **23**:463, 1944.

531. Weinmann, J. P., and Schour, I.: Am. J. Path. **21**:821, 833, 857, 1047 and 1057, 1945.

One of the principal fields of hormone action is the regulation of morphogenesis and structure. The amount of work done in this field is so large, and it has so often been reviewed, that it will not be treated here. Texts of physiology or pathology, as well as specialized accounts of endocrinology, are available for reference. Endocrine influences on tooth development which are not described in detail in many of these works have been reviewed by Schour and Massler [532]; references to original reports may be found there.

A field in which development under hormonal control is particularly active after birth is that of the sex organs and sexually differentiated traits. This is also of great interest, because it demonstrates the interaction of genetic and hormonal controls of development. Certain abnormalities, such as those induced by hormones released by adrenal cortex tumors, are essentially similar before and after birth. Some fundamental aspects of these problems have been discussed in parts I and II.

Inductors are substances of eminent importance in structural development. They differ from hormones in the manner in which they reach their destination in the body: They are directly transmitted from one tissue to an adjacent one and are not distributed to the entire body by way of the blood stream. Abnormal induction will therefore occur when tissues are in contact with one another in a manner in which they would not be in the normal body. This may be brought about by mechanical dislocation of the inductor or of its substrate, as has been illustrated in a previous section with reference to bone induced by transplants of bladder mucosa.

THE EFFECTS OF INFECTION

I shall not discuss here the effects of protracted infectious diseases on general growth and development. The impairment of these processes is not so much a specific effect of the infectious agent as the result of nonspecific unfavorable circumstances, such as malnutrition or fever.

If infection provokes the growth of a tissue which would not usually be present, this is usually in the form of a granulation tissue. A discussion of inflammation and the formation of granulation tissue is not within the scope of this review, even though certain developmental mechanisms are involved in the differentiation of cells in this process and in the determination of the pattern of the lesion. However, brief mention should be made of what has sometimes been called specific granulation tissue. Granulomas of a definite and complex structure are in many cases so characteristic of a causative agent that their presence is considered sufficient evidence for diagnosis in routine practice

532. Schour, I., and Massler, M.: J. Am. Dent. A. **30**:595, 763 and 943, 1943.

of pathology, without the need for demonstrating the causative agent itself. The question thus arises whether these granulomas should be considered as specific developmental responses to the presence of certain micro-organisms. If one analyzes these granulomas, one finds that they are composed of a few elements of nonspecific reaction—for example, to foreign bodies. Various combinations of these elements and the peculiar distributions and biologic properties of the micro-organisms involved account for the characteristic appearances which granulomas may have. This has been analyzed in great detail in the case of tuberculosis, where various fractions of the substance of the bacilli have been isolated and the reactions of mammalian tissues to their presence observed. This work, as well as a large volume of information concerning tissue reactions to various injurious agents, has been reviewed by Forbus.[533]

In a few instances infection provokes structural alterations which do not fall into the group of inflammatory reactions. One example is the contagious pulmonary adenomatosis of sheep (jagziekte); the exact nature of the infectious agent has not been determined.[534] The lungs show an adenomatous nodular growth of columnar epithelium lining the spaces.

In some persons the teeth show evidence of aplasia of the enamel during a limited period of development, and it has been suggested many times that various diseases, among them infections, may be the cause. Sarnat and Schour [535] found no definite evidence of infectious diseases as the cause of chronologic aplasia of enamel in a series of 60 cases, but they admit that this relationship may occasionally exist. The effect on the teeth is a nonspecific one and does not indicate that the dental tissues were actually infected. The same probably holds for the observations of Kreshover,[536] who reported that in experimental tuberculosis of laboratory animals changes occur similar to the aforementioned chronologic aplasia in man.

DEVELOPMENTAL ASPECTS OF CANCER

The most striking peculiarity of cancer is a developmental abnormality concerning its structural differentiation as well as its rate of growth and its relation to adjacent structures. Many data relating to these and other aspects of the problem of cancer have recently been reviewed by Furth,[68b] and many original reports, as well as previous and more specialized reviews, are listed there.

533. Forbus, W. D.: Reaction to Injury: Pathology for Students of Disease Based on the Functional and Morphological Responses of Tissues to Injurious Agents. Baltimore, Williams & Wilkins Company, 1943.

