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Health and Biology

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Director

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INTRODUCTION

The scientific work presented herein has been coded at the program and problem levels according to the scheme given on Pages 7 and 8. In the report all contributions to a given problem have been assembled together without regard to author or to the administrative organization except that the number of the section which did the work is prefixed in each case. By using this number, it can be found on Page 12 what administrative officer can be approached for information about particular work. This does not imply either authorship, or scientific credit which will appear only in final reports issued from this Project. Since only progress in specific scientific problems is being reported herein, the cumulative work of special service units is not given separately. Their contributions appear or are implied in the reports on problems in which they participated.

It should be noted that the Quarterly Technical Reports of The University of Rochester Atomic Energy Project do not attempt to describe progress in all of the research programs but only in those in which some significant results have been achieved.

EXPLANATION OF PROGRAM AND PROBLEM CODES

The scientific work at The University of Rochester Atomic Energy Project has been coded at the program and problem levels. The program, in general, indicate broad fields of investigative or service activities while the problems indicate divisions of these fields. Although no consistent method of division into problems was possible, an attempt was made to achieve a natural division in the sense that each problem would encompass a subject normally written up and generally considered as a unit. The program on chemical toxicity of uranium, for example, has been broken down into problems according to the divisions commonly employed by toxicologists.

The problem codes are not related directly to the administrative organization of the Project. Consequently, the smallest administrative unit, the section, may work on more than one of the coded problems. Conversely, more than one section may work on the same coded problem. The administrative organization will be ignored in making this quarterly report of our research and service activities, all material being assembled according to the program and problem codes. The contribution of each section to a Quarterly Technical Report will be prefixed by the section number, however, to permit reference to the administrative organization if necessary.

It has not been possible to code the problems sufficiently broadly to avoid all overlapping. In cases in which various parts of a given investigation might be coded differently, the whole work was coded according to its principal subject matter as long as the minor subjects were relatively unimportant. Otherwise, the work was divided under appropriate codes.

PROGRAM AND PROBLEM CODES

I. X.R. BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (X-RAYS AND γ RAYS)

- X.R.1 Tolerance Studies (dose levels, survival time, gross and histo-pathology)
- X.R.2 Mechanism of Effects (physiological and biochemical)
- X.R.3 Therapy (measures against radiation effects)
- X.R.4 Hematology
- X.R.5 Genetics (histogenetics)
- X.R.6 Embryology
- X.R.7 Bacteriology and Immunology

II. I.R. BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (INFRA-RED & ULTRA-VIOLET)

- I.R.1 Flash Burns

III. R.M. BIOLOGICAL EFFECTS OF RADIOACTIVE MATERIALS (CONTACT, INGESTION, ETC.)

- R.M.1
- R.M.2 Radon
- R.M.3 Thoron
- R.M.4 Miscellaneous Project Metals

IV. URANIUM

- U.1 Physical and Chemical Properties
- U.2 Toxic Effects (description of acute and chronic toxicity)
- U.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

U.4 Fate (distribution and excretion)

U.5 Mechanism of Toxic Effects

U.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

V. Be. BERYLLIUM

Be.1 Physical and Chemical Properties

Be.2 Toxic Effects (description of acute and chronic toxicity)

Be.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

Be.4 Fate (distribution and excretion)

Be.5 Mechanism of Toxic Effects

Be.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VI. Th. THORIUM

Th.1 Physical and Chemical Properties

Th.2 Toxic Effects (description of acute and chronic toxicity)

Th.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

Th.4 Fate (distribution and excretion)

Th.5 Mechanism of Toxic Effects

Th.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

VII. F. FLUORIDE

F.1 Physical and Chemical Properties

F.2 Toxic Effects (description of acute and chronic toxicity)

F.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

F.4 Fate (distribution and excretion)

F.5 Mechanism of Toxic Effect

F.6 Methods of Detection of Poisoning, Prophylaxis, Treatmand and Protection

VIII. S.M. SPECIAL MATERIALS

S.M.1 Physical and Chemical Properties

S.M.2 Toxic Effects (description of acute and chronic toxicity)

S.M.3 Toxic Limits (respiratory; oral; skin; eye; parenteral)

S.M.4 Fate (distribution and excretion)

S.M.5 Mechanism of Toxic Effect

S.M.6 Methods of Detection of Poisoning, Prophylaxis, Treatment and Protection

IX. I.S. ISOTOPES

I.S.1 Tracer Chemistry

I.S.2 Radioautography

I.S.3 Therapy

X. O.S. OUTSIDE SERVICES

XI. P.H. PROJECT HEALTH

XII. H.P. HEALTH PHYSICS

H.P.1 Research and Development

H.P.2 Service

XIII. C.S. SPECIAL CLINICAL SERVICE

XIV. I.N. INSTRUMENTATION (SPECTROSCOPY, ELECTRON MICROSCOPY, X-RAY AND NUCLEAR RADIATION DETECTORS, X-RAY DIFFRACTION, ELECTRONICS)

I.N.1 Research and Development

I.N.2 Service

I.N.3 Instrumentation for Outside Organizations

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3130	Radiation Physiology	Thomas R. Noonan
3133	Radiation Animals	Thomas R. Noonan
3140	Radiation Chemistry	Kurt Salomon
3150	Spectroscopy	Luville T. Steadman
3160	Radiation Mechanics	Francis W. Bishop
3161	Electron Microscope	Francis W. Bishop
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PROGRAM X.R.

BIOLOGICAL EFFECTS OF EXTERNAL RADIATION (X-RAYS AND γ RAYS)

Problem Code: X.R.1 (Tolerance Studies)

Section Code: 3130

Comparison of Histopathological Effects of Radiation in Adult and Weanling Rats Subjected to 800 r Acute Total Body X-Radiation:

Background: Previous work in this laboratory has shown that rats weighing 50 grams (weanlings) had a mean survival time of approximately 3 days, as compared with approximately 10 days for adult rats, after an acute dose of 800 r whole body x-radiation. This difference in survival time between weanling and adult rats tended generally to decrease as the dosage was increased.

It became of interest, therefore, to investigate possible differences in histopathological effects between weanling and adult rats with a dosage that produces a marked difference in survival time between the two groups.

To this end the following preliminary experiment was carried out. A general summary of the results is presented.

Method: Fourteen adult and twelve weanling male albino rats were subjected to an acute dose (800 r) of total body x-radiation (unfiltered) given at the rate of 70 r per minute and with a target skin distance of about 23 inches. Two adult and two weanling male albino rats were sacrificed for control tissues, and were not radiated.

The weights of the adult experimental rats before radiation ranged from 320 to 458 grams, with a mean of 380 grams. The weights of the weanling experimental rats before radiation ranged from 52 to 64 grams, with a mean of approximately 57 grams.

Histopathological studies were made on selected organs taken from rats sacrificed at various intervals of time after radiation. In the adult group, two radiated rats were sacrificed on the 1st, 2nd, 3rd, 4th, 6th, 8th, and 10th days after radiation. In the weanling group, two radiated rats were sacrificed on the 1st, 2nd, and 3rd days after radiation, and one rat on the 4th day. Five weanling rats died during the night between the 3rd and 4th days and were not suitable for study. No deaths occurred within 10 days in the adult group.

Organ samples taken routinely for histopathological sectioning and examination included generally: submaxillary lymph nodes, abdominal lymph nodes (cecal), spleen, liver, testes, stomach, duodenum, jejunum, ileum, colon, femoral bone marrow (shaft and distal end of the femur), and samples of grossly visible abnormalities. These tissues were fixed in Bouin's solution and stained routinely with Harris' hematoxylin and eosin. Additional samples of liver were fixed in alcohol-formalin and stained for glycogen with Best carmine. Other samples of liver were fixed in 10 per cent formalin and stained for fat with Sudan IV.

Results: Control and radiated weanling rats showed little or no stainable fat in the liver. Control adult rats revealed small amounts of stainable fat in hematic cells. The amounts increased slightly in radiated adults on the 1st and 2nd post-radiation days, became moderate or moderately marked on the 3rd day and in one rat on the 4th day, and decreased thereafter, the adult rats on the 10th day showing little or no stainable fat in the liver.

Amounts of stained liver glycogen were marked in adult and weanling control rats and in all rats sacrificed on the 1st and 2nd post-radiation

days. Marked and moderate reductions in glycogen content were found on the 3rd and 4th days in weanling rats and on the 4th and 6th days in adult rats. One adult rat on the 8th day, and one on the 10th day, revealed marked reduction of glycogen, while their adult partners at these times showed no significant reduction in liver glycogen. The portal regions of the hepatic lobules were those mainly affected in this respect.

The stomach revealed no marked and consistent changes. Mitoses appeared depressed during the first two days after radiation, and a few of the radiated weanling rats revealed small numbers of degenerate mucosal epithelial cells. One adult rat sacrificed on the 2nd day showed a small number of degenerate epithelial cells and mild acute mucosal inflammation, and another revealed a small number of cystic glands. One adult rat on the 6th day showed mild submucosal hemorrhages in the pyloric region of the stomach.

Non-specific changes in the intestines consisted of bacterial infection and marked mucosal necrosis in the small intestine of one radiated adult rat sacrificed on the 8th day, and mild inflammation and submucosal hemorrhage in the large intestine of one radiated adult rat sacrificed on the 6th day.

Radiation changes in the intestines consisted generally of early depression of mitotic activity and subsequent swelling and distortion of basal gland cells, followed by frank degeneration, necrosis, and desquamation of basal epithelial cells, formation of "cystic" glands with disorganization of the mucosal pattern and relative decrease in amount of mucosal epithelium, and finally, return of mitotic activity and regeneration of epithelium. The intestines of weanling and adult rats were essentially or practically normal on the 4th post-radiation day.

In general, the proximal portions of the intestinal tract, such as duodenum and jejunum, showed greater change than more distal portions of the tract such as the ileum and colon.

Intestinal changes were often mildly or moderately greater in degree and extent in the weanling rats than in the comparable adult rats, and progressed more rapidly in the former.

The effects of radiation in the hemopoietic organs involved chiefly the destruction, cessation of production, and decrease in number of parenchymatous cells, with atrophy or reduction in size of the organs.

Hypoplasia and atrophy of the lymph nodes and spleen were very marked in radiated weanling rats by the 3rd day, but were generally never more than moderate in degree in adult rats, with some indication of regeneration in the latter animals on the 6th, 8th, and 10th days.

Moderately marked extramedullary hematopoiesis was found in the spleen of control weanling rats. In the radiated weanling rats these cells were markedly reduced in number along with the lymphocytes by the 3rd post-radiation day, when the bone marrow was also almost extremely hypoplastic. The adult control rats and adult radiated rats sacrificed in the first 6 days after radiation revealed no appreciable extramedullary hematopoiesis in the spleen. Adult bone marrow did not reach the stage of almost extreme hypoplasia until about the 6th day. On the 8th day, foci of extramedullary hematopoiesis were found in the spleen of adult radiated rats, and this increased to a moderate degree by the 10th day. Also, considerable regeneration of bone marrow elements was evident, beginning in the marrow of the femoral shaft, in adult rats on the 8th and 10th post-radiation days. At comparable times, hypoplasia of bone marrow was mildly greater in weanling rats than in adults.

Altered blood pigment, apparently hemosiderin, was present in small and moderate amounts in the spleen of adult control rats and adult radiated rats sacrificed on the 1st and 2nd post-radiation days. The amounts of this pigment increased to moderately marked and marked degrees on the 3rd and 4th post-radiation days, the increase being largely apparent and relative to decrease in the size of the spleen. After this time, when the spleen tended to return toward normal size the amounts of hemosiderin approximated those found in adult control spleens. Small amounts of hemosiderin were found in the spleen of control weanling rats, but this pigment was not found in radiated weanling rat spleens.

Hemorrhagic manifestations were found in lymph nodes and femoral bone marrow. Bone marrow hemorrhage was a relatively early change, apparent on the 3rd day, and was moderate in degree in weanling marrow and only slight in adult marrow. Hemorrhage was preceded by congestion. On the 4th day bone marrow hemorrhage in both groups was mild. The rats sacrificed after this time, all adults, revealed no appreciable marrow hemorrhage, but congestion continued to the 6th day.

Lymph node hemorrhage was a later manifestation and was therefore found only in adults, especially in many of those sacrificed on the 6th day and thereafter, ranging in degree from slight to marked.

Testicular changes following radiation involved chiefly the degeneration and decrease in number of spermatogonia and spermatocytes, and degeneration of spermatids. At comparable times, that is, within the first 4 days after radiation, the total testicular change was considerably greater in weanling rats than in adult rats.

Adult control rats had normal and mature testes. Weanling control rats

had normal but immature testes whose rather small seminiferous tubules were still moderately lacking in relative numbers of spermatids and contained no spermatozoa.

Degeneration and decrease in number of spermatogonia proceeded rapidly in the testes of weanling radiated rats, these cells being markedly decreased in number by the 2nd day. In adult rats a comparable stage was reached by the 3rd day. In the weanling rats there were mild reductions in the numbers of spermatocytes and many of these cells became degenerate. In the adult rats only small numbers of spermatocytes became degenerate and they were not decreased in number appreciably. On the 3rd and 4th post-radiation days degenerate spermatids were rather numerous in weanling rat testes, but were rare at all times in adult rat testes.

Summary and Discussion: Pathological changes compatible with the effects of x-radiation were produced in the stomach, small intestine, large intestine, spleen, lymph nodes, bone marrow, and testes of both weanling and adult rats subjected to 800 r acute total body x-radiation.

Hepatic glycogen was markedly depressed in amount, especially in portal regions of the lobules, in both weanling and adult rats. This depression occurred more rapidly in weanlings than in adult rats.

There was considerable increase in the amount of stainable fat in hepatic cells of adult rats after radiation, followed by a decrease. Weanling rats did not show such an increase.

Radiation changes in the stomach were minimal, and no significant differences between adult and weanling rats were found.

The intestinal, hemopoietic, and testicular changes were considerably greater in weanling rats than in adult rats at comparable times after radia-

tion, these changes generally progressing more rapidly in the former group.

Intestinal change was at a maximum on about the 3rd post-radiation day, after which, in a period of 12 or more hours, all weanling rats not yet sacrificed died, with but one exception. This exceptional case was sacrificed on the 4th day and revealed practically normal intestines, as did all adult rats sacrificed on that day and thereafter.

The spleen of weanling rats showed considerable extramedullary hematopoiesis which disappeared following radiation, along with the parenchymatous bone marrow elements. Not only did the adult rats survive the period of maximum intestinal change, but after the bone marrow became very hypoplastic, on about the 6th day, extramedullary hematopoietic activity began in the spleen and regeneration of bone marrow elements began in the femoral shaft. Regenerative activity was noted in lymphatic tissues also on the 6th day and thereafter in adult rats.

Hemorrhage, apparently a relatively late manifestation of radiation damage, was observed in the lymph nodes of many of the adult rats sacrificed after the 4th day.

It would appear that the vulnerable tissues studied were generally more sensitive in weanling rats than in adult rats to x-radiation at the dosage level employed.

The data suggest also the possibility of differences in the mode of death for weanling rats as compared with adult rats at this dosage. None of the adult rats died spontaneously within the 10-day period of the experiment, however, and no definite conclusions may be drawn until further investigations have been made. Hematological and body weight data will be reported subsequent to analysis.

Problem Code: X.R.2 (Mechanism of Effects)

Section Code: 3150

Measurements on the Blood Plasma Proteins of Irradiated Rabbits:

Background: The present experiments were carried out as part of a general investigation initiated for the purpose of determining whether any significant changes in the organic constituents of blood plasma due to whole body x-irradiation could be detected by spectrophotometric measurements. Since there are a number of organic components in plasma, it is apparent that some amount of chemical separation is desirable before optical measurements are made. The proteins in the plasma comprise a relatively large part of the organic material and measurements both chemical and physical have been made on the different protein fractions after irradiation, but the results of various investigations are not in close agreement. The release of toxic substances from tissue breakdown following irradiation and in traumatic shock has been frequently postulated on the basis of physiological responses but the chemical detection and identification of such materials has not been very satisfactory, at least in the sense that the radiation and traumatic shock syndromes have not yet been completely explained.

One of the most important considerations in conducting measurements on the plasma constituents in small animals is the question of the optimum time for sampling because of the limited number of blood samples that may be taken without seriously influencing the course of the experiment. Some indication of the probable time of maximum effects due to radiation may be obtained from the various biochemical findings in blood that have been reported. For the present purpose, the observations may be roughly divided into two categories, those made during a period of 1 to 2 weeks after a sublethal dose

of radiation, and those made during the acute stage and immediately preceding the death of an animal which has received a lethal dose. It has become customary, however, to consider the biochemical effects in the latter category as not being primarily due to radiation but to secondary systemic effects brought about by infection or other causes.

Some of the supposedly initial changes that have been observed and which may have some bearing on the present experiments may be listed. The blood clotting time (1) in rabbits becomes prolonged at 2 to 3 days after irradiation; this is ascribed to the release of heparin or heparin-like substances from the tissues. The weight loss (2) in rabbits becomes greatest at about 3 days and thereafter, and may be taken as a rough indication of the occurrence of tissue injury. The red cell sedimentation rate (3) for dogs increases rapidly at 4 to 5 days and is ascribed to some as yet undetermined factor in the serum. An increase in plasma histamine (2) in rabbits has been noted within a few hours but the time of maximum increase has not been determined. A temporary decrease of about 25 per cent in the plasma albumin and globulin at 1 to 2 days after irradiation in dogs has been reported by Davy (4). Such changes in dogs were not found by Muntz, et al (5) using electrophoretic methods but no observations seem to have been made after irradiation until the 7th day.

From these reports and from the results of the experiments described below, it is concluded that the optimum time for making measurements on plasma proteins in particular, and on changes in plasma composition in general, is at about 2 days after a sublethal dose. Furthermore, under these conditions any changes observed may be more readily associated with primary effects of radiation rather than to secondary effects developing after 1 to 2 weeks.

However, measurements in the acute lethal period should also be made and the changes, which in general are more dramatic, may advantageously be compared with the sublethal changes.

In the following experiments on measurements of plasma proteins before and after total body irradiation, four conventional protein fractions were obtained by a modified precipitation method employing dialysis. The amounts of protein were evaluated both by nitrogen determinations and by spectrophotometric measurements in the ultraviolet.

Method: Blood is taken by heart puncture using a 2-inch, 20 gauge needle and a 10 cc. Luer-Lok syringe. Before use the dry syringe is moistened with Liquaemin-Roche, an isotonic solution containing 10 mg. heparin per ml. Then 10 mg. of heparin powder (Hynson, Wescott and Dunning) dissolved in 0.15 ml. of 0.85 per cent saline solution are drawn into the syringe. The needle is inserted into the heart and about 12 cc. of blood is removed usually from the left side. The needle is removed from the syringe and the blood is slowly discharged into a 25 ml. Erlenmeyer flask and gently swirled. The blood is then centrifuged in a graduated centrifuge tube for 1 hour and the total volume of blood and cells are recorded. The plasma is removed and the cells discarded.

The procedure for the fractionation of the plasma proteins into several groups was devised for the purpose of overcoming certain disadvantages in the existing precipitation methods. The following objectives were desired:

1. The separations should be made in such a way as to avoid denaturation or other changes from the normal state in the animal body.

2. The precipitating reagent should be one that does not interfere with the subsequent nitrogen determination.

3. The method should be applicable to samples of plasma as small as 3 ml.

4. Besides being reproducible, the method should yield fractions whose protein values in total equal the protein value of the plasma.

It is believed that these objectives have been attained.

The method employs the buffered phosphate reagent of Butler and Montgomery (6) and a dialysis technique modified from McMeekin (7). The reagent is 3.0 M KH_2PO_4-K_2HPO_4 buffered to pH 6.5 prepared by adding 408.5 g KH_2PO_4 previously ground and dried at 100°C to a 1 liter volumetric flask and adding H_2O and 375 ml. of 4.00 N KOH and dissolving with heat. Solution is cooled, made to volume and filtered.

In McMeekin's dialysis procedure the protein solution is placed in a suitable vessel within which is an immersed cellophane bag containing the salt solution for precipitating the protein. The bag is rotated at slow speed until equilibrium is reached and the protein is precipitated. In the present method the dialysis is carried out with the protein solution inside the rotating cellophane bag which is immersed in a bath containing the phosphate solution. The advantage in this arrangement is that the small volume of sample is more easily and more quantitatively handled. Dialysis is continued until equilibrium on both sides of the membrane has been reached. The process is carried out at room temperature which varies somewhat from time to time. Constant temperature has not been deemed essential since Butler, Blatt, and Southgate (8) found temperature ranges as encountered here to be without influence on the amount of protein precipitated.

The equipment consists of stirring devices which rotate at 15 to 20 r.p.m. and to which the dialysis bags are attached. The dialysis vessels are

300 ml. tall form, lipless pyrex beakers and contain 220 ml. of the phosphate reagent. Beakers are covered with watch glasses having a center hole to reduce evaporation. The dialysing bags are prepared from cellophane dialyzing tubing 19 mm. in diameter and are weighted with a stainless steel pendant.

The procedure here described divides the plasma proteins into four groups or fractions. Fraction I contains fibrinogen and some of the globulin of lower solubility. Fraction II consists of euglobulin and some pseudoglobulin. Fraction III consists of pseudoglobulin and some euglobulin. Fraction IV contains the albumin and some pseudoglobulin. This grouping of the proteins is according to Butler et al (8). More fractions than these four could be obtained by employing more dialysing solutions in which the increments of PO_4 concentration were smaller than those given here.

In practice, 3 ml. of plasma are diluted with 9 ml. of 0.75 M PO_4 soln. and transferred to the bag. In the first fractionation, dialysis against 1.07 M PO_4 is begun as soon as possible after collection of the blood sample and proceeds for 24 hours. The precipitated fibrinogen is separated by centrifuging and dissolved in 0.1 M K_2SO_4 solution.

The supernatant is dialysed for 18 hours against 1.53 M PO_4. The euglobulin precipitate is separated by centrifuging and dissolved in water.

The supernatant from Fraction II is dialysed for 18 hours against 2.46 M PO_4. The pseudoglobulin or Fraction III is separated by gravity filtration. It is dissolved from the filter with numerous small quantities of water. Filtration is necessary because the densities of the liquid and precipitate are so nearly the same that centrifuging does not bring down the precipitate.

The supernatant is dialysed for 48 hours against 3.0 M PO_4 solution and

albumin or Fraction IV is removed also by filtration.

The quantity of protein in each fraction is determined from the nitrogen by a micro-Kjeldahl procedure using HgO as catalyst. Protein is estimated as equal to the nitrogen divided by 0.16. The plasma protein is calculated by determining the total plasma nitrogen and subtracting the non-protein nitrogen. The NPN is determined in the filtrate after precipitation of the plasma proteins using 2.5 per cent trichloroacetic acid.

As an example of the application of the fractionation method described above, there are presented in Table 1 below the results from the simultaneous fractionation of 6 samples from the same specimen of plasma. It may be noted that the total protein in the fractions is on the average 88 per cent of the plasma protein. In practice the protein fraction recoveries have often been higher than this.

TABLE 1

PLASMA PROTEIN FRACTIONS

Test No.	NPN Mg %	Protein-G/100 ml. Plasma						% Recovery	A/G Ratio
		Plasma	I	II	III	IV	Sum I to IV		
1	27	6.40	0.46	0.55	1.3	3.2	5.5	86	1.78
2	23	6.34	0.33	0.57	1.3	3.2	5.4	85	1.71
3	21	6.34	0.45	0.77	1.4	3.3	5.8	92	1.55
4	25	6.27	0.28	0.59	1.5	3.3	5.6	88	1.59
5	21	6.38	0.40	0.57	1.4	3.2	5.6	88	1.62
6	25	6.46	0.40	0.64	1.3	3.3	5.7	89	1.65
Av.	24	6.37	0.39	0.62	1.3	3.3	5.6	88	1.65

As a comparison with other methods, it may be observed from Table 1 that the albumin to globulin ratio is 1.65 for this animal. Anson and Edsall (9) give the value 1.48 for the rabbit determined by electrophoretic means.

In Table 2 below is shown the per cent of the plasma protein found in each fraction of the same plasma as given in Table 1.

TABLE 2

PER CENT OF TOTAL PROTEIN IN EACH FRACTION

Test No.	Fraction I Fibrinogen	Fraction II Euglobulin	Fraction III Pseudoglobulin	Fraction IV Albumin
1	7.3%	8.7%	20%	51%
2	5.2%	9.0%	21%	51%
3	7.0%	12.0%	21%	51%
4	4.4%	9.2%	23%	52%
5	6.3%	8.9%	22%	51%
6	6.2%	10.0%	20%	52%
Av.	6.1%	9.6%	21%	51%

The ultraviolet absorption spectra of each plasma and its fractions were obtained with a Bausch and Lomb medium quartz spectrograph and sector photometer. The plasmas and the fractions obtained in solution from the above procedure were suitably diluted with water to give protein absorption curves such that the optical density at the maximum of absorption at 2770 A was between 1.0 and 2.0. The density of the minimum in the protein curve at 2530 A was also measured and the ratio of density of the maximum of the absorption band to the density at the minimum was calculated. This ratio is a rough indication of the character of the protein sample because it is different for different

proteins and is altered if some other material having light absorption is present in the protein sample. The determination of these optical densities on a given sample is reproducible within less than 5 per cent.

The optical density of the maximum of absorption also varies with the type of protein but the wavelengths of the maxima and minima of the bands are very nearly the same for all. Although the optical density is an indication of the amount of protein present, it is found preferable in analyzing the data to calculate the absorption per unit of weight of the protein in each sample. This figure, called D_2 in the following, should be a constant for each protein if the character of the sample does not change.

It is also found that protein measurements on successive blood samples from a normal rabbit show variations which may be ascribed to the effects of blood sampling and to other physiological changes occurring from time to time. Therefore, in the conduct of those investigations on irradiated animals a companion control rabbit was selected for each irradiated animal and blood samples were taken and measurements were made simultaneously on the pair of animals. The results are thus presented in terms of a comparison between the irradiated rabbit and its control. Three quantities are considered, the protein weight, the optical density per unit weight (D_2), and the ratio of maximum to minimum density of the protein absorption curve.

The radiation was given to an adult male rabbit lying on its back, target to skin distance being 50 cm. A single dose was, with one exception, 1000 r from a tube operated at 100 KV with a filtration of 0.5 mm Cu and 1.0 mm Al. This single dose is not lethal for the rabbit. At intervals of about 28 days the dose was repeated. One animal, No. 600, has survived six doses whereas two others, No. 604 and No. 608, have died after two and three

doses respectively. This procedure gives an opportunity for repeat measurements on the same animal during the sublethal period and then just before the death of the animal. Blood samples were taken at various times for the measurements.

Results:

Gross Clinical Observations: In animal No. 600, which has survived 6 irradiations, slight weight losses have occurred following each exposure with a weight increase taking place thereafter. Following the last irradiation in each of the animals that died, the weight loss continued until death and amounted to about 25 per cent of the starting weight. Death was apparently due to the effects of irradiation and occurred 22 and 30 days respectively after the last irradiation. At autopsy no evidences of bacterial infection or other recognizable disease could be found. The hematocrit fell from 41 per cent to 18 per cent in one animal. Common findings of loss of hair and skin injury were seen.

Weights of Protein Fractions: In Table 3 (Page 30) is shown the day and the time after irradiation (R1-R6) that the samples were taken on animal No. 600 and its control No. 599. For a standard size sample of plasma, 3 ml., the ratio of the weight of protein in each fraction compared with the control is listed in the four columns of fractions. It is apparent from these data that there is considerable variation in the ratio values but that the changes after irradiation are not particularly large or on the whole remarkably consistent. However, there is some indication that in the samples taken 1 and 2 days after irradiation there is a rise in the fibrinogen and a fall in the albumin. A decrease in albumin is in agreement with the findings of Davy (4) in dogs. An increase in fibrinogen has been reported by Ham (10) but measure-

TABLE 3

PROTEIN FRACTIONS*

Sample No.	Day	Radiation Day	Sample Time	Fibrinogen	Euglobulin	Pseudoglobulin	Albumin
1	0	-	-	-	-	-	-
2	7	R-1.7	5 min.	0.50	1.55	1.17	1.00
3	11		4 d.	-	-	-	-
4	17		10 d.	0.79	0.88	0.66	0.76
5	41		24 d.	0.52	0.47	1.01	1.23
6	48	R-2.48	5 min.	0.77	0.59	0.99	0.69
9	72		24 d.	0.51	1.03	0.57	1.11
10	111		53 d.	0.92	0.55	0.99	1.14
11	118	R-3.118	3 hrs.	1.17	0.54	0.91	1.13
12	120		2 d.	2.40	0.63	0.72	0.75
13	125		7 d.	0.00	2.06	1.18	0.94
14	160		42 d.	1.22	-	-	1.02
15	162	R-4.161	1 d.	1.81	0.62	1.01	1.18
16	170		9 d.	0.77	0.91	1.10	1.15
17	176		15 d.	0.85	0.75	1.34	1.08
19	191	R-5.189	2 d.	1.08	0.56	1.07	0.95
20	202		13 d.	1.04	0.93	1.07	1.28
21	216		27 d.	0.44	0.84	0.97	1.27
22	219	R-6.217	2 d.	1.60	1.15	1.29	0.86
23	240		20 d.	0.97	0.94	1.18	1.07

* The weight of the protein in each fraction divided by the

ments on fibrinogen after x-irradiation have not been very extensive. Variable behavior in the globulin is also seen in other results (4).

Absorption Spectra Measurements: In Table 4 (Page 32) are shown the measurements by absorption spectra on the same pair of animals. The absorption per unit weight of protein is given as a ratio against the control. These values are not dependent on the recovery in the protein fractions or on the weight of the protein but rather are an indication of the constancy of character of a protein fraction. There is some variability here also, but there is no apparent dependence on irradiation and the changes in the albumin in particular are very slight.

Measurements on the ratio of optical density at the maximum of the band to the density of the minimum of the band are given in Table 5 (Page 33). As before the comparison against the control is listed. The values are for plasma and have the distinction of being measurements on the total constituents of the plasma rather than on proteins only.

The ultraviolet absorption spectrum of plasma is principally that of the proteins since other constituents are present in smaller amounts or have smaller specific absorptions. The data in Table 5 show some variation, more often at 1 or 2 days, which may indicate some transitory change in components other than proteins.

Measurements in the Acute Period: Measurements similar to the above were made on animals No. 604 and No. 608. In the early periods the blood sampling was more scattered than for No. 600 and no data were secured at 1 to 2 days after irradiation. No remarkable changes were found. However, in both animals after the last irradiation changes were noted which increased in magnitude until death. The fibrinogen increased, the euglobulin increased

TABLE 4

ABSORPTION SPECTRA COMPARISONS*

Sample No.	Day	Radiation Day	Sample Time	Fibrinogen	Euglobulin	Pseudoglobulin	Albumin
1	0	-	-	-	-	-	-
2	7	R-1.7	5 min.	0.82	-	0.82	1.11
3	11		40d..	0.89	-	0.91	-
4	17		10 d.	1.00	1.09	1.31	0.89
5	41		24 d..	1.03	1.46	0.98	0.88
6	48	R-2.48	5 min.	1.26	1.04	0.87	1.11
7	51		3 d.	0.89	1.22	0.32	0.80
8	58		10 d.	0.93	1.45	1.19	-
9	72		24 d.	0.83	0.46	0.78	0.87
10	111		53 d.	0.94	1.04	0.87	0.89
11	118	R-3.118	3 hrs.	1.00	1.04	0.97	0.91
12	120		2 d.	1.09	0.63	1.08	1.01
13	125		7 d.	-	0.59	0.89	0.86
14	160		42 d.	0.67	-	-	1.00
15	162	R-4.161	1 d.	0.75	1.11	0.69	0.86
16	170		9 d.	1.54	1.47	0.69	1.00
17	176		15 d.	0.84	0.80	0.68	1.12
19	191	R-5.189	2 d.	0.65	0.95	0.75	1.06
20	202		13 d.	1.18	0.80	0.79	0.86
21	216		27 d.	1.07	1.01	0.53	0.94
22	219	R-6.217	2 d.	1.13	0.39	0.87	1.00
23	240		20 d.	0.74	0.90	0.90	0.92

*The ultraviolet absorption per unit weight for each fraction divided by the corresponding value for the control animal.

TABLE 5

D_{max}/D_{min} FOR PLASMA

Sample No.	Radiation Day	Sample Time	Plasma No 600 / Plasma No 599
1			1.1
2	R-1.7	5 min.	0.9
3		4 d.	1.6
4		10 d.	1.1
5		24 d.	1.1
6	R-2.48	5 min.	1.1
7		3 d.	1.1
8		10 d.	1.1
9		24 d.	1.1
10		53 d.	1.1
11	R-3.118	3 hrs.	1.2
12		2 d.	1.7
13		7 d.	0.9
14		42 d.	1.0
15	R-4.161	1 d.	1.2
16		9 d.	1.2
17		15 d.	1.0
19	R-5.189	2 d.	1.6
20		13 d.	1.0
21		27 d.	1.0
22	R-6.217	2 d.	1.1
23		20 d.	1.0

and then decreased somewhat, the pseudoglobulin markedly increased, and the albumin decreased. These findings are similar to the transitory changes in the sublethal period, Table 3, and also agree with the changes in dogs (5) in the acute period wherein it was found that the albumin decreased to almost half and the globulin-fibrinogen unresolved peak in the electrophoretic pattern increased. No changes are observed in the character of the plasma such as noted in Table 5.

Summary: 1. A reliable plasma protein fractionation method applicable to 3 ml samples is described.

2. Measurements on a few rabbits after successive irradiations indicate that transitory changes in the protein fractions after a sublethal dose are similar to the changes in the acute period. There is an increase in fibrinogen, and decrease in albumin, and an increase in some of the globulins particularly in the acute period.

Bibliography

1. Jacobsen, L. O., E. K. Marks, E. Gaston, J. G. Allen, and M. H. Block, ANL 4163 (1948).
2. Painter, E. E., C. L. Prosser, and M. C. Moore, CH-3727 (1946).
3. Prosser, C. L., E. E. Painter, and M. N. Swift, CH-3738 (1947).
4. Davy, L., Am. J. Roentgenology, 25, 255 (1931)
5. Muntz, J., E. S. G. Barron, and C. L. Prosser, CH-3560 (1946).
6. Butler, A. M. and H. Montgomery, J. Biol. Chem., 99, 173 (1932).
7. McMeekin, T. L., J. Am. Chem. Soc., 61, 2884 (1939).
8. Butler, A. M., H. Blatt, and H. Southgate, J. Biol. Chem. 109, 755 (1935).
9. Anson, M. L. and J. T. Edsall, Adv. in Protein Chem., V.III, 399 (1947).
10. Ham, T. H. and F. C. Curtis, Medicine, 17, 413 (1938).

Problem Code: X.R.2 (Mechanism of Effects)

Section Code: 3350

Preliminary Studies on the Role of the Adrenal in the Radiation Syndrome:

The similarity between the post-irradiation state and the alarm-adaptation syndrome of Selye has been pointed out by both Selye and the physiology group at the Chicago Project. This latter group has studied changes in adrenal size and cholesterol content in the rat after ionizing radiation, and their findings are consistent with those changes in adrenal function associated with the alarm-adaptation syndrome. Briefly, they have shown an early sharp fall in the adrenal cholesterol content a few hours post-radiation followed by a gradual increase in size and cholesterol content over the next 3 to 5 days, after which time a gradual decline in size is noted. They have not reported changes in mortality by the use of supplementary adrenal cortical hormone. Since it is a well-known and often repeated observation in endocrine research that by varying the timing of the administration and the size of the dose almost complete reversals of hormone action can be demonstrated, it seemed of interest to investigate in this laboratory certain of such procedures which might possibly alter mortality and at the same time throw more light on the role of the adrenal cortex in the post-irradiation syndrome.

As a preliminary study, a group of 16 rats were given 1.0 cc of Upjohn adrenal cortical extract subcutaneously 1 hour pre-radiation and 0.5 cc was injected in the same manner on each of the next 3 days. It was hoped that this would block or reduce the increase in adrenal size. A blocking effect of this type has been described by Sayers and Sayers for the adrenal hyper-

trophy which follows cold and other forms of stress. The composite curve for 3 such experiments in which the results were essentially the same are shown in Figure 1. (50 ACH animals and 48 untreated controls.) The animals receiving ACH lost weight rapidly after the 2nd post-radiation day. They died at an earlier time and also had a higher mortality than the other groups. In the control group there was a 50 per cent survival in contrast to a 20 per cent survival of the treated rats. Several showed subcutaneous and gastro-intestinal tract hemorrhages.

To be certain that the increased mortality seen in the treated animals was not due to handling at the time of injection, a fourth group of 16 animals was given saline in the same amounts beginning 1 hour pre-radiation and continuing for 21 days. Their mortality together with that of their controls is shown on Figure 2. These animals actually lost weight at a slower rate than the controls. In this experiment both the control and saline rats showed a 25 per cent survival.

In a second experiment an attempt was made to cause a pre-radiation adrenal hypertrophy by subjecting the animals to a moderate stress prior to radiation. This was done by placing the rats in a cold room at 4°C for 2 hours on each of 5 nights immediately preceding radiation. This amount of stress caused no weight loss and growth was not interrupted. Two groups of 16 rats and controls were studied. Since the control mortality was the same in both groups, they are shown as a single composite curve in Figure 3. The control rats showed a 46 per cent survival, whereas one group of stress animals an 87 per cent and the other stress group a 57.5 per cent survival.

That a pre-radiation stress need not necessarily be the aftermath of a repeated moderate physical exposure in shown in a third experiment where