534. Dungal, N.: Am. J. Path. **22**:737, 1946.

535. Sarnat, B. G., and Schour, I.: J. Am. Dent. A. **28**:1989, 1941; **29**:67, 1942.

536. Kreshover, S. H.: J. Dent. Research **21**:27, 1942; **23**:231, 1944.

Investigators know that all types of causes of abnormal development which have been discussed with regard to embryonic malformations in part I of this review may also produce cancer under proper conditions. They include hereditary factors and influences of the environment of a mechanical, chemical, radiant, thermic or infectious nature. As is often the case in embryonic maldevelopment, several of these factors (e.g., hereditary and environmental ones) may combine their effects also in carcinogenesis.

Hereditary factors may appear in two ways. In one of these, cancer develops in individuals carrying a certain gene or combination of genes either by virtue of these genes alone or when an environmental agent is added. In the latter event the genetic constitution determines what is often called the susceptibility to the environmental agent. The second way in which genes are concerned with cancer relates more directly to the cancerous tissue itself; this is somatic mutation. The fact that the essential abnormality is apparently passed on from one cancer cell to its descendants without the necessity of the continued presence of a carcinogenic agent has led many workers [537] to the conclusion that cancer cells are genetically different from the cells of their host, which constitutes a somatic mutation. In support of this hypothesis it has been pointed out that agents which are known to increase the mutation rate (e.g., roentgen rays) are also carcinogenic. Strong [68c] reports that one of the most commonly used chemical carcinogens, 20-methylcholanthrene, also increases the rate of germinal mutations.

Among environmental agents, virus infection has been held by Oberling [538] to be responsible for all cancers. Actually, it has been found in regard to certain cancers of the mammary glands of mice, which were previously believed to be purely hereditary, that one of the causative agents is a substance transmitted to the young by the mother's milk.[539] The properties of this substance resemble those of a virus as far as they are known. However, Oberling's theory has not been accepted to its full extent.

The nature of the basic abnormality of cancerous growth has been defined in various manners, each depending largely on the line of approach of the investigator. Pathologists, biochemists, immunologists, geneticists and others have found differences between cancerous and noncancerous tissue. Within the scope of the present review are only

537. (*a*) Bayne-Jones, S., and others: Pub. Health Rep. **53**:2121, 1937. (*b*) Berrill, N. J.: Physiol. Rev. **23**:101, 1943. Furth.[68b]

538. Oberling, C.: The Riddle of Cancer, New Haven, Yale University Press, 1944.

539. Bittner, J. J., in Research Conference on Cancer, American Association for the Advancement of Science, 1945, p. 63. Shimkin, M. B., and Andervont, H. B.: ibid., p. 97.

those facts and theories which relate to cancer as a phenomenon of abnormal development. All those[540] who have discussed in recent years the problem of cancer from the point of view of developmental mechanics have justly emphasized the obvious fact that cancer cells are not subject to those regulatory mechanisms which normally control growth and differentiation and assure that each part keeps within the limits of the structural pattern of the entire organism. There are indications that in some cases cancerous growth is initiated when a tissue is removed from the regulatory influences of its surroundings. This interpretation might be used in the cases in which carcinoma originates in repeatedly transplanted mammary glands[541] or in those in which explanted and reimplanted cells show malignant growth.[542] Lack of proper regulation may also be claimed for cancer developing in regenerating tissue—for example, in cirrhotic livers, or after subtotal orchiectomy in birds.[543]

In the great majority of instances, however, there is no evidence that a lack of organic control is the cause of cancer. It is quite possible, if not probable, that the lack of response to existing control mechanisms is a fundamental property of cancer cells rather than the cause of their cancerous behavior. The possibility that this change within the cells may be determined by genes, has already been mentioned.

Not in all cases of cancer is there a complete lack of response to normal controls. A well studied example of the effectiveness of such control and its therapeutic application is the carcinoma of the prostate. In many cases the tumor shares with its parent tissue the susceptibility to hormonal influences and regresses when the level of testicular hormone is lowered—for example, by orchiectomy.[544]

The function of cancer cells is well preserved in many cases. These cells may elaborate secretions (e.g., mucus, bile) or hormones, or undergo cornification, or produce a characteristic ground substance (e.g., bone). The function may be much better developed than the structural differentiation would make one believe; this has been observed in hormone-producing cancers.