FIGURE 1

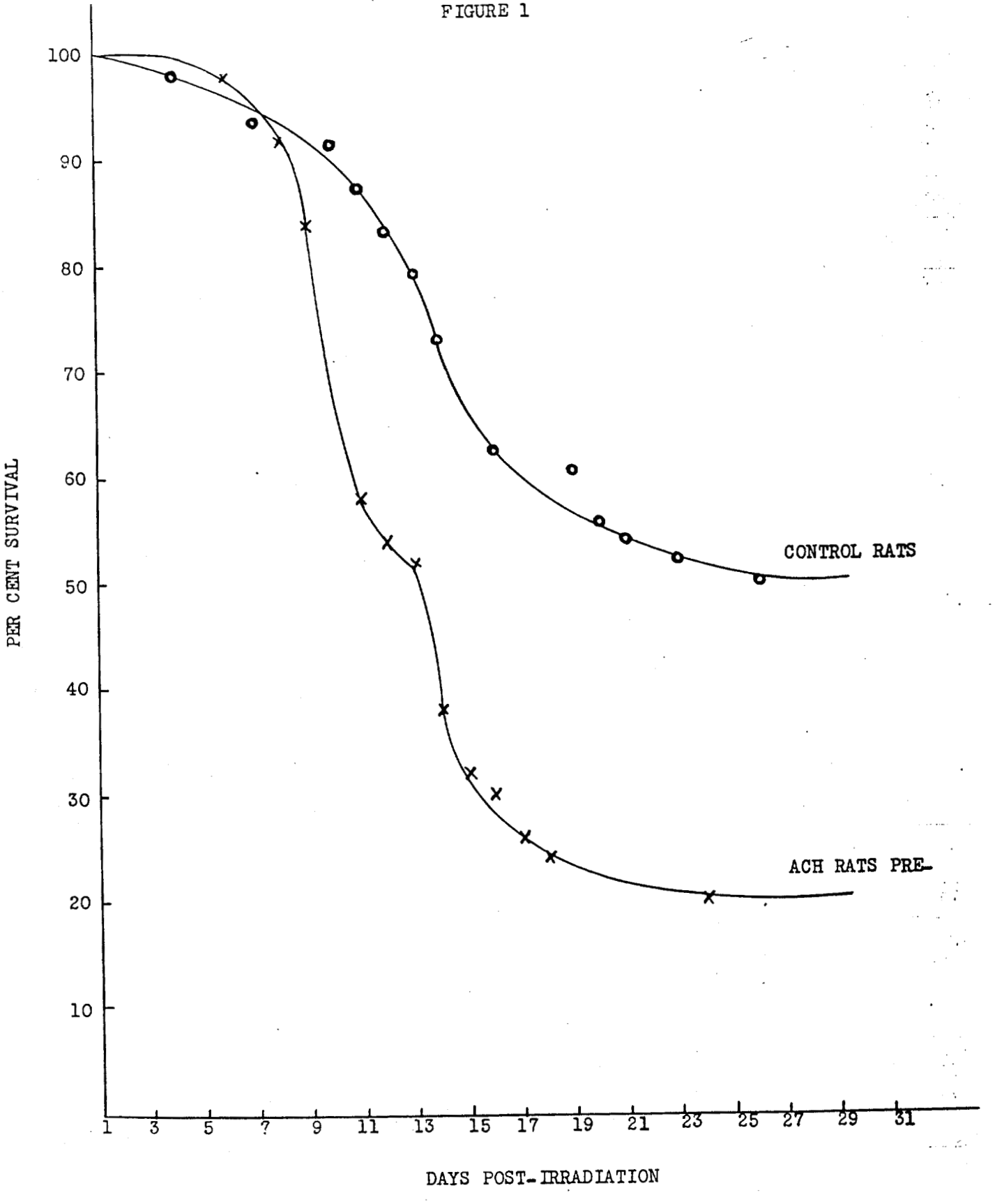

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FIGURE 2

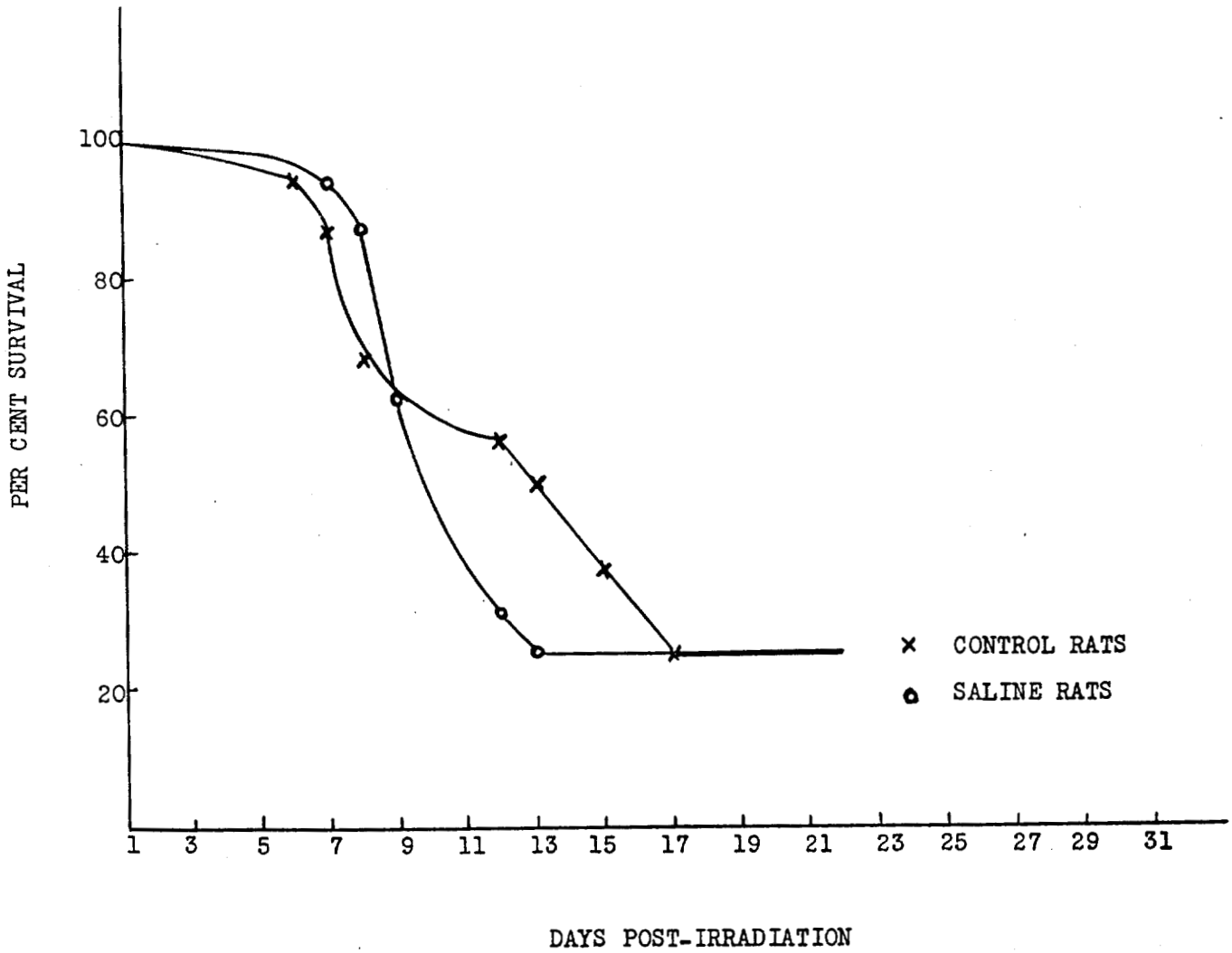
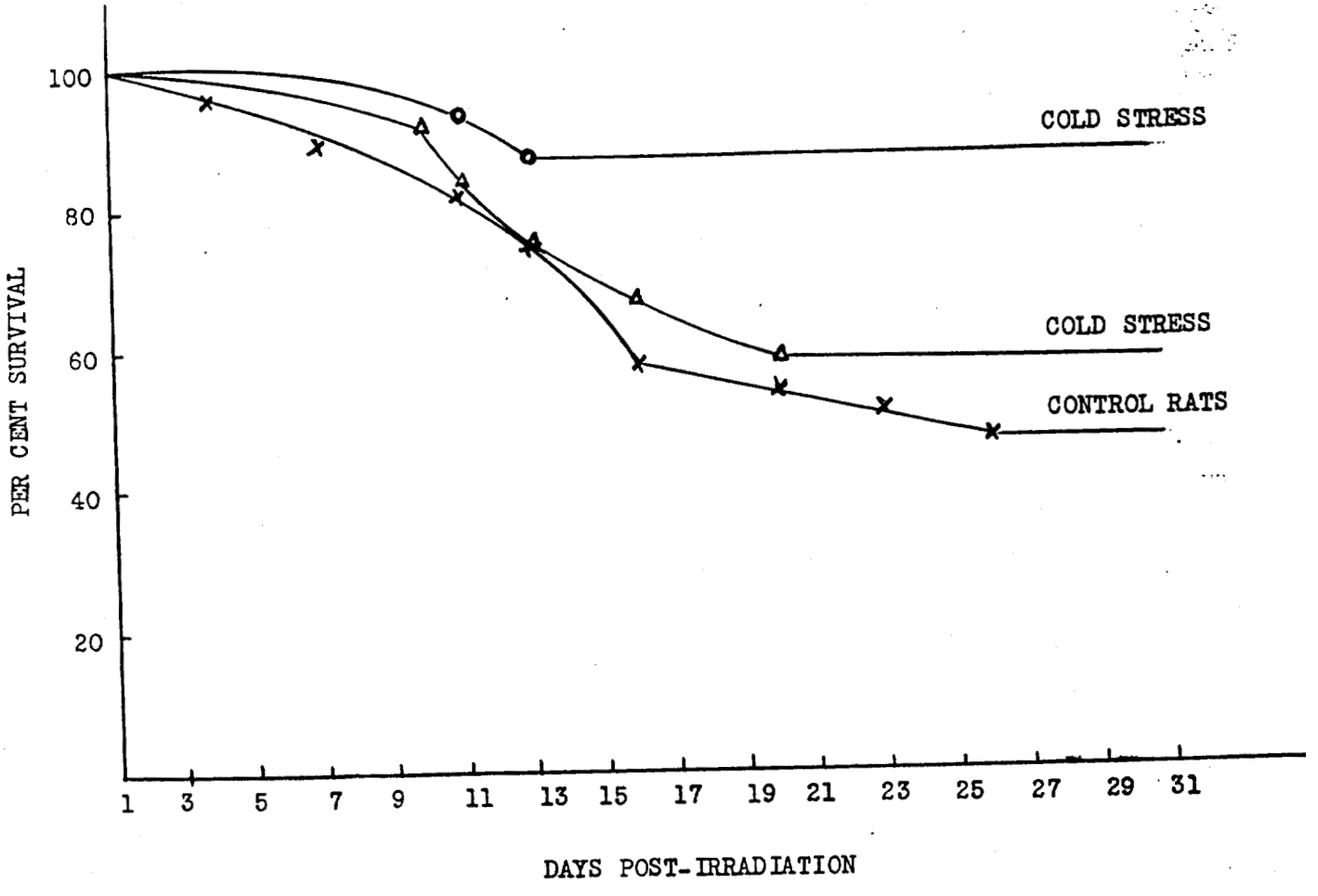


FIGURE 3



the mortality was markedly decreased by the probable acute stress resulting from the administration of a single injection of 300 mgs/kg. of phloridzin in propylene glycol 6 hours pre-radiation, or simply by the injection of propylene glycol as shown in Figure 4. (Propylene glycol was used as the solvent for phloridzin.) While this protective action of propylene glycol, with or without phyloridzin, may be a result of the same mechanism as that following cold stress, its exact nature is unknown and is in the process of investigation. In these groups, the control animals showed a 50 per cent survival in contrast to a 91 per cent survival in the phloridzin rats and an 80 per cent survival in those animals treated with propylene glycol.

Since these studies seemed to indicate that those procedures which commonly cause adrenal hypertrophy also produce a beneficial effect in reducing radiation mortality, it was decided to investigate the possibilities of supplementing the animals' own stress response. This was done by giving the rats 1.0 cc of ACH at 72 hours post-radiation and 0.5 cc daily for 21 days. The control animals showed a 52 per cent survival, while the treated rats showed a 75 per cent survival. Only one variation in the time of dose has been tried, namely, beginning treatment 24 hours post-radiation. In the single group studied, the mortality was similar to that seen in the pre-radiation experiment with a 5 per cent survival of the control and a 27 per cent survival of the treated rats. (Figure 5) No variations in dose have been studied, nor have other ACH preparations been studied.

All rats used in these experiments were females of the Wistar strain weighing between 125 - 175 grams, except in the phloridzin, glycol experiment where male Wistar rats weighing 200 - 250 grams were used. All experimental

FIGURE 4

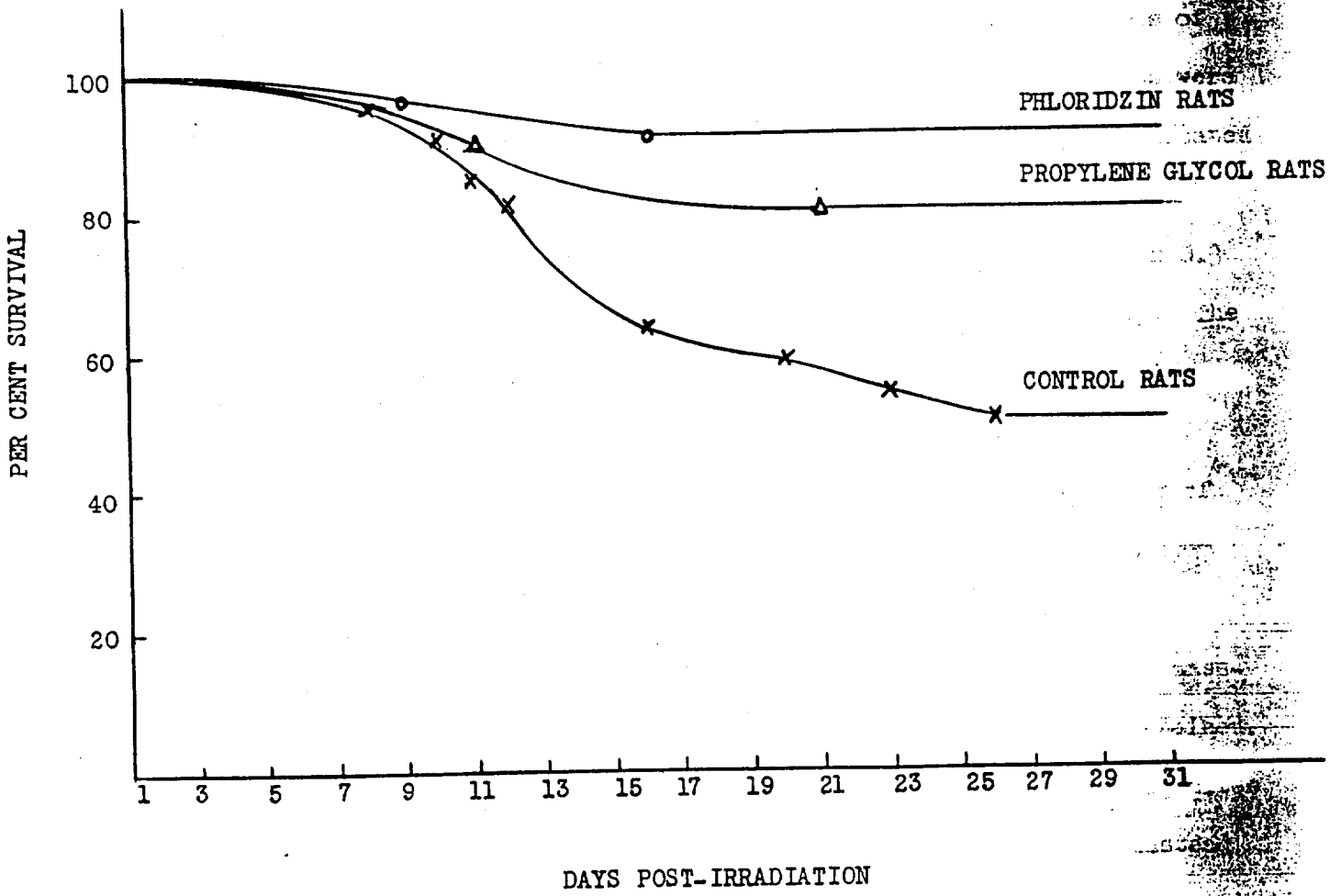
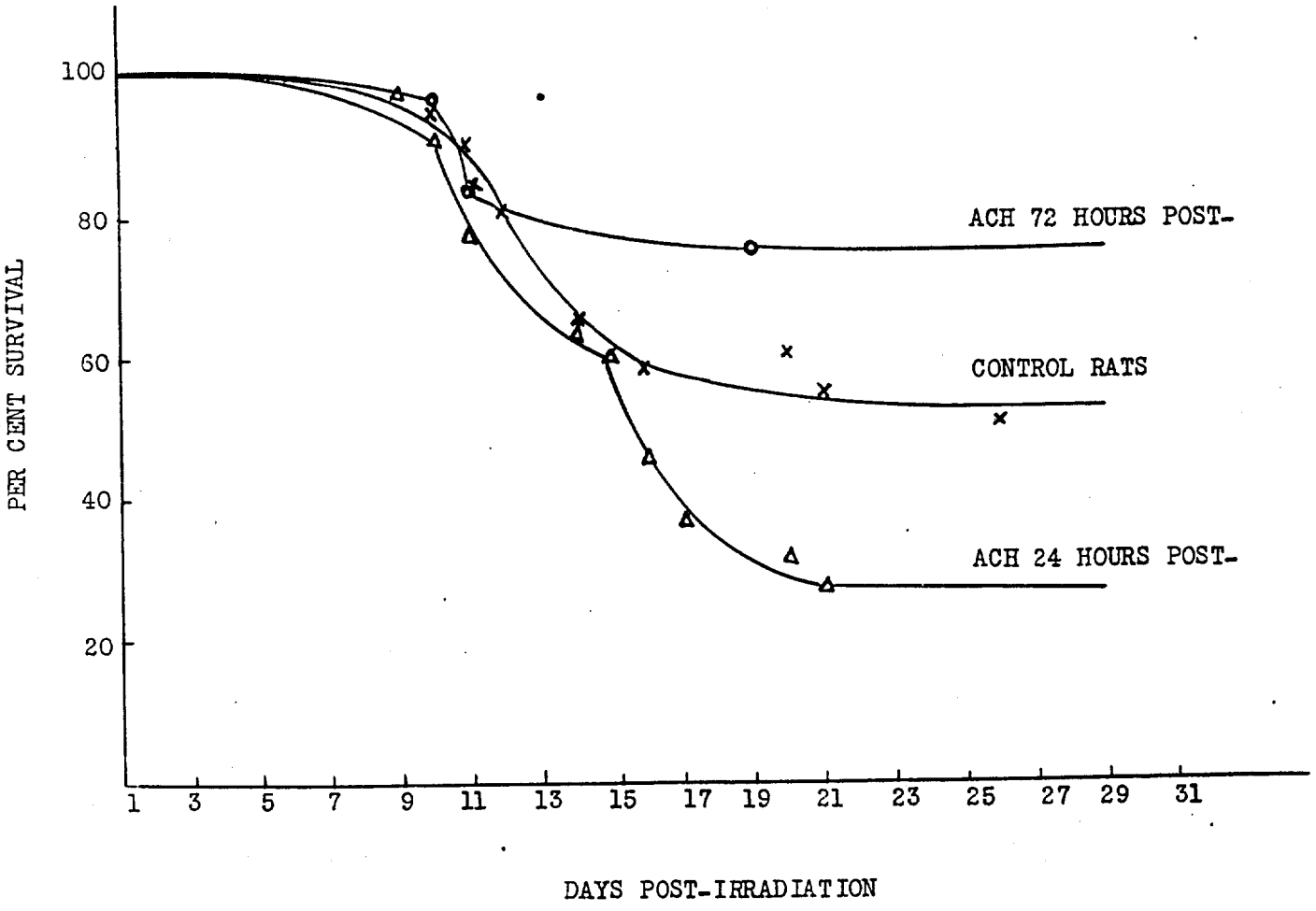


FIGURE 5



rat groups, together with a control group of identical numbers, were radiated simultaneously at the uniform dosage of 625 r, the mid-lethal dose previously determined for the Wistar strain.

Problem Code: X.R.7 (Bacteriology and Immunology)

Section Code: 3442

Preliminary Studies to Determine the Effect of the Route of Inoculation on the White Blood Cells in Mice:

Groups of standard three-week-old Swiss mice were infected with virulent Hemophilus pertussis intracerebrally, intranasally, and intraabdominally. Total and differential white blood cell counts were carried out on a representative member of the subjects. Highest counts (51,980) were obtained in mice infected intranasally with 0.25 billion organisms. Mice infected by the intracerebral method showed increases in the total counts (41,175). Inoculation by the intraperitoneal method, in this group, resulted in counts only slightly above the normal range of 11 to 15 thousand.

Conclusion: Inoculation of mice with H. pertussis by the intracerebral and intranasal routes resulted in similar increases of the white blood counts as well as the mortality rate. Inoculation by the intraabdominal route gave results close to those obtained in the control group of subjects.

Effect of X-ray Irradiation on Experimental Murine Pertussis:

(1) Influence of irradiation on immunized mice: Standard groups of mice were actively immunized with intraabdominal injections of a suspension of 2 billion organisms of Phase 1 H. pertussis per cc. Eleven days later, the test groups received a single exposure of 200 r. Two days after the

x-ray treatment, all groups were challenged with intracerebral injections of the same organism from the growth of which the immunizing vaccine was prepared.

Results of this experiment in terms of the 50 per cent endpoint mortality dose were as follows:

Immunized: Group I No x-ray treatment - 14.2 m. (dose of organisms)
Group II X-ray treatment - 3.9 m.

Non-immunized: Group III No x-ray treatment - 0.49 m
Group IV X-ray treatment - 0.86 m.

Conclusion: X-ray treatment appeared to decrease protection in the immunized mice when given eleven days after active immunization.

The influence of new drugs on experimental murine pertussis is also being determined. In the original experiment, aureomycin given in a dosage of 1 mg for three days, 0.1 mg one day, gave a 50 per cent endpoint of 31 million organisms. Polymyxin B, given in doses of 100 micrograms daily for three days protected 50 per cent of mice against more than 500 million organisms. 0.2 gm per kilogram of Darvisul given daily for three days protected 50 per cent of the mice against 37.2 million organisms. Streptomycin given as 1000 micrograms daily intraabdominally for three days protected 50 per cent of the mice against more than 500 million organisms. There were no fatilities with Streptomycin. The infection in the control mice produced an M.L.D. of 5.6 million organisms.

In a repeat experiment, the controls did not react, and another series is projected.

PROGRAM U.

URANIUM

Problem Code: U.1 (Physical and Chemical Properties)

Section Code: 3210

Sampling and Measurement of Aerosols: UO_3 Particle Size Studies:

A series of 51 Cascade Impactor samples were taken on atmospheres of an animal exposure chamber in which aerosols of different particle sizes were being dispersed during the pre-exposure testing period. The results indicated that a concentration from 10 to 15 mg/m^3 could be maintained with large or with small particles of UO_3. The smaller size UO_3 particles ranged from 0.3 to 0.4 micra with a geometric standard deviation averaging close to 2.5. The larger size ranged from 1.5 to 2.0 micra with geometric standard deviations ranging from 2.0 to 2.8.

A series of 16 Cascade Impactor samples were taken on atmospheres of the particle-size chamber during the subsequent animal exposure period from 7/12/48 to 8/2/48. This atmosphere contained a fine particle size dust comparable to the uranium dioxide dusts previously studied. An average mass-median value of 0.29 micra with a range from 0.23 to 0.34 micra were obtained. The particle-size ranges were thus somewhat lower than the corresponding sizes of uranium dioxide (0.45 micra). The size distributions were normal instead of being atypical as were the uranium dioxide aerosols; this result was expected because the uranium trioxide had been freshly precipitated. The average value for the geometric standard deviation was 2.5 with a range from 2.3 to 2.7. The consistency of results indicated that the aspirator method of dispersing suspensions was as successful for uranium trioxide as for uranium dioxide.

Problem Code: U.1 (Physical and Chemical Properties)

Section Code: 3220

Uranium Complex:

Reinvestigating the polarography of uranyl ion in citrate medium, it has been found that uranium catalyzes the destruction of citric acid under the influence of light. The considerable wide variation in polarographic results in earlier studies may be attributed to the variable amount of destruction of citrate ion. Much of the earlier work has been repeated employing freshly-made solutions giving a much improved precision.

Problem Code: U.1 (Physical and Chemical Properties)

Section Code: 3260

Dissociation Constants of Various Uranium Complexes:

Uranium is known to inhibit sugar metabolism of yeast by complexing with certain groups on the surface of the cell. The dissociation constant of the yeast-uranium complex is very small, i.e., of the order of 3×10^{-7} (see Rochester Reports UR-8 and UR-17). In the present report the complexing ability of various substances is compared with that of the yeast cell groups in the hope that some light may be thrown on the nature of the latter.

After a number of methods were tried, the following procedure was adopted: A fixed amount of uranium was added to a suspension of washed yeast containing 10 mg of cells per ml of suspension. A sample of the supernatant fluid was taken for analysis: Then various concentrations of substances under test were added and each time, samples of the supernatant were analyzed for uranium. In the case of the phosphorylated compounds, molybdate was added to

inhibit the cell surface phosphatases (detailed report in preparation). Uranium analyses were made by isotope technique, using U_{233} and an alpha counter after electroplating on silver foil (see Rochester Report UR-17).

Redistribution of uranium between cells and supernatant is shown in Figure 1 (Page 48) for different concentrations of various substances. The dissociation constant of the uranium complex with each substance can be approximately calculated from the concentration of that substance necessary to keep 50 per cent of the uranium in the supernatant.

The calculated constants are given in Table 1 (Page 49). The equation used in these calculations was derived from the Law of Mass Action and for the particular condition of this experiment could be simplified in the following:

$$\frac{K_y}{K_x} = \frac{y}{x}$$

where K_y = dissociation constant of yeast-uranium complex
K_x = dissociation constant of substance X with uranium
y = concentration of yeast cell groups
x = concentration of substance x

The dissociation constants varied from greater than 10^{-3} to less than 6×10^{-7}. The ester and ortho-phosphate complexes have high constants. Polyphosphates and metaphosphates have very low constants. Proteins, hexose diphosphate and citrate are intermediate. The only substance with a constant similar to that of yeast is hexametaphosphate. It is interesting to note that this compound has recently been shown to be intimately connected with carbohydrate metabolism.*

*Juin, Kamen, Reiner, and Spiegelman, Arch. Biochem., 18, 387, (1948).

Figure 1. The distribution of uranium between yeast cells and supernatant in the presence of various concentrations of uranium complexers

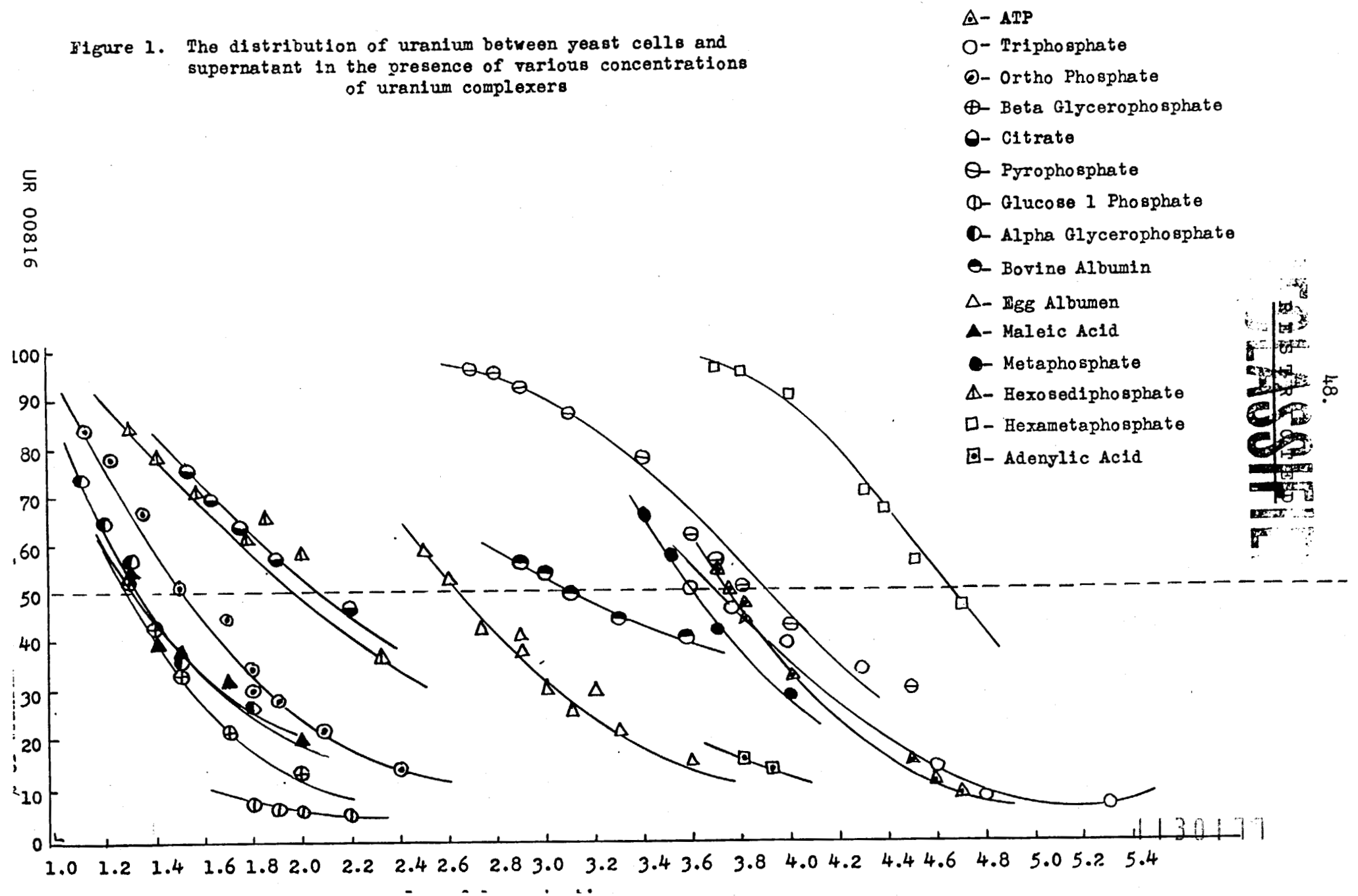


TABLE 1

Dissociation Constants of Various Complexes of Uranium at pH 3.5

Yeast surface groups*	2 to 5 x 10^{-7}
Hexametaphosphate	6 x 10^{-7}
Inorganic pyrophosphate	4 x 10^{-6}
Adenosine triphosphate	5 x 10^{-6}
Inorganic triphosphate	6 x 10^{-6}
Inorganic metaphosphate	8 x 10^{-6}
Bovine serum albumin**	3 x 10^{-5}
Egg albumen**	8 x 10^{-5}
Adenylic acid	1 x 10^{-4}
Hexose diphosphate	3 x 10^{-4}
Citrate	3 x 10^{-4}
Inorganic orthophosphate	7 x 10^{-4}
Alpha glycerophosphate	1 x 10^{-3}
Maleic acid	1 x 10^{-3}
Beta glycerophosphate	1.5 x 10^{-3}
Glucose 1 Phosphate	1.5 x 10^{-3}

*For calculations, see Rochester Report UR-17.
**Molarity calculated on the basis of free carboxyl groups.

Problem Code: U.3 (Toxic Limits)

Section Code: 3210

The Relation of Particle Size of UO_2 to Toxicity Following Inhalation by Rabbits and Rats:

A series of four studies to determine the effect of particle size of UO_2 aerosol on the retention, distribution and toxicity in animals has been completed. The series tested was:

Test	Particle Size	Average Aerosol Concentration mg UO_2/m^3	Mass Median Particle Size μ
I	Small	22	0.45
II	Small	80	0.45
III	Large	80	1.0
IV	Large	80	(approx) 2.0

Comparison of clinical chemical responses of rabbits in all four studies clearly indicated that toxicity increases sharply as the particle size of the inhaled dust decreased below 1 μ. In particular, it was indicated that the concentration of urinary protein in the rabbit was almost entirely a function of the mass of inhaled particles below 1 u in size, and was little if at all related to the total concentration of all sizes.

Tissue analyses and results of histologic examinations of tissues from experiments I and IV are now completed. At 80 mg/m^3 concentration and 2 μ mass-median particle size, no evidence of histopathologic damage was found in the rat. In the rabbit, typical renal tubular necrosis was slight in 3 of 16, and moderate in 1. At this same concentration (80 mg/m^3) but at mass-median

size of 0.45 μ nearly all rats and many rabbits showed renal damage varying from slight to moderate.

Tissue analyses did not indicate any significant correlation between particle size and amount of uranium in femur or kidney of either species. In the lung, however, deposition of uranium from dust of 0.45 μ mass-median size was of the order of ten times as great as that from dust of approximately 2.0 μ mass-median size at the same exposure concentration. Mean values of uranium deposits in the tissues of animals of the complete series are found in Table I (Page 52). The deposits of uranium in the lungs of both species are roughly proportional to the concentration of sizes below 1 μ in the exposure atmosphere and bear little relation to concentration of the dust as a whole (Figure 1 -- Page 53).

The Effects of Exposure of Rabbits and Rats to Inhalation of Hydrated UO_3 at Approximately 3.6 mg/m^3 Concentration and 0.3 μ Mass-Median Particle Size:

Previous studies* of inhaled UO_2 dusts of different particle size have shown that retention, distribution and toxicity of UO_2 are altered with change in particle size. An associated cause of this phenomenon was the insolubility of the UO_2 dust. It now becomes of interest to determine whether such effects still obtain with a uranium dust of intermediate solubility. UO_3 was selected because of its special property of being relatively insoluble in water but appreciably soluble in body fluids.

Rabbits and rats were exposed in a chamber to an aerosol of $UO_3 \cdot 2H_2O$ ($H_2UO_4 \cdot H_2O$, $UO_2(OH)_2 \cdot H_2O$). Use of this hydrate instead of UO_3 was necessitated

* Reported in Rochester Report No. UR-21 and UR-38.

TABLE I

Deposition of UO_2 in Tissues of Rabbits and Rats

Micrograms of Uranium per Gram of Wet Tissue

		I	II	III	IV
Mass-Median Particle Size, μ		0.5	0.5	1.0	2.0
Exposure Concentration (all sizes) mg/m^3		22	80	80	80
Concentration of sizes below 1 μ (approx.) mg/m^3		19	68	30	14
Exposure hours		140	191	191	192
LUNG:	Rabbit	76	2180	373	46
	Rat	305	1233	510	157
KIDNEY:	Rabbit	1.0	1.2	2.6	1.1
	Rat	2.0	3.0	3.2	1.2
FEMUR:	Rabbit	1.8	2.2	4.8	2.7
	Rat	1.2	4.0	3.1	1.9

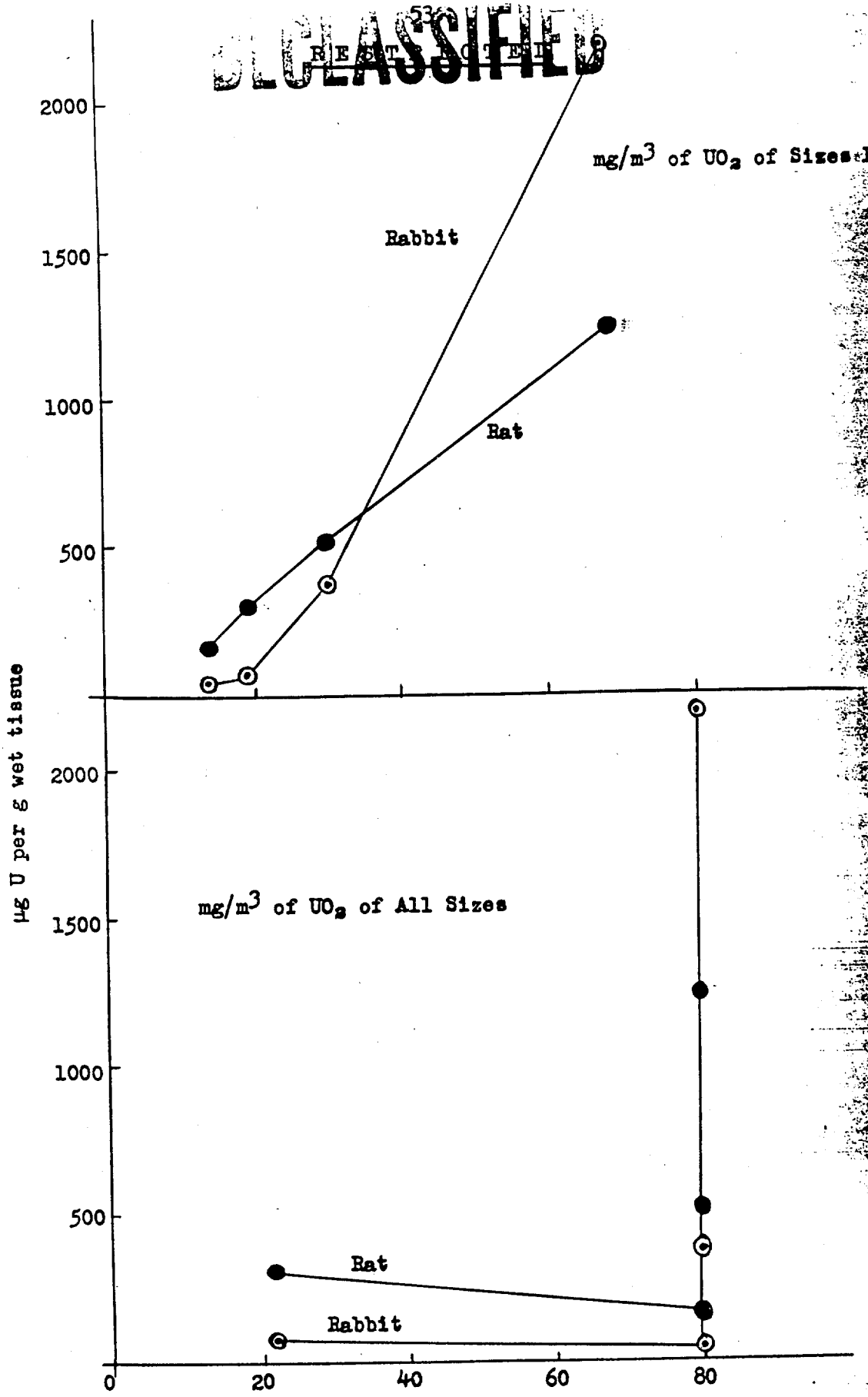


Figure 1. Deposition of UO_2 in Lung in μg U/g of Tissue vs. Exposure Concentration in mg. UO_2/m^3

by a combination of circumstances: 1) a dry dust feed dispersing dusts of graded particle size has not been developed, and 2) UO_3 in aqueous suspension gradually takes up water, the end product being the dihydrate, the stable hydrate under ordinary atmospheric conditions. The dry $UO_3 \cdot H_2O$ aerosol was produced by "atomization" of a water suspension of the compound by means of the aspirator feed that had been used in previously reported UO_2 particle-size studies. Attempts to prepare a suspension of suitable particle size by grinding UO_3 in aqueous suspension were not successful. A suspension of the desired particle size was finally prepared by dissolving UO_3 in hydrochloric acid and reprecipitating with NaOH. After repeated washings a pure suspension of $UO_3 \cdot 2H_2O$ was obtained, the only impurity present in more than a trace being sodium. About 1 per cent of Na remained.

Ten rabbits and 24 rats were exposed daily for 22 calendar days (16 exposure days or 96 cumulative exposure hours) to $UO_3 \cdot 2H_2O$ at a mean concentration of 3.6 mg/m^3 at a particle size of 0.3 μ. The only toxic effects observed were traces of urinary protein in two of ten rabbits near the end of the second week and a slight but definite growth reduction in the rats during the first and second weeks.

Problem Code: U.4 (Fate)

Section Code: 3201

Ca^{45} Adsorption:

Using radioactive calcium, it has been shown by in vitro studies that a sizable fraction, that is 1/4 to 1/5, of the total calcium of bone may enter into an exchange reaction with calcium ions in a solution to which the bone is exposed for a period of two weeks. This lability of bone calcium is exactly analogous, even numerically comparable, to the phosphate exchange previously described. These studies are basic to the problems of explaining the bone deposition of uranium, beryllium, and perhaps other ions. Bone may act like an ion exchange resin in the fixation of these elements.

PROGRAM Be.

BERYLLIUM

Problem Code: Be.1 (Physical and Chemical Properties)

Section Code: 3220

Analytical Procedure:

Because of the difficulty in obtaining alkanin (the color agent present in alkanet root), attempts have been made at the synthesis of quinizarin, a structural analogue of alkanet. Sufficient synthetic material has been obtained to show that this compound will be equivalent to pure alkanin as an agent for the quantitative determination of beryllium. In the meantime, an investigation has been made of the mechanism of color formation when beryllium is mixed with anthraquinone compounds. Beryllium combines with dye in a 1:1 molecular ratio. The dissociation of this complex is extremely low; the complex is not soluble in water or solvents; and, when formed in solution, usually remains colloidal. From the results of direct analyses and x-ray diffraction studies it appears that beryllium is present as a hydroxide, but due to the fact that the complex shows a definite combining ratio, the reaction cannot be considered as the formation of a "lake". This information, though seemingly of an academic nature, may provide the basis for obtaining important information regarding the efficacy of various complexing substances of biological significance.

Qualitative studies on the isolation of beryllium using flurosilicate have been undertaken.

Solution Chemistry:

Conductivity titrations of beryllium and various physiologically important compounds have continued. Approximate solubility measurements have

been made on a number of beryllium hydroxide preparations. The ability of beryllium to pass a membrane when present in various media has been studied utilizing an ultrafiltration apparatus of our own design. A beginning has been made on the study of the state of aggregation of beryllium hydroxide in various solutions utilizing high speed centrifugation.

Problem Code: Be.3 (Toxic Limits)

Section Code: 3210

The Inhalation Toxicity of Beryllium Sulfate Hexahydrate Mist in Animals at a Concentration of 10 mg/m^3 for 95 Days:

The acute toxicity of beryllium has been studied in 8 species of animals exposed daily for 95 days (426 exposure hours) to an aerosol of beryllium sulfate hexahydrate at a concentration of 10 mg/m^3 and at a mist particle size (size mass-median) of 1.3 micron. This study is the third of a series of inhalation studies to which successively lower concentrations of toxic agent have been employed in an attempt to establish beryllium concentrations producing borderline or no toxic response. In previous study at 100 mg/m^3 injury had been produced to the lung in all species exposed; in another at 50 mg/m^3, a moderately severe response had been produced in most but not all species. Specifically, the rabbit at 50 mg/m^3 showed lesions most like those of human lungs in the acute phase. The present study at 10 mg/m^3 (0.4 mg Be/m^3), in which more detailed serial histologic examination has been performed, showed that lung injury was produced in certain of the more susceptible species, the cat, dog, monkey, and rabbit, but this response developed more slowly, requiring approximately one month's exposure before fully developed lesions appeared.

Whereas, at the higher concentrations, the overall mortality approximated 30 per cent an in many species attained values as high as 100 per cent, at the 10 mg level, overall mortality was reduced to 14 per cent with only a single species, the rat, showing an appreciable mortality (50 per cent). Moreover, at this level, the weight loss that in the previous studies has been general among the majority of exposed animals, was now noted only in the monkey and dogs, did not occur in the cat and rabbit, and gains were noted in the goat, guinea pig, and rat. Similarly, biochemical constituents that had given evidence of severe toxic response at the higher levels, namely, changes in protein of the urine and possibly those of the serum, now showed only doubtful changes in an occasional member of a species. There was noted, however, at the 10 mg level a possible trend toward depressed blood phosphorus in the rabbits with no concomittant change in blood calcium. The leukocytosis that was rather marked the third week in dogs and rats exposed at the 50 mg level, still was observed at the 10 mg level. The slight thrombocytosis in rabbits and rats at the higher level was not seen at the 10 mg level, although a slight decrease may have occurred in the red blood count of dogs, and there was a questionably increased fragility of the red blood cells in the rats. Serial histologic examinations performed on 4 of 8 species showed pulmonary injury in 3, namely, the rabbit, monkey, and dog. These animals examined after the first 3 weeks showed pulmonary injury marked by distortion of lung architecture, edema, congestion, and monocytic infiltration; animals sacrificed at the 95th day, however, showed a chronic response in which the pulmonary exudate was changed from a monocytic to a phagocytic character and a thickening had occurred in the alveolar walls. Another outstanding characteristic of the response at the 10 mg level was the increase

in severity of the pulmonary injury with increased exposure, e.g., in the cat lung, effects were minimal at the 73rd day but had become moderately severe by the 95th day. This change in character of the lung lesion was noted also in the dog, monkey and rabbit, although not at the same interval of exposure. Of all species exposed to date, the rabbit, dog, cat, and monkey show pulmonary changes most closely resembling those seen in man in the acute phase following beryllium exposure. Moreover, the rabbit showed a remarkably fine correlation between pulmonary disease and duration of exposure. Rating the degree of lung injury from 1 to 3+, indicating progressive injury, animals exposed from 30 to 66 hours showed a 1+ response; from 96 to 132 hours, a 2+ response; and from 336 to 408 hours, a 3+ response with an occasional individual showing minor variations. On the other hand, lung pathology in the rat following beryllium exposure became typical only after several weeks of exposure and then was minimal in degree, and in the guinea pig that injury found during the first 6 weeks of exposure tended to disappear by the 95th day. The goat showed no changes of consequence throughout exposure. The analysis of the animal tissues for beryllium content showed surprisingly that the lung of the rat contained the most beryllium per gram of fresh tissue, the guinea pig next in order, the dog least; the amounts of beryllium averaged 11, 6, and 4 μg/g, respectively, in the lungs of these species. These amounts of deposition do not accordingly correspond to the histologic response, although differences in deposition are not great. These results would indicate either that once beryllium has entered the lung, the amount is of lesser consequence in producing injury or that species vary greatly in the susceptibility to the amount deposited. Deposition of beryllium among the various tissues decreased in amount in the following order: lung, pulmonary lymph nodes, liver, tooth, kidney, and femur. No accumulation of beryllium

occurred in either the lung or femur of the monkey at day 19 over that found at day 5.

The Combined Effect of Inhalation of Hydrogen Fluoride Gas and Beryllium Sulfate Aerosol in Rats:

Industrial exposures to beryllium are commonly not to beryllium alone but include mixtures of other potentially toxic agents. In some processes in the manufacture of beryllium, hydrogen fluoride (HF) or other soluble fluorides have been shown not only to be present with the beryllium but to exist in amounts several hundred fold greater than that of beryllium (Rochester Report M-1997). Toxic exposures have been shown to occur among plant personnel at sites where these mixed exposures existed. The study described below was performed to determine whether HF had a potentiating effect on beryllium poisoning by inhalation. The results obtained in rats confirm this hypothesis.

Eighty rats were exposed in groups of 10 and 20 each to either beryllium sulfate or to HF, separately or in combination, on alternate days or daily for one month, a period of sufficient duration to determine completely the effects of exposure. The concentration of beryllium sulfate hexahydrate approximated 10 mg/m^3. This concentration had previously been established (Rochester Report M-1844) as giving 50 per cent mortality in rats in a period of 3 weeks. Exposure concentrations of HF were approximately 7 mg/m^3, a concentration that had been previously shown (Rochester Report soon to be issued) to produce either inconsequential pulmonary changes in rats or no response at all.

The 20 rats that were submitted to both agents were exposed on alternate days for 6 hours, first to the beryllium aerosol and then to the HF gas for a total exposure period of 62 hours for each. This procedure was adopted partly

because of expediency* and partly because a definite interpretation of toxicologic results would be made more certain if exposure to each agent was less than that of the controls on the reasoning that if mortality occurred from reduced exposure (lowered C.T. values), the HF presumably would have potentiated beryllium toxicity (HF certainly was not lethal at the C.T. values employed). Serving as controls for this study were 4 additional groups of rats; one group of 20 was exposed daily to HF at concentrations approximating 7 mg/m^3 (124 exposure hours), another group of 20 rats was exposed daily to beryllium sulfate hexahydrate mist at approximately 10 mg/m^3, and third and fourth group of 10 rats each were exposed on alternate days to the above concentrations of either HF or beryllium sulfate.

The weighted mean concentration of beryllium sulfate hexahydrate dust for the entire one-month study was 9.2 mg/m^3 with a standard deviation of 2.7; similarly the weighted mean concentration of HF gas was 8.5 mg/m^3 with a standard deviation of 4.9. Gravimetric analysis of filter paper dust samples with an occasional check by the spectrographic method was used for the determination of beryllium concentration in the atmosphere; analysis for fluoride was made on chamber-air samples absorbed in 0.005 normal KOH and titrated directly with chrome azurol-S according to the Torton method (Rochester Report M-1694).

The toxicologic findings were clear-cut and unequivocal. Results of mortality, weight response and histologic examination all were consistent for any given group and were sharply different between the group exposed to the two agents as compared with the groups exposed to the agents separately for the

* A simultaneous exposure to both beryllium sulfate and HF as a mixed aerosol was not resorted to because a mixed exposure would present analytical difficulties for determining one constituent in the presence of the other. An exposure chamber was available and in operation with an atmosphere containing beryllium sulfate at 10 mg/m^3 at the time the study was planned.

same exposure time. As for mortality, precisely the same number of rats died that were exposed to both agents on alternate days as died from beryllium sulfate alone with daily exposures. Mortality began on the 13th and 10th days in each group, respectively, but both groups reached the identical rate of mortality on the 20th day (40 per cent), and no more deaths occurred thereafter. Thus, the same mortality was produced from one-half the total exposure to beryllium plus HF as was produced by double the amount of exposure to beryllium alone. No deaths occurred in rat groups exposed to HF alone daily, or to beryllium sulfate alone on alternate days. One rat of a group of 10 exposed to HF on alternate days died; however, from some cause unrelated to the exposure. Substantiating this evidence of potentiating toxicity of HF for beryllium were the weight-response data. The only rat groups to show weight changes lower than pre-exposure mean values were the same 2 groups showing the 40 per cent mortality. Plots of the weights of the other 3 groups never dipped below this pre-exposure mean and 2 groups showed progressive increase in weight upon continued exposure. Similar evidence substantiating the main conclusion was obtained from histologic examination of the animals dying from exposure and those sacrificed at its termination. Rats exposed to HF showed no changes attributable to exposure. Rats exposed to beryllium sulfate showed minimal to moderate pulmonary disease, and those exposed to the combination of both beryllium and fluoride showed pulmonary changes similar in character and degree to those exposed daily to beryllium alone. Exposure to beryllium on alternate days resulted in definite pulmonary lesions in only a few instances, none in a few cases and equivocal effects in the remainder.

Thus, it may be definitely stated that since pulmonary injury and associated effects were as great in rats receiving one-half the exposure of beryllium

[redacted]

TABLE I

Table of Mortalities Following Intraperitoneal Injections of Rats with Fluorescent Grade of Beryllium Oxide

Fluorescent Grade

Dose (mg/kg)	Sex	Age	24 Hrs.	Days 7	Days 14	Days 21	Weeks 8	Weeks 9	Months 3	Months 4	Months 5	Months 6	Months 7	Months 8
3000 (S)	♂	50-days	0/10	0/10	3/10	4/10	6/10	6/10	7/10	7/10	7/10	7/10	7/10	7/10
3000 (A)	♂	50-days	0/10	0/10	2/10	2/10	4/10	5/10	3/10	3/10	3/10	3/10	3/10	3/10
2000 (A)	♂	50-days	0/10	2/10	3/10	4/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10
2000 (S)*	♂	50-days	4/10	6/10	7/10	8/10	8/10	8/10	9/10	9/10	9/10	9/10	9/10	9/10
1000 (A)	♂	50-days	0/10	0/10	0/10	1/10	1/10	1/10	1/10	1/10	2/10	2/10	2/10	2/10
1000 (S)	♂	50-days	0/10	0/10	1/10	1/10	1/10	1/10	1/10	2/10	2/10	2/10	2/10	2/10
1000 (A)	♀	weanlings	0/50	2/50	2/50	3/50	5/50	5/50	5/50	6/50	7/50	9/50	10/50	12/50
Controls (no injection)	♀	weanlings	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	0/25	1/25	1/25

* These injections were made with a Saline suspension approximately 4 months old.