The resemblance of cancerous and embryonic cells is superficial. It consists in rapid multiplication and a low degree of structural differentiation. On the other hand, one of the fundamental properties, namely, the lack of organization, distinguishes cancer very definitely

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540. Waddington, C. H.: Nature, London **135**:606, 1935. Needham.[4] Berrill.[537b]

541. Fischer, A.: Am. J. Cancer **31**:1, 1937.

542. Earle, W. R., in Research Conference on Cancer, American Association for the Advancement of Science, 1945, p. 139.

543. Champy, C., and Lavedon, J. P.: Compt. rend. Acad. d. sc. **207**:99, 1936.

544. Huggins, C.: Stevens, R. E., and Hodges, C. V.: Arch. Surg. **43**:209, 1941.

from embryonic tissue, which shows the effect of organizing influences to a high degree. In cancer, both the rapid growth and the poor differentiation are related to the lack of control. The poorly differentiated cancer cell is too abnormal to differentiate properly; the embryonic cell, on the other hand, is normal and may have several possible ways of differentiation depending on its environment. The cancer cell therefore differs more from the embryonic cell with its multiple potencies for differentiation than from the adult cell which may have undergone a reduction of these potencies and is less accessible to organizing influences. Therefore, a poorly differentiated cancer cell should be called anaplastic and not embryonic.[545]

The just mentioned resemblance of embryonic and cancerous cells accounts for the great interest which many workers have taken in the rare cases of cancer of the fetus. The rarity alone is significant, and it illustrates the statement that actually embryonic and cancerous cells are not closely related. After careful scrutiny, Wells [546] reviews and discusses a limited number of unquestionable cases of prenatal cancer. Among these are no typical cases of carçinoma. A more recent report of bilateral ovarian carcinoma occurring in a premature infant [547] must be discarded; it deals with normal ovaries in which some of the sex cords are not yet divided into follicles. These cords and their transitions into follicles were described as carcinoma cords arising from follicles. I have seen many similar ovaries in newborn infants.

In conclusion, the meager information concerning the fundamental developmental aspects of cancerous growth can be summarized as follows: Developmental abnormalities, particularly the lack of integration into the pattern of the organism and the poor structural differentiation, are properties rather than causes of cancer. As Berrill [587b] points out, the incompleteness of present knowledge of the fundamental mechanisms "is due to the standards of reference also being problems as challenging as malignancy itself." Among the possible causes of cancer which include all types of agents that may produce malformations, two are now being investigated as more universally active than others: somatic mutation and virus infection.

There is one fundamental difference between cancer and most of the other developmental abnormalities which have been discussed in this review. In the latter, the teratogenic factors act during a short period of development and produce certain changes. Subsequently, development proceeds by normal mechanisms, and the result is abnormal only because the substrate of these mechanisms is abnormal, owing to

545. Ewing, J.: Neoplastic Diseases: A Treatise on Tumors, Philadelphia, W. B. Saunders Company, 1941. Gruenwald.[452]

546. Wells, H. G.: Arch. Path. **30**:535, 1940.

547. Ziegler, E. E.: Arch. Path. **40**:279, 1945.

earlier changes. In cancer, on the other hand, the abnormal mechanism is perpetually active; it has been mentioned that this is one of the facts suggesting a genetic change in the cancer cells by somatic mutation.

CLOSING COMMENT

The present review is based on excerpts from a huge volume of pertinent information scattered throughout the literature of all divisions of medicine, as well as zoology, genetics, veterinary medicine, agricultural research and other fields. In the past, research in developmental pathology has been conducted from many different points of view, and few workers in the field have had a knowledge of previous work in other parts of this discipline, and of some of the basic problems involved. Developmental pathology deserves to be recognized as a portion of biology and pathology in which much has already been accomplished. If further work is to be successful, and if it is to be done in a rational manner, it is necessary to take stock now and endeavor to correlate whatever information is at hand. The present review is an attempt in this direction.

It is obvious that all those disciplines which have contributed to developmental pathology will in turn benefit from the progress made in this field. This holds particularly for practical medicine. Only a few years ago the knowledge of abnormal development had little of practical value to offer to the physician. This changed when within a few years great advances were made in such subjects as erythroblastosis fetalis, cystic fibrosis of the pancreas, malformations caused by rubella during pregnancy and the surgical treatment of several previously hopeless malformations. A stage has been reached in which developmental pathology is bound to make valuable contributions to practical medicine.

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