(A) = Aqueous suspension - 50 mg/cc.

(S) = Saline suspension - 50 mg/cc.

TABLE II

Dosage	Date of Injection	Age	24 Hrs.	Days 7	Days 14	Days 21	Weeks 8	Weeks 9
2000 mg/kg (S)	7/28/48	50-days	0/10	0/10	0/10	0/10	2/10	3/10
2000 mg/kg (S)	8/27/48	50-days	0/10	0/10	1/10	1/10		
2000 mg/kg (S)	9/28/48	50-days	0/10	1/10				

Intraperitoneal Toxicity of Beryllium Oxide in Rats:

In a breeding experiment a group of twenty-five female rats (weanlings) were injected intraperitoneally with a single dose of beryllium oxide (S.P. grade) at a level of 1000 mg/kg; a second group of twenty-five was injected with the same dosage of fluorescent grade beryllium oxide; the third group was left untreated as controls. The male rats were not injected. The rats were paired (one pair to a cage) at weaning. Data on litter production are summarized in Table III below. A small reduction in the number of litters may have taken place and the total number of pups is reduced by about 20 per cent. There seems to be no difference in the effects on reproduction by the two grades of beryllium oxide.

TABLE III

Summary (at end of 8 months)	Total Number of Litters	Total Number of Pups
Controls (no injection)	118	1014
BeO (refractory grade)	96	794
BeO (fluorescent grade)	101	761

NOTE: When the death of a rat in one group occurs, its littermate brothers or sisters are prevented from mating.

Pilot Experiments on the Oral Toxicity in Dogs of Beryllium Oxides:

In a preliminary experiment to measure the oral toxicity of the beryllium oxides, 3 dogs were used. One animal (No. 643) was fed normal dog chow; the second dog (No. 983) was fed beryllium oxide (Brush S.P. Grade) at a dosage level of 10 g/kg of body weight; the third animal (No. 1063) was fed beryllium oxide (Clifton fluorescent) at a dosage level of 10 g/kg of body weight.

The beryllium oxides were added to basic dog chow and mixed thoroughly into a mass, placed on a food pan from which the dog was permitted to feed at will. The 10 g/kg dosage level was offered for two days and when it was noticed that the dogs refused the food, the dosage level was decreased to 5 g/kg of body weight, a level apparently tolerated. This regimen was maintained for the duration of the experiment (62 days). Gross observations on these dogs were negative; there were no significant weight changes (See Table IV below).

TABLE IV

Weights of Dogs Fed Beryllium Oxide

Dates of Weighing	Controls	Be Oxide (S.P.)	Be Oxide (Fluor)
6/16/48	11.3	12.2	14.1
6/24/48	11.6	12.3	13.7
7/7/48	11.7	12.2	14.0
7/16/48	11.6	12.1	14.3
7/29/48	11.8	12.5	14.4
8/6/48	11.8	12.5	14.0
8/13/48	12.1	13.1	13.8
8/27/48	11.6	13.2	13.3

Problem Code: Be.4 (Fate)

Section Code: 3220, 3250

Distribution and Excretion of Beryllium Using Be_7 as a Tracer:

During the past three months, the work on the distribution and excretion of soluble beryllium using Be_7 as a tracer has continued. This is the first time we have had isotope of sufficient activity to carry out a large number of experiments with one sample. Because of this, some experiments were duplications of previous work and were confirmatory. It has been shown that the distribution and excretion are affected by the amount of beryllium administered to the animal. Also, it has been shown that the urinary excretion following intravenous administration is much faster in rabbits having an acid urine than in those with an alkaline urine; this also seems to alter somewhat the deposition of beryllium in the organs. More beryllium is deposited in the bones in growing rats than in adult rats.

Problem Code: Be.5 (Mechanism of Toxic Effect)

Section Code: 3210

Changes in Blood Lipid Ratios as an Index of Beryllium Poisoning:

It has been shown previously in these reports that the intravenous injection of $BeSO_4.4H_2O$ into rabbits altered the ratio of phospholipid to free cholesterol concentration in the red cells to an extent greatly outside the probability of error, or of individual variation. An experiment with dogs exposed to the same compound by inhalation gave results that were definitely encouraging although not so conclusive (Rochester Report UR-38). As an additional investigation, the concentration of these lipids has been measured in

the red cells of dogs injected intravenously with $BeSO_4 \cdot 4H_2O$. Because no figures were available concerning the intravenous dosage of this compound required to produce tissue damage or death in dogs, the acquisition of such data became an added objective.

Five mature dogs, 8.2 to 10.5 kg in weight, were injected with graduated amounts of $BeSO_4 \cdot 4H_2O$ via the femoral vein of the right rear leg. The compound was dissolved in distilled water at a concentration of 10 mg/ml and injected at dosage levels of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg of body weight. This represents 0.25 to 4.0 mg of beryllium per animal (average weight, 10 kg). In a few days, deep ulceration of the leg was observed in two dogs, even though the injection had been made very carefully into the vein. Aside from the refusal of food by two dogs and a general malaise for two days following the administration of $BeSO_4 \cdot 4H_2O$, however, no ontoward systemic effects were observed. By contrast, the intravenous injection of 2 mg. of this compound/kg of body weight killed 5 of 6 rabbits, although this species is approximately comparable to the dog in rate of mortality when exposed by inhalation. This experiment suggested that for intravenous administration of $BeSO_4 \cdot 4H_2O$ to dogs, a dosage greater than 8 mg/kg of body weight may be used and that a dilute solution be given by intravenous drip.

Phospholipid and free cholesterol analyses were made on the red cells from 25 ml of blood taken from each dog immediately previous to the injection and on the 4th, 7th, 14th, 21st, 28th, and 42nd days following. In general, triplicate or quadruplicate determinations were made and a total of 107 lipid-partitions and 214 separate analyses were carried out.

As in the previous inhalation experiment with dogs, the ratio of phospholipid to free cholesterol concentration in the red cell decreased in all animals by the 4th day after injection. The changes, however, were small and were not followed by the constant downward trend noted previously in dogs continuously exposed to the compound; changes noted in this experiment were cyclic in nature.

Although abnormalities in the ratio of phospholipid to free cholesterol in the red cell were not of sufficient magnitude to be conclusive, one fact was of interest. All dogs almost invariably showed parallel changes. This appears to indicate a common causative agent acting similarly on all animals and that individual variations of the phospholipid to free cholesterol ratio within the red cells of any one animal must be less than the changes observed in this experiment. Otherwise, the ratio changes would have been random rather than uniformly parallel. It is felt, however, that these data are merely suggestive, since the degree of response elicited was in no way correlated with the amount of $BeSO_4 \cdot 4H_2O$ administered. The dosages used in this experiment wereapparently not sufficient to produce clearly-definitive toxicity as the result of a single injection.

Problem Code: Be.6 (Methods of Detection of Poisoning, Prophylaxis, Treatment, and Protection)
Section Code: 3210

Rutin as a Prophylactic and Therapeutic Agent in Beryllium Poisoning in Dogs:

One of the greatest needs in the current beryllium exposure problem is that of an effective prophylactic or therapeutic agent. Attempts to fulfill this need were focused on rutin, a plant flavonol, because of its particular

chemical configuration and physiologic properties. One of these latter properties is its ascribed capillary antifragility action. This action should prove beneficial in beryllium poisoning which is characterized in part by hemorrhage and edema of the lungs. A second property of potential beneficial value in beryllium poisoning is its phenolic structure permitting complex formation with beryllium. Indeed an especially sensitive microcolorimetric method employing rutin as the analytical reagent has been developed in this laboratory (Rochester Report soon to be issued). Further evidence on the potential effectiveness of rutin in beryllium disease was furnished by an experiment in which beryllium combined with serum was shown to be capable of removal by dialysis in the presence of rutin; removal of beryllium from the serum proteins did not occur in the absence of rutin. There was thus a number of indications that rutin might prove effective as a therapeutic or prophylactic agent in the treatment of beryllium poisoning.

Accordingly, an inhalation exposure experiment was performed employing 10 dogs, 5 of which received daily by capsule 100 mg of rutin in 2 daily divided doses for 2 weeks prior to the beginning of exposure to beryllium sulfate mist at a concentration of 25 mg/m^3. A closely similar group of dogs, some of which were litter mates of those in the untreated group, received the regular diet without rutin but received the exposure to the beryllium sulfate simultaneously with the treated dogs. Exposure to beryllium sulfate mist was continued daily for 6 hours for a period of 35 days. During this time, rutin was administered daily, at first in 100 mg amounts, later (during the last 2 weeks of the experiment) in 300 mg daily doses.

There was no significant difference in the weight response of the animals of the 2 groups at the termination of the beryllium sulfate exposure. During

the course of exposure, however, animals receiving no rutin showed a decided loss in weight from the first to the third week, but after this period the weight of the 2 groups was essentially indistinguishable. Differences in mortality in the control and treated groups were not remarkable. Two dogs of the beryllium-exposed group died, one on the 17th, and the other on the 35th day of exposure; but one of the 5 rutin-treated dogs died on the 22nd day of exposure. This difference though indicating a possible favorable response to rutin treatment was not considered significant.

Inasmuch as rutin has been claimed to maintain the integrity of the capillary wall, a special attempt was made to determine differences in the amount of edema or hemorrhage in the lungs of the dogs receiving rutin compared with those of the controls. No such effect was observed; rather the lungs of the animals receiving rutin showed somewhat more edema than the controls. Furthermore, the character of the inflammatory response was similar in both groups and no difference in the degree or extent of the pulmonary lesions could be demonstrated. Rating the degree of injury from 1 to 4, indicating progressive change from mild to extensive pulmonary involvement, of 5 beryllium-exposed but not rutin-treated animals, 2 each gave a 1+ and 2+ reaction and one, a 4+ reaction; of the four animals examined in the rutin-treated group, no animal showed a 1+ response, one each showed a 2+ and a 4+ response, and two showed a 3+ response. Thus, rutin instead of improving the condition of the pulmonary lesion from beryllium did appear to have exacerbated the condition. Further evidence bearing out this observation was furnished by spectrographic analysis for beryllium content of certain tissues of the exposed and treated dogs. Results of these analyses appeared to show that rutin, rather than ridding the tissues of beryl-

lium, tended to prevent elimination of this element. Amounts of beryllium in the lung, bone, liver, and kidney were for the beryllium-exposed animals respectively 4.9, 0.56, 0.24, and 0.08 µg/g. For the same tissues of the rutin-treated dogs, values of beryllium were correspondingly 5.6, 0.72, 1.2, and 0.09. Thus, in each tissue somewhat higher averaged values for beryllium were found in the rutin-treated dogs.

It is therefore concluded that from the bases of histologic evaluation and results of spectrographic analysis of the tissues for beryllium that rutin is ineffective either as a prophylactic or as a therapeutic agent in the treatment of beryllium injury in dogs.

PROGRAM Th.

THORIUM

Problem Code: Th.3 (Toxic Effects)

Section Code: 3210

Acute Toxicity of Inhaled Thorium Nitrate Dust:

A series of 3 pilot experiments has been completed in which 85 laboratory animals, comprising 6 species, were exposed to thorium nitrate tetrahydrate dust. The purpose of these experiments was to determine whether acute toxic effects are produced by inhalation of thorium nitrate at relatively high concentrations. In the first experiment, 25 female mice, 20 rats, equally divided with regard to sex, 10 male guinea pigs, and 3 rabbits were exposed 6 hours daily for a total of 68 hours during 11 calendar days. The mean concentration of thorium nitrate dust in the chamber was 91 mg/m^3. The mean concentration of thorium nitrate dust was 79 mg/m^3 in the second experiment in which 3 rabbits and 20 male hamsters were exposed 6 hours daily during 10 calendar days for a total of 60 hours. In the third experiment, the mean concentration of thorium nitrate was 92 mg/m^3; 4 female dogs were exposed 6 hours daily during 10 calendar days for a total of 60 hours in this experiment.

The overall mean concentration of thorium nitrate dust in these experiment was 87 $\pm$ 6 mg/m^3. The thorium nitrate was dispersed as a dry dust prepared from material purchased from the Maywood Chemical Company. A sample of the Maywood product was analyzed spectrographically by the National Bureau of Standards and was reported to contain 47.28 per cent of thorium dioxide by weight. The overall mean concentration of dust as thorium in these experiments, therefore, was 36 $\pm$ 2 mg/m^3. Since the thorium nitrate had been processed more

than two years ago, it contained appreciable amounts of mesothorium. The dust was prepared for dispersion in the chamber by grinding it twice through a Bantam Micropulverizer. The ground thorium nitrate was dispersed by means of a ball-mill feed, using dry nitrogen for aspirating since the material proved to be quite hygroscopic.

The concentration of thorium nitrate dust in the chamber was followed by taking filter paper dust samples periodically. The samples were weighed and the concentration of dust in the chamber was calculated from the weight of the sample and the known sampling rate. Four spot samples were taken daily for quantitative chemical analysis employing the newly-developed colorimetric method in which thorium is detected through the formation of a colored complex with carminic acid. The results obtained by chemical analysis were in satisfactory agreement with the chamber dust concentrations determined gravimetrically.

In each experiment, the period of exposure was preceded by a 2-week conditioning period during which the animals were placed in a chamber which was held at the same temperature and relative humidity as the exposure chamber, but which contained no thorium nitrate dust. The animals were conditioned in this manner 6 hours daily for 10 days. During both the conditioning period and the period of exposure, the animals were weighed at scheduled intervals, and blood and urine samples were taken for determination of the blood NPN, blood urea nitrogen and urinary protein, and blood samples were taken for hematologic study.

There were no deaths among any of the animals during the period of exposure. All of the animals were sacrificed terminally with the exception of 2 dogs which are being held for further observation. The dogs were the only species in which toxic signs were observed during the exposure period.

Beginning on the 3rd day of exposure, the dogs were nauseated. Retching, gagging, and, occasionally, vomiting, were observed periodically. There were no significant changes in weight among any of the animals and anorexia was not observed even in the dogs. The clinical chemical determinations revealed no significant changes in blood NPN, blood urea nitrogen or urinary protein at any time during the exposure period or during the period of observation following exposure in the 2 dogs which were held for this purpose. The hematologic findings were negative except in the dogs. All of the dogs exhibited leukocytosis at some time during the exposure period of immediately thereafter. The average increase in the white blood cell count for all 4 animals was 50 ± 13 per cent. The peak of the response occurred at different times in different individuals. In the 2 animals which were sacrificed, the highest leukocytic count occurred during the second week of exposure, whereas in the 2 animals that were held for observation, the peak of the response was observed during the first week following exposure. In 3 of the animals, a decrease in the white cell count was observed after the peak had been attained. In 1 of the 2 animals held for further observation following exposure, the reticulocytes disappeared entirely from the peripheral circulation for a period of 2 weeks beginning about one month after the termination of exposure. Coincident with the disappearance of reticulocytes, a very low white blood cell count of 6000 was recorded for this animals. These findings were not associated with any change in the red blood cell count.

Gross pathologic findings on the animals which were sacrificed terminally indicated moderate pulmonary damage of hemorrhagic nature in a few of the animals; in most, however, the lungs were grossly normal. All of the other organs presented a normal appearance in all of the animals.

PROGRAM F.

FLUORIDE

Problem Code: F.3 (Toxic Limits)

Section Code: 3210, 3230

Acute and Pilot Feeding Studies:

The ingestion experiment with weanling and mature rats at dietary levels of 0.1, 0.2 (mature rats only), and 0.4 per cent sodium fluoride was terminated after 83 days' duration; suitable controls were also included. Because of the high mortality in the weanling rats, urinary fluoride excretion was followed only in the mature rats. The results obtained, together with the blood fluoride contents are listed in the following table:

Blood and Urine Fluoride Levels in Rats on Diets Containing Added Sodium Fluoride

Time on Diets - 83 Days

Per Cent NaF in Diet	Mean Urinary Fluoride, mg F/l	Blood Fluoride µg F/100 ml
Control	0.16	45
0.1	1.60	65
0.2	3.38	163
0.4	5.25-9.10*	---**

* Range of four determinations.
** Insufficient number of survivors for adequate blood samples.

It is seen that a two-fold increase in dietary fluoride results in an approximately two-fold increase in mean urinary fluoride excretion, and in a 2.5-fold increase in blood fluoride.

Problem Code: F.4 (Fate)

Section Code: 3210

Protective Action of Fluoride in Uranium Poisoning:

As reported last quarter, the ingestion of 15 ppm F in the drinking water of rabbits appeared to offer some measure of protection against the toxic effects of uranyl nitrate administered intraperitoneally. These observations have been extended now to include data on rats fed a stock diet containing 0.2 per cent sodium fluoride for intervals of three and six months. No protective effect of the ingested sodium fluoride was noted, however, when 2.5 mg U/kg, as uranyl nitrate (21-day LD50 dose), was injected intraperitoneally; on the contrary, the mortality was slightly greater in those animals receiving sodium fluoride in the diet. The mortality was less after three months than after six months. It has been known for some time that older rats are more susceptible to acute uranium poisoning than are younger rats. It has also been known that male rats require larger doses of uranium than females to produce death. The results obtained are shown in the following table:

Mortality in Sodium-Fluoride-Fed Rats Following Intraperitoneal Injection of Uranyl Nitrate

Time on Diet	Dietary Level NaF, Per Cent	Mortality	
		Male	Female
3 months	Control	0/5	3/5
	0.2	1/5	4/5
6 months	Control	1/6	4/6
	0.2	4/6	5/5

PROGRAM S.M.

SPECIAL MATERIALS

Problem Code: S.M.3 (Toxic Limits)

Section Code: 3210

Experimental Standard Chamber:

Although many of the problems involved in the maintenance of dust atmospheres of constant composition with respect to concentration, distribution, and particle size have been recognized; the difficulties involved in their solution and urgent need for toxicologic data have been so great that little progress has been made in their solution and admittedly imperfect experiments have been accepted. Thus, variations in aerosol concentrations as great as 20 per cent and particle-size variations of two-fold have been characteristic of some of the best studies to date.

An examination of our experience has shown that very little was known about the behavior of various dusts in the atmosphere and that the problem involved both the nature of the feed and the exposure chamber itself. The introduction of the aerosol-type feed solved one of these problems by dispersing both solutions and suspensions with consistent results. Under properly controlled conditions, uniformity of the atmosphere, particularly with respect to particle size were shown for example in the uranium dioxide size studies. The elimination of dead space and excessive turbulence in the present chamber was also shown to be of considerable value.

During the past year, work has been in progress on the design and construction of a new type of dust exposure chamber. This unit has been designated the experimental standard chamber because of the uses to which

it may be put. These include the maintenance of standard reference atmospheres of known composition for the comparative testing and calibration of various types of sampling instruments, the study of the methods of producing aerosol atmospheres of constant composition, as well as a study of the behavior of aerosol atmospheres. During the past quarter, work has been completed on the installation of this chamber and its accessory control equipment. Several range-finding pilot atmospheres have been investigated with respect to concentration, distribution, and concentration maintenance. The results to date have been very encouraging and the significance of several factors such as feed, operating pressures, chamber-air flow and the specific nature of the material to be dispersed have been recognized.

The design of the chamber illustrated in Figure 1 (Page 79), although representing a radical departure from conventional types, is based on aerodynamic principles, modified by the practical limitations of space and available materials. The construction is in the form of a vertical cylinder with inlet and outlet regions modified to give a minimun of turbulence and maximal uniformity at any point within the cylinder section. The mixing chamber is a cylinder 18" in diameter, 6" high, constructed of galvanized iron and coated with a corrosion-resistant paint. Air and feed inlets enter this chamber tangentially and diametrically opposed. The resulting turbulence thoroughly mixes the concentrated aerosol with the diluting air. For the present studies, aerosol units of the type described previously as the "stainless steel submerged aerosol unit" were used as feeds. The air entering the chamber is under slight positive pressure produced by a 300 ft.^{3}/min. I.L.G. centrifugal fan. The air is filtered and adjusted to temperature by means of an automatically controlled

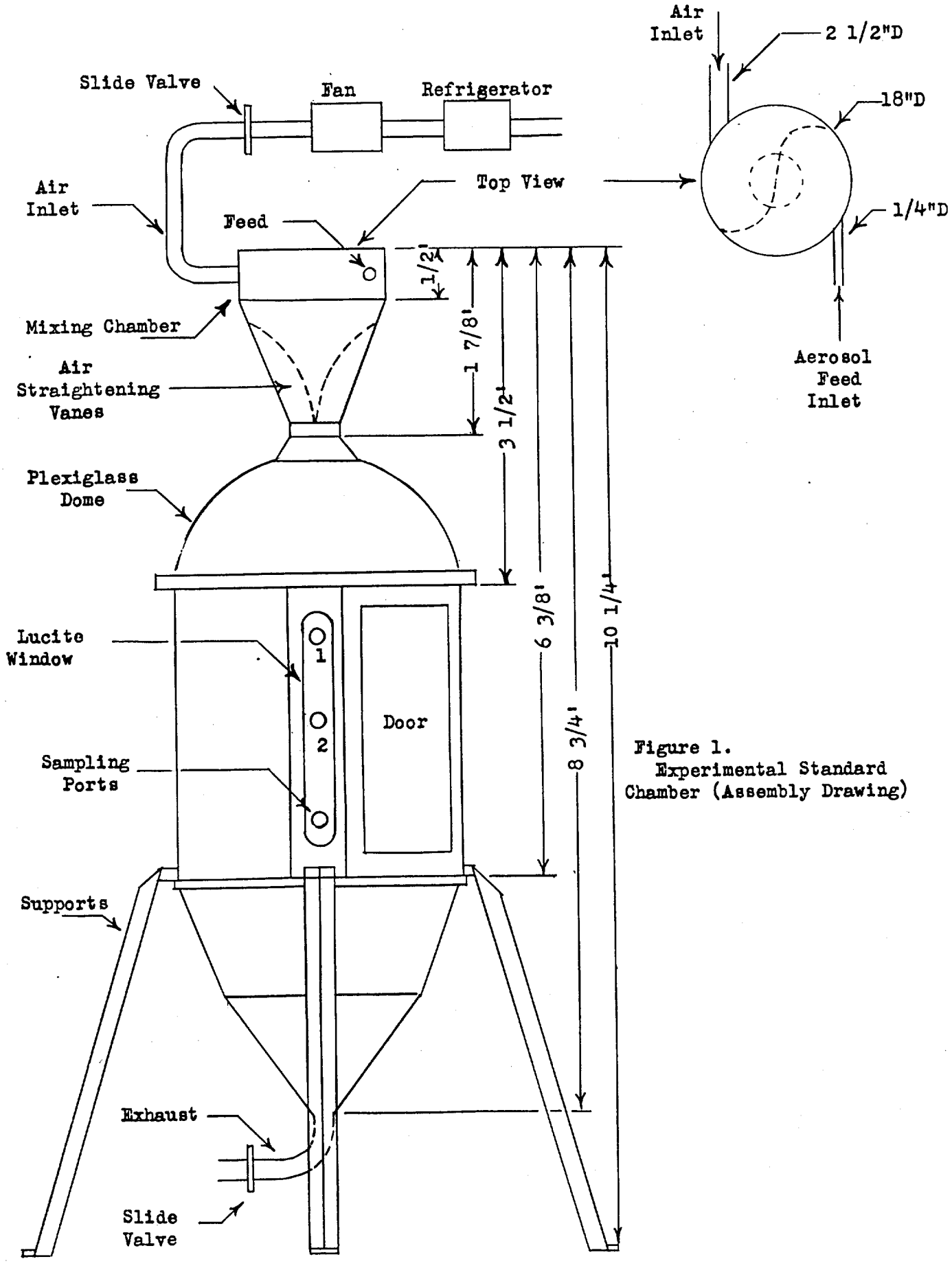


Figure 1.
Experimental Standard Chamber (Assembly Drawing)

refrigerating unit. After mixing, the air stream within the chamber enters a converging section where straightening vanes give it a vertical linear flow. This section terminates in a throat 6" in diameter. Directly beyond the throat, the air stream is under negative pressure produced by a rotocline at the exhaust end. The stream distributes itself uniformly within the plexiglass dome by the time the main chamber is reached.

The main chamber section is constructed of stainless steel, forming a cylinder 3 ft. in diameter and 3 ft. high having a calculated volume of 20 ft^3 of 0.57 m^3. A curved door, 19 x 33", is fitted to the front side of the main section. Four lucite windows measuring 3 x 30" are set into the outside wall at equidistant positions and numbered counter clockwise from 1-4 starting at the left side of the door. Window No. 2 has 3, 1 1/2" sampling ports numbered from 1 to 3 from the top of the section down. Sampling port No. 2 is at mid position of the chamber, whereas 1 and 3 are 6" each from their respective ends of the cylinder.

The chamber is exhausted through a conical section of stainless steel leading into a 2" exhaust pipe which in turn is connected to a 120 ft^3/min. rotocline.

All internal surfaces of the chamber were buffed smooth and all fittings were adjusted to the contours of the internal walls to prevent the occurrence of local areas of turbulence. The construction design was also modified to permit the removal of various sections for any future changes.

The control equipment for the chamber is mounted on a panel board located approximately 4' behind the rear section in order to permit easy access of the windows from all sides and simplify operations. The present

equipment is conventional including air and nitrogen feed pressure lines, pressure regulating valves and meters, and 2 high-rate and 1 low-rate sampling manometers. In addition, a pitot tube was located in the first section of the exhaust pipe to measure air velocities. Readings are taken on an inclined kerosene manometer having a ΔH range equivalent to 1 to 250 $ft^3/min.$ was adopted. At this rate the velocity through the chamber is 2.03 $ft^3/min.$, representing an air change of once every 2 1/2 minutes.

Several preliminary studies of the flow characteristics within the chamber were made by introducing phosphorous smoke. No samples were taken but distribution conditions were observed visually. An initial period of from 5 to 10 minutes was required to produce visual uniformity within the entire chamber. After this period uniformity was maintained for the entire period of testing. Flow characteristics appeared to be satisfactory and no immediate change in design is contemplated.

Several types of preliminary range-finding experiments were made to determine the operating characteristics of the chamber and the aerosol feed. These included the maintenance of atmospheres of oil and also of sodium chloride aerosols. Oil was dispersed because it represented the prototype of a non-volatile homogeneous liquid and did not involve the additional problems of aqueous solutions (see following paragraph). Sodium chloride was used as inexpensive innocuous prototype of a water-soluble metal salt. The results given in the following paragraph for concentration and distribution studies are typical of those obtained and are presented to indicate the direction and progress of these studies.

Oil Aerosols: For production of an oil aerosol, No. 20 grade motor oil was dispersed by a stainless steel submerged aerosol unit having 0.040" orifices

of both liquid and air jets. The unit was mounted in a one liter, round-bottom flask as described previously and the oil level was set at 1" below the jets. This particular unit was later found to be improperly adjusted and operating at a level of only approximately 50 per cent efficiency. The nature of the results obtained, however, were the same as those at conditions of maximal efficiency. For the distribution and concentration studies, simultaneous filter paper samples were taken through window No. 2 at position 2 and 3. The samples at position 2 were fixed at a distance of 1 1/2" within the chamber. Samples taken at position 3 varied with respect to the distance and provided a comparative measurement of the distribution uniformity at the lower level of the chamber. An average sampling rate of 14.3 liters per minute was used and concentrations were determined gravimetrically with an analytical balance sensitive to 0.1 mg.

The results listed in Table I (Page 83) are typical of a continuous exposure for 3 hours at 11 pounds feed pressure. Simultaneous samples were taken at 1/2-hour intervals after allowing a period of 15 minutes for equilibrium to occur within the chamber. In the sampling time of 10 minutes, filter paper weights increased from 7.9 to 9.1 mg. A comparison of the concentrations and distribution values obtained with most previous types of chamber atmospheres shows remarkable consistency. For the fixed sampling position, the value of 54.0 mg/m^3 obtained with the first sample indicated that equilibrium near the sides of the chamber was not achieved within a period of 1/2 hour. The rest of the values at this position, however, show a variation within the limits of maximal error of the analytical method (±1.3%). With respect to the samples taken at varying positions, results show that equilibrium toward the center of the chamber is reached more rapidly than at

TABLE I.

Oil Aerosol Concentration and Distribution Study at a Feed Pressure of 11 lbs/in^2

Time	FIXED SAMPLING POSITION		VARIABLE SAMPLING POSITION	
	Concentration	Distance Within Chamber	Concentration	Distance Within Chamber
hr-min.	mg/m^3	in.	mg/m^3	in
9.30*	--	--	--	--
9.45	54.0	1½	58.0	25
10.15	59.2	1½	58.0	25
10.45	59.2	1½	59.3	34
11.30	59.2	1½	60.5	20
12.15	58.5	1½	56.8	12

*Start of experiment.

the sides. Thus, the value of 58 mg/m^3 was obtained for both the first and second samples at positions 25" within the chamber. The variations between the distribution samples and the corresponding ones taken at fixed positions at the same times showed that the uniformity of distribution was in the order of analytical error. Maximal variations from the total mean 59 mg/m^3 after equilibrium had been obtained were negligibly small and in the order of twice the analytical error (or 2.6%). Similar results were found at a range of concentration levels from 20 to 90 mg/m^3 representing respective range of feed pressures from 8 to 15 pounds.

Figure 2 (Page 85) shows a plot of the chamber concentration against the pressure in pounds per square inch. The determinations reported are the average of at least 4 samples at varying positions taken from data collected on 2 consecutive exposure days. A concentration range of from 616 - 27.5 mg/m^3 was maintained over a range in pressures of from 5 - 30 pounds. The plot shows that above pressures of 8 pounds a linear relationship between concentration and pressure exists for the particular aerosol feed system. For the entire range of data, the average variation from mean values of each point was less than 2 per cent. The extreme variations occurred at the low pressure of 5 pounds and at values above 25 pounds, the largest percentage deviation being 7.2 per cent at 30 pounds. In terms of distribution, the variation of the samples taken at varying positions in the chamber in comparison with those taken at fixed positions showed an average percentage deviation of 2.7 per cent, the extreme variations again occurring at 5 pounds and above 25 pounds. The average percentage deviation values for the range between 8 and 15 pounds were all below total average values. The maximal percentage deviation was found to be 6.6 per cent at 30 pounds.

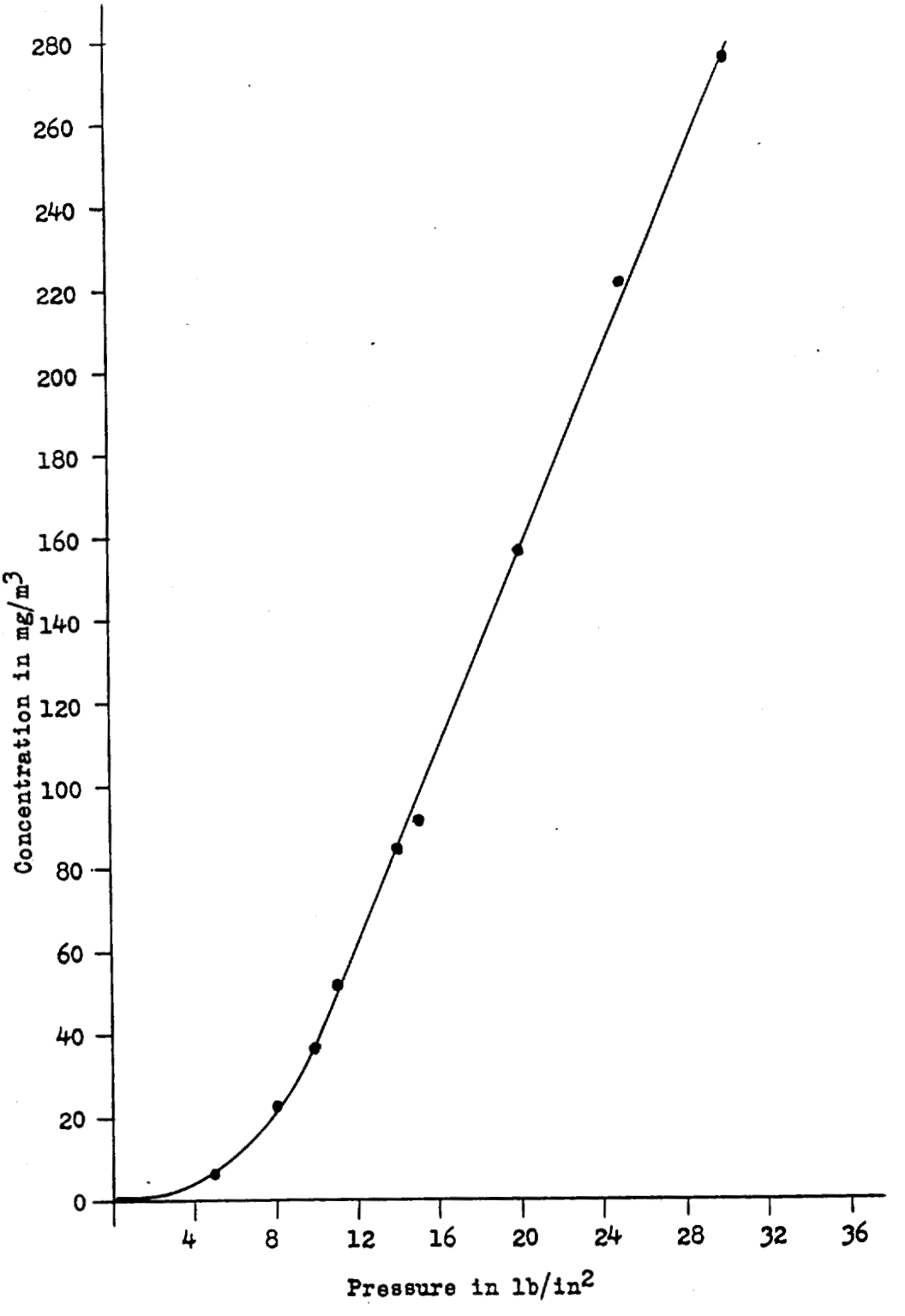


Figure 2. Experimental Standard Chamber
-- Oil Aerosol Atmospheres --
Chamber Concentration as a Function
of Feed Pressure

The critical nature of the feed pressure was the most significant finding of this study. The rate of increase above 8 pounds pressure of chamber concentration was found to be 1.2 mg/m^3/pound of pressure. Since the available pressure-regulating devices were not better than ± 1/2 pound, an error in the order of ± 0.6 mg/m^3 could be expected. At a 50 mg level this is equivalent to an error of 1.2 per cent. At pressures within the range of 8 - 20 pounds, the summation of the feed pressure and the analytical errors are sufficient to explain all variations in concentrations found. Larger variations at the 5-pound pressure level were attributed to the pressure-regulating valve and a lag in this system. At pressures above 20 pounds, the larger variations are explained in terms of liquid-level variations. Several preliminary tests have shown that the exact level of liquid above the orifices of the aerosol nozzle is of critical nature in terms of concentration. At higher concentrations, the rate of output of the feed was sufficient markedly to change this level during a single study. In addition, excessive turbulence created at the higher pressures within the feed container caused continuous variation of the set level.

Sodium Chloride Aerosols: For production of sodium chloride aerosols, 10 per cent aqueous solutions of sodium chloride were dispersed by the same feed as that used for oil. In addition to the known variables of feed pressure and liquid level, a third factor was found to be of importance in maintaining concentration levels. Individual tests with the feed showed that in the case of solutions, water was removed at a more rapid rate than the solute. Thus, both the concentration of the feed material and the concentrations within the chamber progressively increased. Small variations of solution concentration as measured by specific gravity were found to cause marked changes in chamber concentration. In addition a direct relationship between particle

size and the initial solution concentration was found with concentrated solutions resulting in larger particle sizes. In order to eliminate the problem of liquid level of variation and the changes in solution concentration, a constant leveling apparatus, illustrated in Figure 3 (Page 88) was designed for the feed. The aerosol unit used for this apparatus was one of the more recently-developed types having 0.027" orifices for the liquid and air jets. The apparatus operates as a continuous circulating system activated by an automatic hypodermic syringe pump (Brewer Automatic Pipetting Machine). The solution is continuously pumped into the feed container from a level 1 3/4" above its base. An overflow opening set at the height of the top of the aerosol unit allows excess liquid to flow off by gravity. The liquid then returns to a large-capacity reservior (4 liters) through a 21" glass air-trap.

Using the constant leveling apparatus several studies similar to those made with oil were made with salt aerosols. The sampling rates and chamber conditions were the same as for the oil aerosol studies. The results listed in Table II (Page 89) are typical of a continuous exposure for 6 1/2 hours at 20 pounds feed pressure. Simultaneous samples were taken at approximately 1-hour intervals after allowing an initial period of 30 minutes for equilibrium to be reached. The sampling time was 20 minutes and a range of filter paper weight increases of from 2.6 to 3.5 mg was obtained.

Although variations in concentration and distribution values obtained in this study were larger than those obtained with oil aerosols, the marked consistency is still outstanding. For the fixed sampling position, a mean concentration of 10.4 mg/m^3 was obtained, with a range in values of from 9.6 to 11.1 mg/m^3. The average percentage deviation from the mean was 4.3 per cent with the maximal value being 7.7 per cent. For the distribution data, the

Figure 3. Constant Leveling Apparatus for Aerosol Feed

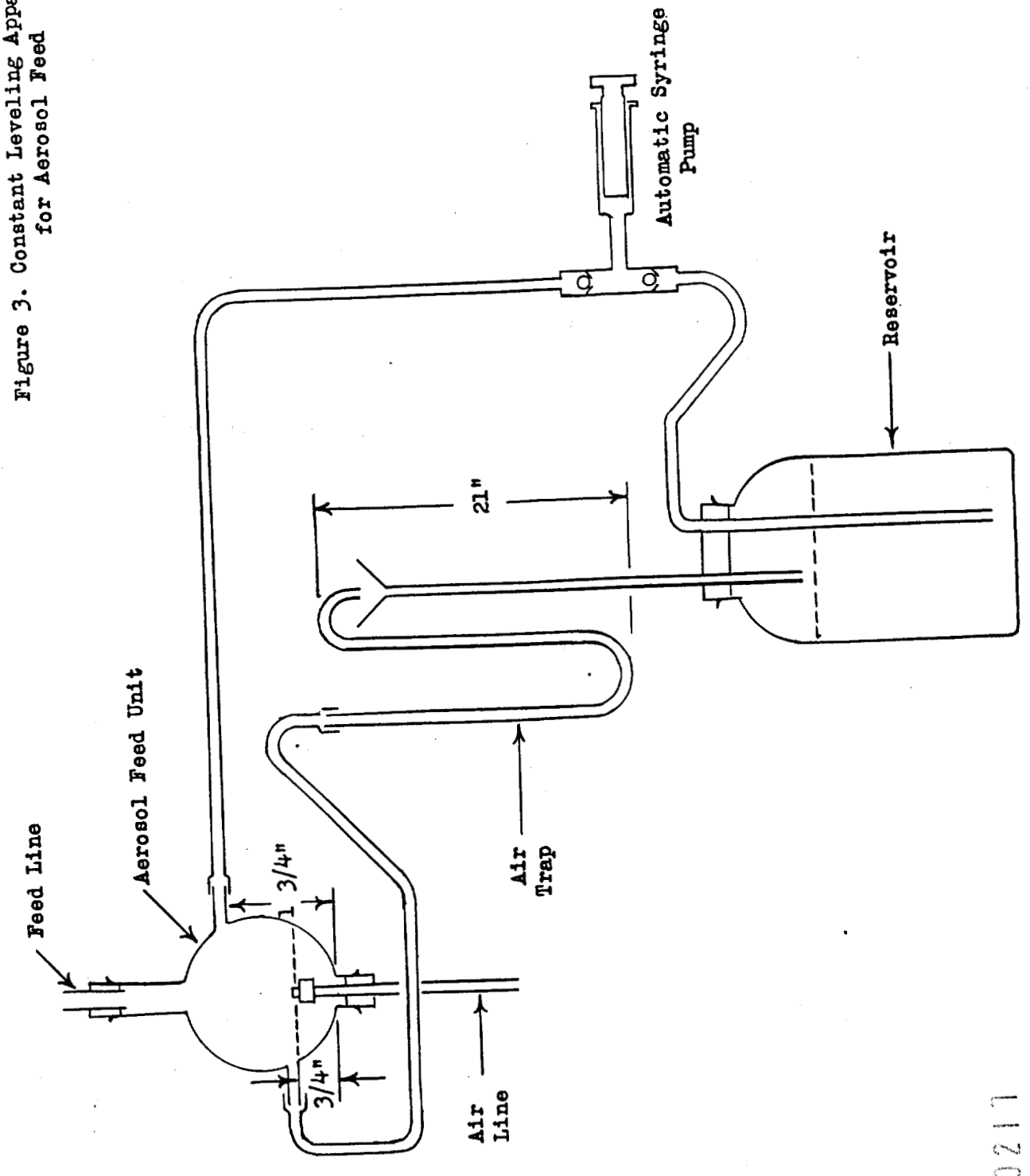


TABLE II.

Sodium Chloride Aerosol Concentration and Distribution Study at a Feed Pressure of 20 lbs/in^2

	FIXED SAMPLING POSITION		VARIABLE SAMPLING POSITION	
Time	Concentration	Distance Within Chamber	Concentration	Distance Within Chamber
hr-min	mg/m^3	in	mg/m^3	in
9.30*	--	--	--	--
10.00	9.6	1½	9.8	20
10.30	10.0	1½	10.1	32
11.30	11.1	1½	11.4	26
13.00	10.7	1½	10.5	14
13.30	11.1	1½	10.2	5
14.15	10.0	1½	9.2	5
15.00	10.4	1½	10.1	14
15.30	10.0	1½	10.4	22

*Start of experiment

average percentage deviation between the samples taken simultaneously at fixed and variable position was 3.8 per cent with a range in variations from 1.0 to 8.1 per cent. The average percentage variations with respect to the total mean for all samples of 10.3 mg/m^3 was the same as that found for the samples taken at the fixed position (4.3 per cent). The average analytical error was of the order of $\pm$ 3 per cent, indicating that most of the variations could be attributed to this factor.

Similar results were obtained for concentration ranges between 2.4 and 30 mg/m^3. It was found, however, that the 4 liter reservoir did not completely solve the problem of solution concentration by more rapid removal of water than solute. Further work is in progress on the solution of this problem and in the development of more adequate methods of feed pressure regulation.

Problem Code: S.M.3 (Toxic Limits)

Section Code: 3230

Intraperitoneal Injection of Zirconyl Nitrate:

Zirconyl Nitrate (Source: Rohm and Haas) when injected intraperitoneally in rats (group of ten) has given an LD50 of 225 mg for rats of 190 grams average weight, or an LD50 of approximately 1185 mg/kg of body weight.

PROGRAM I.S.

ISOTOPES

Problem Code: I.S.1 (Tracer Chemistry)

Section Code: 3120

Studies in Protein Metabolism in the Dog Using C^{14}-Labeled DL-Lysine. I. The In Vivo Conversion of Lysine to Glutamic and Aspartic Acids:

Background: The intermediary metabolic fate of the carbon chain of L-lysine is not known. A brief note by Borsook, H., et al (1) reports the probable conversion of ϵ-C^{14} labeled lysine to α-aminoadipic acid by guinea pig liver in vitro.

The last quarterly report (Rochester Report UR-38) presented data showing that L-glutamic acid, isolated from the liver of a dog previously fed ϵ-C^{14}-DL lysine, had about 17 per cent of the C^{14} activity of the L-lysine on a millimolar basis. Likewise the L-aspartic acid from the same liver had about 5 per cent of the C^{14} activity of an equimolar amount of L-lysine from the same liver. On this basis alone, it appears very unlikely that the dicarboxylic acids' C^{14} activity arose entirely from a CO_2 assimilation reaction (2) whereby CO_2 reacts with pyruvate to produce oxalacetate, and ultimately glutamic and aspartic acids.

By selective degradation of glutamic acid with ninhydrin it is possible to determine exactly the C^{14} activity of the α-carboxyl group of glutamic acid. Thus, ninhydrin releases the CO_2 of the carboxyl group α to the amino group and it is this carboxyl which has been proved to arise from CO_2 assimilation (3). Hence, any activity remaining after ninhydrin treatment must originate elsewhere.

Likewise, ninhydrin releases the CO_2 from both carboxyls of aspartic acid, and makes possible the assignment of C^{14} activity to the carboxyl groups or the inner two carbon atoms of aspartic acid.

Method: The glutamic and aspartic acids were isolated from liver and plasma protein hydrolysates as glutamic acid hydrochloride and copper aspartate by methods previously described.

Weighed amounts of the amino acids were placed in the reaction flask of the C^{14} assay apparatus (4) and treated with ninhydrin by the method of Christensen, et al (5). After washing through with nitrogen the carbon dioxide released by ninhydrin, the residue in the reaction flask was evaporated to dryness at 30 - 35°C and subjected to a separate C^{14} assay in the usual manner.

Results: The results of the above reactions are given in Table 1 below along with the total C^{14} activity of the amino acids determined on separate samples in the usual way.

TABLE 1

C^{14} Activity in Volts*/Minute/Millimol

		Total	Released by Ninhydrin	Released from Ninhydrin Residue
LIVER	Glutamic Acid	.399	.077 .078	.251 .241
	Aspartic Acid	.110	.82	.01
PLASMA PROTEIN	Glutamic Acid	.323	.080	.226
	Aspartic Acid	.18	.125	.01

*Volts/min. x (1.88×10^4) = disintegrations/minute
Volts/min. x (8.54×10^{-3}) = microcuries

[redacted]

from the same tissues has the same order of activity as the glutamic acid (6) it appears very unlikely that ornithine could have been the glutamic acid precursor. It seems more probable that both are desivable from a common precuror. The α-amino-adipic acid described by Borsook, et al (1) may be such a precursor, but we have found no evidence to support this notion.

It will be noted that the total C^{14} activity as determined directly on the amino acids, particularly glutamic, exceeds the sum of the ninhydrin released and ninhydrin residue activities. It seems likely that much of this discrepancy is referable to losses of $C^{14}O_2$ occurring during the evaporation of the ninhydrin residue solutions. Christensen (5) has pointed out that as much as 15 per cent excess of CO_2 may be released from glutamic acid of the ninhydrin treatment is prolonged. This is essentially what occurs during the prolonged evaporation of the ninhydrin residues prior to the wet oxidation of the dried residue for C^{14} assay.

Summary: Glutamic and aspartic acids isolated from liver and plasma proteins of a dog fed ε-C^{14} labeled DL lysine have radioactivity which can be accounted for only on the basis of a direct conversion of the carbon chain of lysine to that of the dicarboxylic acids.

BIBLIOGRAPHY

1. Borsook, H., et al; J. Biol. Chem. 173, 420 (1948).

2. Wood, H. B., and Werkman, C. H.; Biochem. J. 30, 48 (1936).

3. Wood, H. G., Werkman, C. H., Hemingway, A., and Nier, A. O.; J. Biol. Chem. 139, 365,377,483 (1941). Evans, E. A., Jr., and Slotin, L.; J. Biol. Chem. 136, 301 (1940) and 141, 439 (1941).

4. Bale, W. F. (Unpublished)

5. Christensen, B. E., West, E. S., and Dimick, K. P., J. Biol.Chem. 137, 735 (1941).

6. Part II. The Conversion of Lysine to Arginine (this report).

Problem Code: I.S.1 (Tracer Chemistry)

Section Code: 3120

Studies in Protein Metabolism in the Dog Using C^{14}-Labeled DL-Lysine. II. The Conversion of Lysine to Arginine:

Background: The last quarterly report (Rochester Report UR-38) presented data showing that the C^{14} activity of the basic amino acid fraction (lysine, arginine, and histidine) of liver protein from a dog fed ϵ-C^{14}-labeled DL lysine, could not be accounted for on the basis of C^{14} activity in lysine alone.

Furthermore, it has been long recognized that the adult dog and rat could produce enough arginine to maintain nitrogen and weight balance in the absence of dietary arginine; however the exact metabolic precursors of arginine have not been defined.

We have isolated arginine from liver and plasma proteins of a dog fed ϵ-C^{14}-labeled lysine, as arginine monoflavianate. This amino acid has been found to contain C^{14} activity not only in the guanidine moiety, but also in the carbon chain of the ornithine moiety.

Method: The basic amino acid fraction obtained from the protein hydrolysate with the aid of the ion exchange resin Amberlite IRC-50, is treated with excess flavianic acid at PH 4.5 and the precipitated flavianate is converted to the monoflavianate by dissolving with the aid of a few drops of concentrated ammonium hydroxide, and reprecipitating near the boiling point by adding 20 per cent hydrochloric acid to bring the PH to 4-4.5. This process of solution and reprecipitation was repeated twice again with the addition of about 15 mg of L-lysine to "wash out" any contaminant of radioactive lysine, and then repeated at least once again (without added lysine).

The crystalline monoflavianate was then assayed for C^{14} activity. Another portion was treated with aqueous barium hydroxide to remove most of the flavianic acid. An excess of crystalline barium hydroxide (enough to make a 10 per cent solution) was added to the solution of arginine, and the mixture was heated in a sealed tube for 18 hours at 100 - 104°C. The precipitate of barium carbonate was filtered off, washed with water, alcohol and ether, and assayed for C^{14} activity. The remaining alkaline solution was treated with excess paratoluenesulfonyl chloride in ether with stirring and a crystalline diparatoluensulfonyl derivative isolated and recrystallized at least twice from dilute ethyl alcohol. This derivative had mp 171-172 and showed no depression of melting point when mixed with a specimen prepared in identical fashion from an authentic specimen of arginine. The diparatoluenesulfanyl derivative was then assayed for C^{14} activity.

Results: The table below presents the data obtained from the arginine monoflavianates isolated from liver and plasma proteins:

C^{14} Activity in Volts*/Minute/Millimol

	Arginine Monoflavianate	Guanidine Moiety	Ornithine Moiety
Liver	.175	.07	.10
Plasma Protein	.172	.08	.07

*Volts/min x (1.88×10^4) = disintegrations/minute
Volts/min x (8.54×10^{-3}) = microcuries

It is clear that the activity present in the ornithine moiety represents a large or major portion of the total activity present in the original arginine and is of the same order as the activity found in the glutamic acid from the same tissues (liver protein glutamic acid .399 volts/min/millimol, plasma protein glutamic acid .323 volts/min/millimol). Because these amino acids have activity of the same order, it appears unlikely that the ornithine activity was derived from a conversion involving glutamic acid and proline such as described by Shemin and Rittenberg (1).

It seems more likely that the arginine carbon chain was derived from lysine (liver lysine, 2.39 volts/min/millimol) by a more direct metabolic path, possibly from the same precursor as glutamic acid.

The finding of conversion of the carbon chain of lysine to that of arginine represents the first definite identification of an amino acid as a metabolic precursor of the carbon chain of arginine (i.e. ornithine), and may represent a major metabolic pathway for the formation of arginine in the absence of dietary arginine, especially when the diet contains only the essential amino acids.

The activity in the guanidine moiety is, of course, referable to the incorporation of carbon dioxide in the synthesis of urea through arginine as noted by others (2).

Summary: Arginine has been isolated as the monoflavianate from hydrolysates of liver and plasma proteins, obtained from a dog fed ϵ-C^{14}-labeled DL lysine. The activity of the diparatholuenesulfonylornithine obtained from

(1) Shemin, D. and Rittenberg, D., J. Biol. Chem., 158, 71 (1945).

(2) Delluva, A. M., Wilson, D. W., J. Biol. Chem., 166, 739 (1946).

degradation of the arginine is best explained by a conversion of the carbon chain of lysine to that of arginine.

This may represent a major metabolic pathway for the formation of arginine in the absence of dietary arginine.

Problem Code: I.S.2 (Radioautography)

Section Code: 3130, 3140, 3171

Autoradiographs of C^{14} Incorporated in Individual Blood Cells:

Background: Since it has been demonstrated (1) that the alpha-carbon atom of glycine labeled with C^{14} is incorporated into the hemin and globin moieties of hemoglobin, it was thought that the incorporated C^{14} in an individual blood cell could be demonstrated by an autoradiograph.

Method: To this end a male rat weighing 120 grams was given a total of 3 μc of glycine containing C^{14} in the alpha-carbon atom*. The specific activity of this glycine was 1.83 μc per mg. The glycine was administered by means of three intraperitoneal injections of one each, given at hourly intervals. Blood was taken from the tail veins 25 hours after the first injection, was diluted with serum made from dog blood, and was smeared directly on an Eastman NTB emulsion. The smears were dried in air and fixed in methyl alcohol. After an exposure period of 67 days the emulsion plates were developed in Kodak D-19 and cleared, and the cells were stained with Wright's

*This sample of glycine was kindly supplied by Dr. B. M. Tolbert of the University of California.

(1) Altman, K., Casarett, G., Masters, R., Noonan, T., and Salomon, K.; Fed. Proc., 7, 2 (1948) and J. Biol. Chem., (In Press).

stain. The blood smears were made sparsely cellular to insure clearcut, well-defined autographs and minimal cell clumping.

In order to prove that the autographs are not the result of chemical fogging, similar smears of blood from a control rat were exposed under identical conditions. The exact details of the technique for preparing blood smears on a photographic emulsion will be described in another paper (2).

Result: The photomicrographs on Page 100 show autoradiographs resulting from beta emissions from C^{14} incorporated into blood cells.

Discussion: In photomicrograph 1 it can be seen that the silver grains are non-uniformly distributed, being concentrated focally about certain individual cells to form autoradiographs. Other cells, such as the erythrocytes in this particular field, produced no autoradiographs. In order to make the autoradiographs prominent at this magnification (X440) the NTB plate from which this photomicrograph was made was developed for a longer time in D-19 than that used for photomicrographs 2, 3, and 4. This procedure enhanced the visibility of the beta radiation effects, but obscured cellular detail. Silver grains between the autoradiographs on the test plates, and in all regions of the control plates, are relatively very small in number per unit area and randomly distributed, as is extraneous background fog.

The identifiable cells on the test plates include lymphocytes, polymorphonuclear leucocytes, and erythrocytes. Although this technique is not at present completely quantitative, it is apparent that the percentage of cells of each type associated with definite autoradiographs declines in the order:

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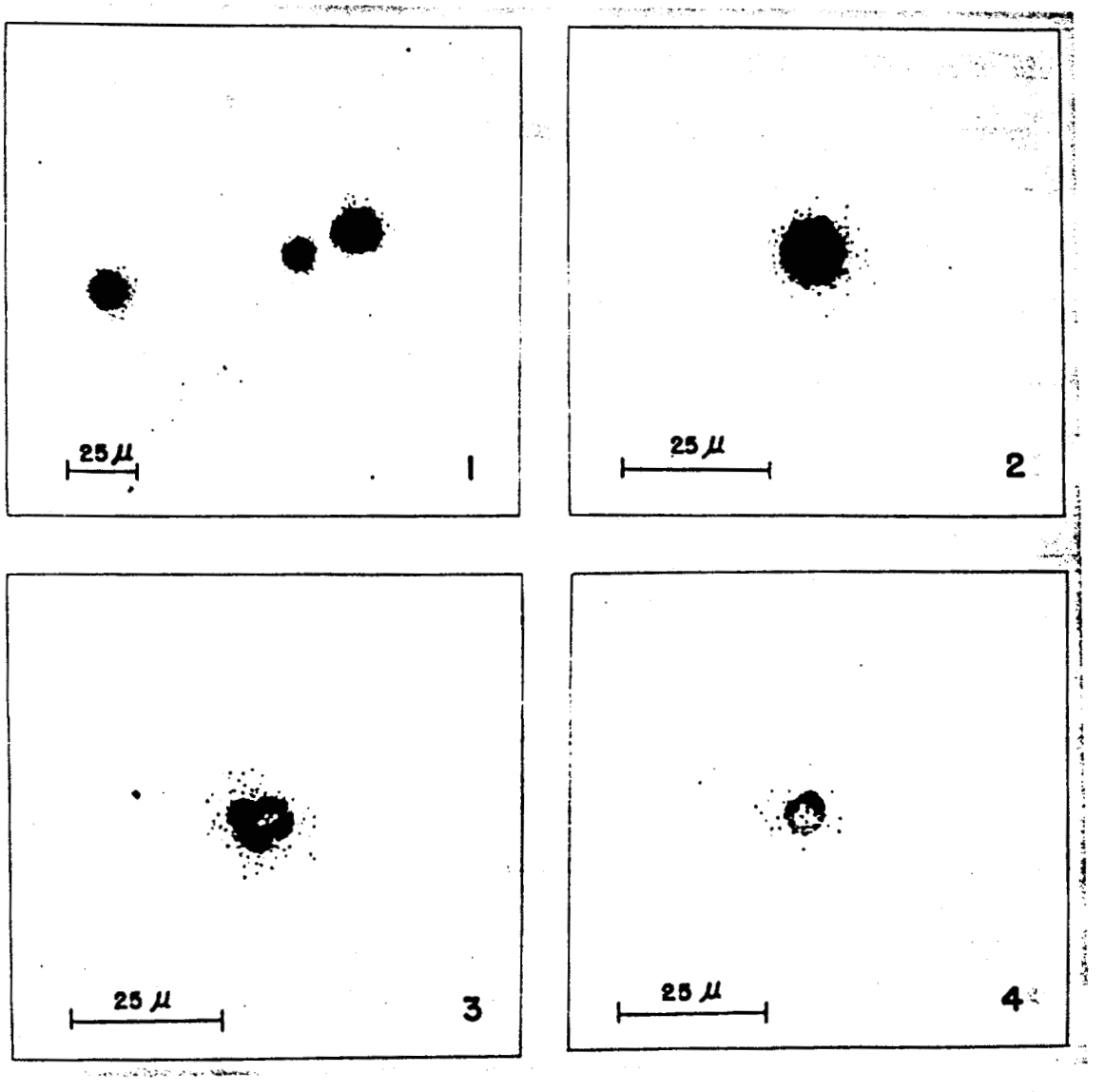
(2) Boyd, G., Williams, A., and Casarett, G. (To be published).

AUTORADIOGRAPHS OF C^{14} INCORPORATED IN INDIVIDUAL BLOOD CELLS OF A RAT AFTER INJECTION WITH GLYCINE LABELED IN THE α-CARBON ATOM



1. FIELD OF TEST BLOOD SMEAR ILLUSTRATING NONUNIFORM DISTRIBUTION OF SILVER GRAINS AND CONCENTRATION OF GRAINS AROUND CERTAIN CELLS. CELLS WITHOUT AUTORADIOGRAPHS ARE ERYTHROCYTES. 440X
2. LYMPHOCYTE. 950X
3. POLYMORPHONUCLEAR LEUCOCYTE. 950X
4. ERYTHROCYTE. 950X

lymphocytes, polymorphonuclear leucocytes, erythrocytes. One possible explanation for this phenomenon lies in the difference in the rate of formation among the three types of cell under consideration. Although the exact life spans of circulating rat blood cells are not known, it is agreed that the erythrocyte has a much longer life span than the leucocytes, and it is probable that the polymorphonuclear leucocyte has a slightly longer life span than the lymphocyte. It is to be expected, therefore, that the percentage of new cells of each type in the circulating blood of a normal rat at a given time would decrease in the order mentioned above.

Thus, most of the lymphocytes are associated with autoradiographs. The polymorphonuclear leucocytes are associated in several cases with autoradiographs, despite the fact that these are the least numerous of the three cell types in the rat blood. The erythrocytes rarely produced an autoradiograph under our experimental conditions, despite their relatively large numbers in the circulating blood.

The grain concentration, i.e., number of silver grains per unit area in the autoradiographs, which is a measure of the relative amounts of C^{14} incorporated in the cells, varies in each cell category. There are cells of each type which reveal no C^{14} incorporation detectable by this technique. Of the cells which yield autoradiographs, however, the concentration of silver grains is generally greatest in the case of the lymphocytes. Photomicrograph 2 represents approximately the average grain concentration in autoradiographs associated with lymphocytes. The maximum grain concentration in autoradiographs associated with polymorphonuclear leucocytes, represented in Photomicrograph 4, is less than that of most of the definite autoradiographs given by leucocytes.

The concentration of silver grains appears to be higher in the case of those cells containing relatively larger amounts of nuclear material. It seems reasonable to assume that the cells which show the presence of C^{14} have incorporated the labeled materials in their proteins. Since, according to Abrams, et al (3), glycine is a specific precursor for purines of the nucleic acids of yeast, much of the C^{14} activity may reside in the purine moiety of the nucleoproteins. Inasmuch as the concentration of nucleoproteins is highest in lymphocytes, this offers a possible explanation for the variation in grain concentration among the three cell types.

It is probable that glycine is incorporated into the hemoglobin of the red cell in the bone marrow and not in the circulating blood. This contention is supported by in vitro studies of London, et al (4) which showed that the synthesis of heme from glycine does not occur to a detectable extent in normal human peripheral blood incubated with glycine labeled with N^{15}, and by the finding that rabbit bone marrow homogenates incorporate appreciable amounts of C^{14} labeled alpha-carbon of glycine in hemin within 3 hours of incubation (5). The present experiments strongly suggest, therefore, that the red blood cells associated with autoradiographs are cells which were recently formed and introduced into the circulating blood within the 25 hour period of the experiment.

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(3) Abrams, R., Hammersten, E., and Shemin, D., J. Biol. Chem., 173, 429 (1948).

(4) London, I. M., Shemin, D., and Rittenberg, D., J. Biol. Chem., 173, 797 (1948).

(5) Altman, K. I., and Salomon, K. (To be published).

Problem Code: I.S.2 (Radioautography)

Section Code: 3171

Technique for Making Autoradiographs of Individual Blood Cells:

Background: The production of autoradiographs of individual blood cells with absolute registration of the cells and the images of the beta particle paths requires the making of permanent smears of the blood on the emulsion. This raises three major problems. One is that of smearing on the emulsion which, containing gelatin, imbibes the fluid of the blood and makes smearing with good distribution of blood cells difficult. A corollary to this difficulty is that of a thickly populated smear. As beta particles from an atomic nucleus within a blood cell can penetrate a photographic emulsion for several micra, the range increasing with the energy, the cells must be widely separated to prevent overlapping of the autographs. Both of these problems were solved by diluting the blood with dog serum.

Since the blood cells were taken through the photographic developer and fixed before staining, the third major problem was to determine the solution concentrations which would not change the staining properties of the cells or injure the cell walls by osmotic pressure. This was accomplished by using Eastman Kodak D-19 developer and a 10 per cent solution of sodium thiosulfate (hypo).

While we were able to find conditions by which single cell autographs could be made, we do not wish to imply that they are optimum. This report is made at this time more as an indication of the general approach instead of a final method. Since this work involves both the arts of staining and of photo graphic development, which are difficult to report quantitatively, it should

serve only as a guide for others to learn the technique by experience.

Description of Method: The smear was made in the following manner: A small drop of ammonium and potassium oxalate solution was put in the center of a watch glass and approximately 6 drops of blood were added and mixed thoroughly. Hemoglobin-free dog serum, which had been stored in the deep freeze at about -15°C and allowed to thaw just prior to use, was added until a little over twice the volume of blood plus oxalate solution was reached. The proportions of blood and serum were not accurately measured. Experience was the best instructor, the guide being the distribution and the separation of cells desired on the photographic plate. For higher-energy beta particles a greater dilution is needed to give greater separation of the cells in order to prevent the overlapping of the autographs.

Several minutes after the serum was added, some of the blood cells formed reversable clumps -- they did not clot -- and settled out of the mixture. The diluted blood was stirred just prior to taking a drop for smearing on the plate in order to break up some clumps and to bring others into suspension. Very few clumps were found when the smear was examined microscopically.

The suspension of blood cells was taken into the dark room to be smeared on the photographic plates. The usual dark room precautions were observed and a Wratten Series II safelight with a 10 watt bulb was used at a distance of about 18 inches from the working space. With the photographic plate lying flat on the table, an optimum angle of the eye to the plate can be found by which reflection makes possible the observation of the smearing process.

Eastman Kodak NTB plates were used throughout the work. These have extremely low background fog and of all plates tested are the most sensitive

for their grain size to beta particles.* The emulsion thickness was 6 micra. Thicker emulsions can be used but this increases the probability of excessive surface grains on development and increases the fixing and washing time. These factors can be detrimental, as will be discussed later.

A drop of dilute blood was picked up with a flat, wire spiral. The drop was larger than one used for ordinary smearing since the serum does not spread as readily over the emulsion as it does over glass due to imbibition by the gelatin.

The smear was made on the emulsion in the usual manner by placing one end of a glass slide against the photographic plate at an angle over the drop and between it and the center of the plate. The thickness of the smear, and hence the mean distance between the cells, was controlled by the angle of the slide: The more acute the angle, the greater the mean distance.** After the slide is drawn back into the drop permitting it to spread along the edge, it is pushed forward. To eliminate scratches, a smooth-edged glass slide was used and pushed with as little downward pressure as possible.

The smears were air-dried and then fixed by flooding with absolute methyl alcohol for two minute. They were again air-dried before sealing without a dessicant in light-tight boxes. These were placed in a refrigerator for the exposure period.

The plates were developed in D-19 for two minutes at 20°C; rinsed in tap water for one half minute, fixed in freshly made 10 per cent sodium thiosulfate solution for 6 minutes, washed for 4 minutes in tap water, and dried in air

*Data obtained in this laboratory and to be published soon.

**Lillie, R. D., Histopathologic Technique, p. 204, The Blakiston Co., Phila., 1946.

before staining. To develop weaker latent images, i.e., latent images with lower potential of development to visability, and thus increase the number of visable grains per beta particle, the smear-artograph was, in some cases, developed by first soaking for five minutes in water followed by 25 minutes in dilute D-19 (1 part D-19 to 3 parts of water).

The blood cells were stained with Wright's stain. This was first filtered with No. 42 Whatman filter paper to remove the maximum amount of precipitate, which might be confused with photographic grains if allowed to settle on the surface of the emulsion. A cover slip was sealed on with Permamount.

Comments: Physiological saline was tried as a diluent. However, saline smears on the emulsion showed many more injured leucocytes than smears using dog serum.

The photographic-fixing step is the most critical in the technique. Two variables which influence fixing, viz., the nature of the silver bromide grain and the emulsion thickness, were constant for our experiment. The former will probably be held constant by the manufacturer. The thickness of the emulsion, however, depends upon the customer's request, and the above fixing conditions should be considered only for emulsions of 6 micra. We do not know how they should be changed for thicker emulsions.

In order to obtain the best results with staining, there must be a careful balance among various factors, such as the concentration of the sodium thiosulfate concentrations from 1 per cent to 35 per cent were tested. The 35 per cent solution cleared the 6 μ emulsion in about one minute while it took the 1 per cent solution 90 minutes to clear. Both extremes damaged the cells. A 10 per cent solution cleared the plate in 3 to $3\frac{1}{2}$ minutes, leaving cells in a satisfactory condition for study.

As it is, the usual photographic procedure to fix for twice the length of time required for clearing, our first work was done by fixing for 6 minutes and washing for 4. Longer washing can take the place of a portion of the fixing after clearing. We now feel that it is better to fix until clear and then wash for 20 to 30 minutes. It is probable that this rule would be satisfactory for emulsions of 12 to 15 micra in thickness. However, we have not fully explored this and merely suggest it as a guide for future improvement of the technique.

Problem Code: I.S.2

Section Code: 3220

Partition-Chromatography of Lipids:

Using P_{32} as an analytical agent the partition chromatography of phospholipids has been studied. It has been possible to select those solvents or solvent mixtures which permit a separation of at least seven chemically different individual phospholipids on a micro scale. Currently, the phospholipid preparations are being hydrogenated to permit an even better separation.

Problem Code: I.S.3

Section Code: 3110

Effect of X-radiation on the Uptake of Iodine:

Background: The sensitivity of the thyroid gland to injury by radiation is a matter of some interest in view of the present widespread use of I^{131} in the treatment of hyperthyroid disease as well as malignancy of the thyroid.

In order to obtain information on this matter, experiments are being done in which rats receive x-radiation and at varying intervals post-radiation, thyroid function is tested by administering I^{131} and studying uptake. Since this work was initiated, Bender (1948) has reported studies showing that the oxygen consumption of rats was unaffected by local doses of 50 to 5000 roentgens of x-radiation administered to the thyroid. Earlier results had variously reported stimulation of the B.M.R. of rabbits (Takayama, 1947), increased activity of the thyroid of guinea pigs (Torentin and Watrin, 1933), and little or no effect on thyroid activity of rabbits (Ziminitsky, Baskina and Devirz, 1936), no effect on dogs (Walters, Anson, and Ivy, 1931), and no effect on guinea pigs (Eckert, Probstein and Galinson, 1937). The report that follows is confined to the results obtained with an x-ray dose of 1000 r. Work in progress at other dose levels will appear in future reports.

Method: Male and female albino rats were used. They varied in weight from about 200 to 300 grams. A total of 53 animals were used, of which 11 were controls. The control rats were handled the same as the experimental rats except that they were not irradiated.

The x-irradiation was carried out at 15 ma, 250 K.V. with an aluminum plus copper filter such that the half-value layer was 2.15 mm of copper. The tube distance was 38 cm. The rate of irradiation was 42 r/min. The animal was shielded with lead except for an area about 2.5 cm. in diameter directly above the thyroid.

Measurements with an r-chamber were made which showed little or no scattered radiation to other parts of the rat's body. During the irradiation the rats were anesthetized with nembutal.

The rats were fed on a synthetic iodine deficient diet (less than 1 μg I per gram diet), and maintained on this diet for at least 18 days before sacrifice. With the object of increasing the preliminary iodine depletion of the gland, four days before sacrifice one-half unit of thyrotropic hormone was injected intraperitoneally, and equal doses were given on the two succeeding days allowing one day of rest before sacrifice. Later information showed that this dose of thyrotropin was too small to be effective, but in order to keep conditions the same, the above procedure continued throughout the experiment.

On the day of sacrifice, a small dose of I^{131} (3.5 to 8 μc) was injected into the tail vein. Four hours later the animals were sacrificed and the thyroid glands dissected out. Samples of blood and muscle were also collected for analysis.

Analyses were made by homogenizing the tissue (muscle as well as thyroid) in a blender with the addition of a few ml of KI containing sol. at pH 12 - 13. This was made up to volume and a suitable small aliquot was deposited as small droplets on a silver foil and evaporated to dryness. The beta emission of the sample foil was then counted in a calibrated G-M counter.

A further aliquot of the homogenized thyroid as well as the blood was dialyzed in a continuous flow apparatus and counts were made on this dialysate and the undialysable material.

Results: The results of the thyroid uptake are presented in Table I (Page 110). It was further found that at four hours after administration of the iodine dose, the I^{131} in the thyroid was more than 95 per cent in the bound form, (i.e., non-dialyzable). The contrary was true with regard to the blood

TABLE I

Effect of 1000 r of X-radiation on I^{131} Uptake of Thyroid at Various Times Post-Radiation

Days after Irradiation	No. Rats	Dose	% Dose in Thyroid
(Controls)	11	3.5 - 7.0 μc	17.1 ± 6.0*
5	5	7.0 μc	24.0 ± 8.7
7	7	7.0 μc	14.4 ± 4.0
9	6	7.0 μc	12.3 ± 7.0
11 - 12	7	7.0 μc	16.4 ± 10.9
16	5	7.1 μc	20.3 ± 7.3
20 - 25	7	7.0 μc	11.8 ± 4.4
35	2	7.0 μc	13.9 ± .1
51	2	7.0 μc	12.8 ± 2.2

*The errors calculated are standard deviations.

which showed more than 95 per cent of the iodine 131 in the inorganic or dialyzable form.

Discussion: Early results in this experiment seemed to show a decrease in I^{131} uptake at about seven days with a compensatory increased uptake at about sixteen days. On the basis of the more complete data available now and in view of the large variation between individual experiments as shown by the large standard deviations, it appears that 1000 r of local x-irradiation has little or no effect on the uptake of I^{131} by the thyroid gland of the rat. Further work at higher x-ray dose levels is planned.

PROGRAM O.S.

OUTSIDE SERVICE

Problem Code: None

Section Code: 3310

1. (a) 1176 industrial film badges from Brookhaven, Columbia, California, and the University of Rochester Physics Department.

(b) 144 radon atomspheric analyses (Dr. Hursh)

(c) 25 uranium dust analyses (Dr. Smith)

(d) 567 beryllium dust analyses (Dr. Steadman)

(e) 171 fingerprint analyses (Dr. Harvey and Mr. Hay)

(f) 4 beryllium soil analyses (Dr. Steadman)

(g) 8 uranium liquid analyses (Dr. Steadman)

(h) 26 fluoride liquid analyses (Dr. Smith)

(i) 17 SO_4 liquid analyses (Dr. Thompson)

(j) 19 miscellaneous (tissues) Be (Dr. Steadman)

(k) 124 urine samples of which:

16 analyzed for Be (Dr. Steadman)
108 analyzed for U (Dr. Smith)
70 analyzed for F (Dr. Smith)

2. Receiving, allocating of samples for analysis, tabulating and reporting of results, as well as providing sample containers for the above samples was performed by this section.

PROGRAM H.P.

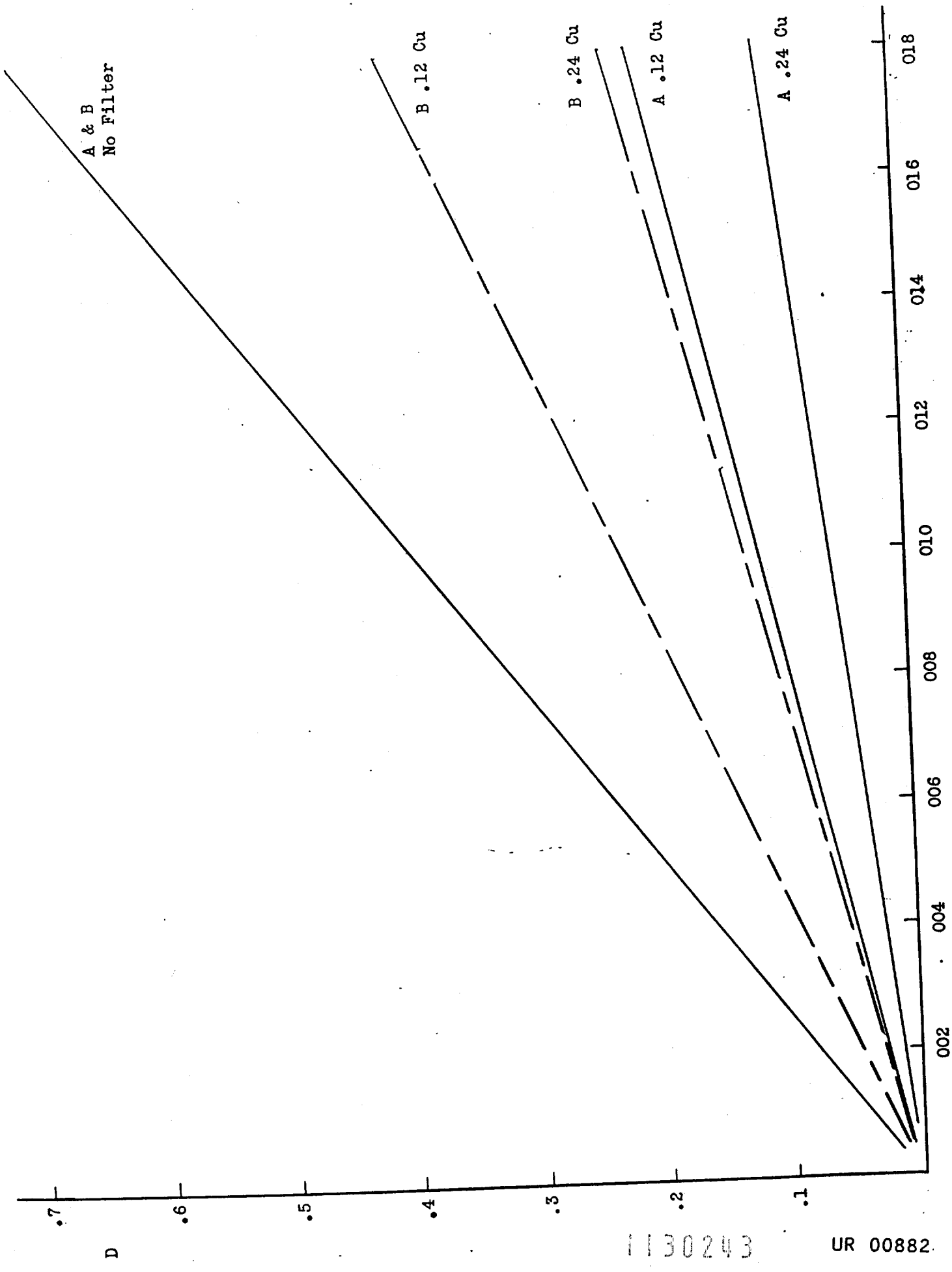
HEALTH PHYSICS

Problem Code: H.P.1 (Research and Development)

Section Code: 3320

Background: In order to test the method outlined in the last Quarterly Technical Report, concerning measurements of scattered x-ray radiation, systematic investigation was carried out in cooperation with 41 employees (physicians, technicians, and clerical personnel) in the Department of Radiology of The University of Rochester School of Medicine and Dentistry.

Methods: Special film badges were distributed weekly which were carried by the personnel during each working day. The badges contained a dental packaged EK type K film equipped with a dual filter of .12 and .24 mm copper, leaving three separate areas for density measurements. Films were developed each week rather than every two weeks, since it was desired to find the accuracy of the lower sensitivity limit for this type of film. Densities versus radiation intensities were plotted and the graph reveals linear relation between these quantities in a density range of 0-.7 for the three regions on the films, (a) direct exposure, (b) exposure through .12 mm Cu, and (c) exposure through .24 mm Cu. This graph is shown on Figure I (Page 114). On this figure, the density values recorded on the ordinate represent densities from which the fog density has been subtracted. Two families of lines are shown marked "A" and "B" and these represent characteristic curves of the film when exposed to 100 and 200 KV x-rays, respectively. From such families, it was possible to differentiate grossly the radiation quality of the monitoring films. This is



accomplished by the fact that three densities found on monitoring film will lie on a line parallel to the ordinate if this film was exposed to a quality of radiation similar to that of a characteristic family of curves. (See Rochester Report UR-38).

Table I (Page 116) shows the accumulated dosages of the exposed personnel. From this table it is seen that the quantities of radiation received have not approached the tolerance of .1 r per 8 working hours.

Since the values recorded in Table I constitute only the results of film density measurements in terms of radiation received, and since many of these values appear unduly low, a spot check of the values was made with calibrated ionization chambers in form of pencil chambers during a period of two weeks. These chambers were worn simultaneously with the film badges and their readings recorded daily. A further check was made by means of integrating proteximeters located at fixed positions and comparison made with film density readings from film at the same positions. The pencil chambers and the proteximeter were investigated for wavelength dependence and after that were calibrated by means of the standard r chamber. These calibrations were performed for 100 and 200 KV unfiltered x-radiation.

Illustrative data are given in the Tables II and III (Page 117). As seen from Table II, the pencil chamber readings were made daily and summated at the end of the six working days. The fair comparison of r values as read by ionization chamber and film badge seem to indicate the validity of the method. Measurements with the proteximeter were also made daily. However, due to the position of the instruments within the exposure rooms, (6 feet from scattering center) the quantities recorded are greater than those of operating personnel.

TABLE I

Personnel Number	Number of Weeks	Total "r"	Max. "r" received in a week	"r" per week	KV
1	20	.485	.300	.024	100
2	9	.105	.030	.011	100
3	18	.160	.060	.009	200
4	17	.105	.015	.006	200
5	17	1.455	.090	.085	100
6	14	.265	.045	.019	100
7	16	.500	.090	.031	100
8	16	.060	.020	.004	100
9	13	.385	.160	.030	200
10	16	.290	.015	.018	200
11	12	.045	.020	.004	100
12	19	.305	.090	.016	100
13	18	.280	.110	.015	100
14	6	.175	.050	.029	100
15	4	.000	.000	.000	
16	12	.000	.000	.000	
17	3	.010	.010	.003	100
18	19	.195	.045	.010	100
19	6	.245	.100	.041	100
20	19	.280	.095	.015	100
21	19	.395	.095	.021	100
22	20	.125	.035	.006	100
23	14	.195	.045	.014	100
24	19	2.345	.400	.124	100
25	6	.005	.005	.001	100
26	14	.055	.025	.004	100
27	3	.095	.085	.031	100
28	14	.050	.025	.004	100
29	13	.035	.020	.003	100
30	18	.035	.040	.002	100
31	21	.225	.045	.010	100
32	7	.005	.005	.001	100
33	19	.160	.035	.008	100
34	14	.045	.020	.003	100
35	19	.680	.320	.036	100
36	16	.075	.010	.005	100
37	7	.105	.060	.015	100
38	11	.010	.005	.001	100
39	11	.000	.000	.000	
40	15	.175	.060	.012	100
41	11	.080	.030	.007	100

TABLE II

Days Worn	Pencil Chamber "r"	Film "r"
1	.003	
2	.005	
3	.010	
4	.007	
5	.003	
6	.006	
1 week	.034	.031
1	.008	
2	.023	
3	.000(not worn)	
4	.012	
5	.031	
6	.005	
1 week	.079	.085
1	.011	
2	.007	
3	.007	
4	.003	
5	.010	
6	.002	
1 week	.040	.041

TABLE III

Proteximeter "r"	Film "r"
.026	.023
.030	.028
.010	.013
.065	.070

PROGRAM C.S.

SPECIAL CLINICAL SERVICES

Problem Code: None

Section Code: 3312

Experimental results reported previously had shown failure of one patient with chronic beryllium poisoning to retain significant amounts of nitrogen under the influence of testosterone propionate. One possible explanation for this failure to store nitrogen is that hepatic disease which may be associated with beryllium poisoning prevented the acceleration in synthesis of body protein usually brought about by testosterone propionate. For this reason a patient with early cirrhosis of the liver was given testosterone propionate and the nitrogen, calcium and phosphorous balances were determined. The purpose of the experiment was to determine whether or not liver disease of a mild degree would prevent the protein anabolic effect of testosterone.

The patient, E.M., was a 46 year old white female with a history of chronic alcoholism of six years' duration. She had been admitted to the hospital on three occasions during the past six years, primarily because of peripheral neuritis. On all occasions the liver was felt to be enlarged and evidence of a mild active hepatitis was present. At the time of the present study the patient was not jaundiced but had a 1 - 2+ cephalin flocculation, a thymol turbidity ranging from 18 - 34, and a bromsulphalein retention of 5 - 10 per cent. The electrophoretic pattern of the plasma proteins was considered typical of cirrhosis and was partitioned as follows:

Albumin	3.21
α 1	1.47
α 2	.70
B	.96
ϕ	.89
γ	1.05
	7.28

The patient was considered to have a cirrhosis of mild degree. She was placed on a diet containing approximately 2500 calories, containing a daily intake of 10.50 gms. of nitrogen. The nitrogen balance data are tabulated in Table I (Page 121).

One should note that the first nine days, (Periods 1 and 2), are control periods. In Periods 3 and 4, 25 mg of testosterone propionate were given daily. Periods 5 and 6 are post-control periods.

From the data in the table one notes a good storage of nitrogen during the control period and under the influence of testosterone propionate a large increase in storage occurred. This should be considered a normal protein anabolic effect of testosterone propionate and it may be concluded that liver disease of the type which this patient had did not inhibit the above effect.

Of interest were the serial electrophoretic patterns which follow:

8-9		8-19		8-30	
Albumin	3.21	Albumin	3.09	Albumin	2.68
α 1	1.47	α 1	.45	α 1	.38
α 2	.70	α 2	.67	α 2	.67
B	.96	B	.90	B	.89
ϕ	.89	ϕ	.72	ϕ	.65
γ	1.05	γ	1.05	γ	.82
	7.28		6.88		6.09

A comparison of these patterns taken at approximately ten-day intervals reveals several rather unusual findings. It is apparent that the albumin and gamma globulin progressively decreased throughout the experimental period. This finding is somewhat unexpected in view of the large storage of nitrogen. According to the figures of Allison and others, approximately 1 gm of serum protein should be stored when 30 gms of tissue protein are stored. One explanation is that the effect is one of simple dilution since it is known that testosterone propionate causes retention of sodium and chloride. However, other possibilities need to be considered. The decrease of gamma globulin may have been due to improved hepatic function resulting from the dietary regimen. The findings in regard to the serum albumin may mean that while she was unable to manufacture serum albumin in a normal manner she was still able to accelerate syntheses of tissue protein. Needless to say, much additional information is required to establish whether the data obtained in this patient are significant and if so what the explanation may be.

The calcium and phosphorus balances done on this patient will be given in a subsequent report.

TABLE I

Nitrogen

Period	Date	Diet	Urine	Stool	Balance
1	8/9-10	10.50	6.55	1.26	+2.69
	10-11	10.50	6.50	1.26	+2.74
	11-12	10.50	5.89	1.26	+3.35
	12-13	10.50	6.47	1.26	+2.77
	13-14	10.50	6.97	1.26	+2.27
	14-15	10.50	5.77	1.26	+3.47
					+17.29
2	15-16	10.50	6.46	1.26	+2.78
	16-17	10.50	6.39	1.26	+2.85
	17-18	10.50	7.05	1.26	+2.19
					+7.82
3	18-19	10.50	6.43	1.26	+2.81
	19-20	10.50	5.25	1.26	+3.99
	20-21	10.50	4.69	1.26	+4.55
	21-22	10.50	4.46	1.26	+4.78
	22-23	10.50	4.51	1.26	+4.73
	23-24	10.50	3.92	1.26	+5.32
					+26.18
4	24-25	10.50	lost	1.26	+5.44?
	25-26	10.50	3.69	1.26	+5.55
	26-27	10.50	3.52	1.26	+5.72
	27-28	10.50	2.75	1.26	+6.49
	28-29	10.50	3.87	1.26	+5.37
	29-30	10.50	4.35	1.26	+4.89
					+33.46
5	30-31	10.50	3.56	1.26	+5.72
	31-1	10.50	4.66	1.26	+4.58
	9/1 -2	10.50	lost	1.26	+4.44?
	2-3	10.50	4.94	1.26	+4.30
	3-4	10.50	6.01	1.26	+3.23
	4-5	10.50	6.14	1.26	+3.10
					+25.37
6	5-6	10.50	7.14	1.26	+2.10
	6-7	10.50	8.01	1.26	+1.23
	7-8	10.50	7.84	1.26	+1.40
	8-9	10.50	8.62	1.26	+ .62
	9-10	10.50	8.44	1.26	+ .80
	10-11	10.50	7.39	1.26	+1.85
					+8.00

PROGRAM I.N.

INSTRUMENTATION (SPECTROSCOPY, ELECTRON MICROSCOPY, X-RAY AND NUCLEAR RADIATION DETECTORS, X-RAY DIFFRACTION, ELECTRONICS)

Problem Code: I.N.1 (Research and Development)

Section Code: 3161

Preparation of a Rugged Film for Mounting Materials for Electron Microscopy:

Background: There has been routine and sometimes special examination of dusts, powders, and other materials submitted for electron microscopy. Considerable work was done with beryllium oxide, and this is being recorded in a separate paper under the probable title of "Toxicity Studies of Beryllium Oxide of Various Grades".

It has always been obvious that the specimen supporting films of collodion, Formvar, etc., which are almost universally used, leave much to be desired when their instability in the electron beam is considered. Formvar films often contain many small holes and when sterilization of the film-covered screens is essential, as in the culture of living cells on such films, heat or chemical sterilization procedures are fairly destructive.

Silica (SiO_2) is often used in the production of replicas in electron microscopy. It is extremely stable under electron bombardment and will withstand high temperatures and appears to be structureless in the electron microscope.

It seemed to be desirable to attempt to devise a method whereby silica films could be produced on a large scale and handled with ease with which collodion films on water can be manipulated.

Method: The technique of preparing collodion films has been discussed in a previous report, but a brief review will be given here. A drop of a

2 per cent solution of Parlodion in amyl acetate is allowed to fall on the surface of clean distilled water in a suitable container. When the solvent evaporates after a few seconds, the film is either picked up on a specimen screen or the water is withdrawn and the film allowed to settle on the specimen screens, and after drying is used in the usual manner. It was hoped that silica films could be prepared on the surface of the liquid and thereafter handled by the methods used for collodion, Formvar, etc. Silica films of suitable thickness can be produced by vacuum evaporation from a heated filament and will deposit on surfaces prepared for it. Accordingly, a dish of mercury was placed in the vacuum chamber and the silica film deposited on it. Although the films were excellent in every other respect, it was found that there was contamination of the specimen screen by the mercury and this method had to be discarded. Octoil was substituted for mercury but it was difficult to remove traces of the oil from the specimen screen afterwards. It was finally decided to try to use collodion film as a support for the silica during its formation and subsequently dissolve the collodion leaving the silica. This method has proved to be very successful.

A much more dilute solution of collodion is employed than is necessary for the usual specimen support. The very thin film formed is much too light to support ordinary specimens but is useful to serve as a base for the deposition of silica. When the composite film is examined in the electron microscope, it is found that it will withstand any available beam intensity with little damage, intensities that would instantly destroy the ordinary collodion or Formvar film and moreover the very thin collodion component is invisible or difficult to detect. The double film is, therefore, often used without any further treatment.

The collodion can be removed, however, by suitable solvents. These films can be sterilized in the autoclave, by dry heat or other methods, making them very suitable for use in tissue culture work. A dozen or more screens may be coated at one time on a single glass slide so that large numbers can be prepared with ease. The permanence of the film and the relative simplicity of the method makes it possible to produce a larger number of usable specimen mounts in a given period of time than the older technique with collodion alone.