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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 but which are not sufficiently complete to be written up as final reports.

Submitted by: Henry A. Blair
Director

Date of Report: 2/26/53

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CENTRIFUGE TYPE OF ULTRAFILTRATION APPARATUS

by
T. Y. Toribara

ABSTRACT

A one-piece all-glass centrifuge-type ultrafiltration apparatus has been designed. This equipment has been in continuous use for over three years and has proven to be especially valuable for the study of the clearance of various ions in biological work.

* * * * *

In the study of the clearance of various ions in biological work, ultrafiltration is a very valuable technique. Feldman et al., (1) have described an all-glass centrifuge type of ultrafiltration apparatus which eliminated a number of undesirable features of other types (2,3,4.5). The apparatus described here maintains all the desirable features of the all-glass apparatus, and in addition its one-piece construction makes it simpler to use.

Apparatus: One end of a borosilicate glass straight sealing tube with coarse fritted disc (Corning No. 39570 - 25 mm. diameter with 20 mm. disc) is sealed off and a 6 mm. glass tube is sealed on at an angle near the disk (Figure 1 A). This tube serves as the outlet for the removal of the filtrate and as a means of maintaining the same atmosphere above and below the fritted disk when connected to the glass tube at the top with tygon tubing during centrifugation. The rubber stopper to fit on top should be cut out with a shoulder as shown. The apparatus is centered in the largest cup (Cat. No. 373) for an International centrifuge, size 2 by use of the ring shown in Figure 1 B. This consists of a brass ring made from sheet brass with the proper size wooden blocks attached to keep the ring in place.

The solution to be filtered is placed in a piece of Visking seamless cellophane dialyzing tubing as has been previously described (1). It was found most convenient to place in the apparatus the previously soaked tubing doubled so that both open ends are on top. After filling the tube, the open ends are tied or doubled and closed by means of rubber bands. The filtered solution is withdrawn through the sidearm by means of a pipet with the tip drawn out to fit.

This apparatus is in regular use for filtering dog blood plasma at speeds up to 2000 r.p.m. In actual operation, the material collected during the first 15 minutes is discarded — this eliminates any dilution which might be caused by surface moisture on the cellophane. The size shown will handle conveniently up to 20 ml. input, and for larger quantities a larger size sealing tube may be constructed in the same manner. If an apparatus very much larger than the one shown is desired, it would be preferable to start with a fritted disc funnel (Corning No. 36060) which is made of much heavier glass. In the latter case, a bulb is sealed onto the stem which is thickened and enlarged for strength. This apparatus is also used for drying precipitates by centrifugation.

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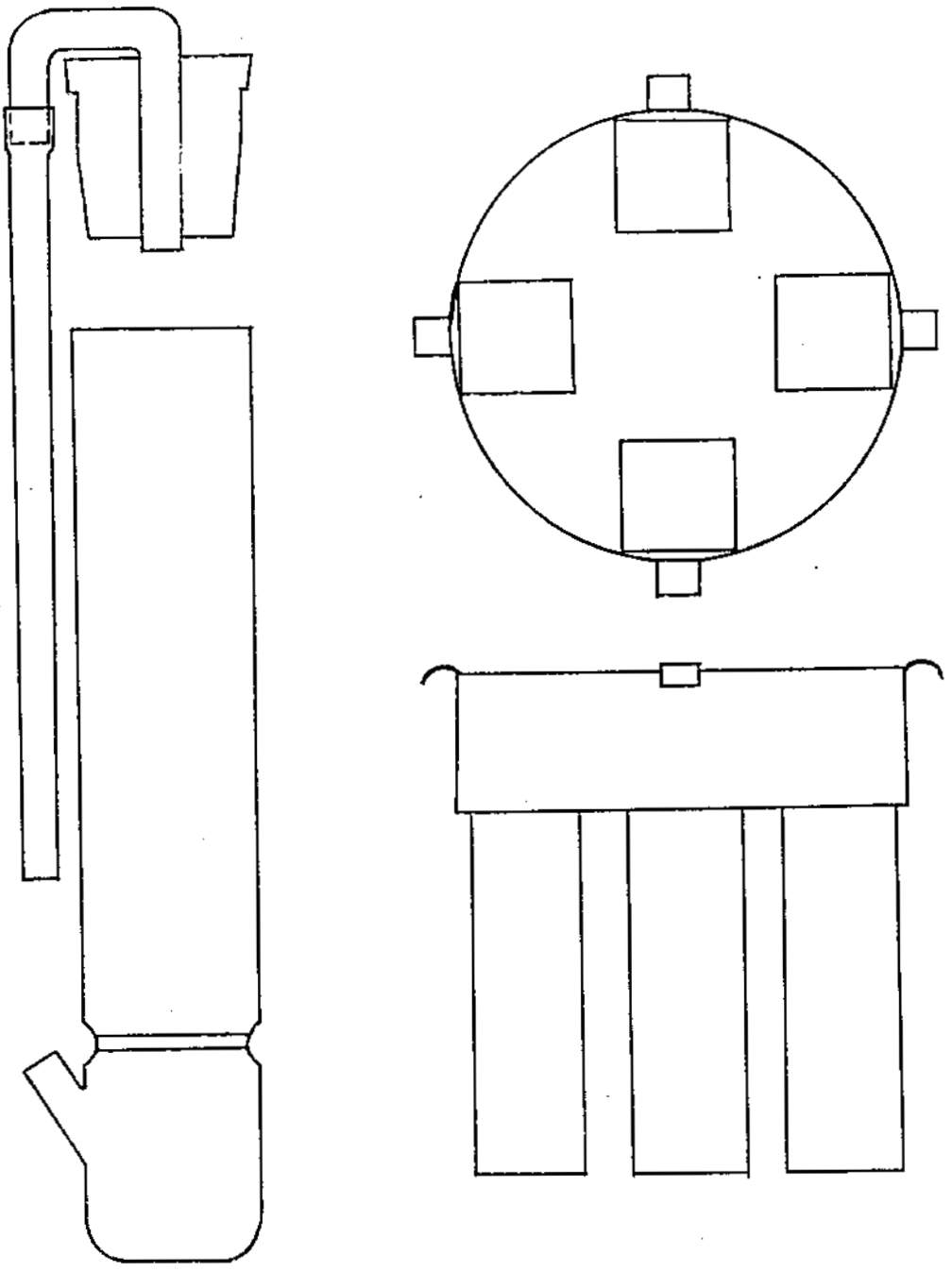

Figure 1. (A) Filtration apparatus made from straight sealing tube, 25 mm. diameter with 20 mm. fritted disc.

(B) Top and side views of centering device.

A PARTIAL EVALUATION OF THE HAZARD FROM RADON AND ITS DEGRADATION PRODUCTS *

by

Jacob Shapiro and William F. Bale

ABSTRACT

Radon in the air is accompanied by radioactive daughter substances of a few minutes half life, usually attached to dust particles. Experiments have been performed in which rats were exposed to radon and these accompanying daughter products. Results indicate that most of the radiation dose to the lungs is produced by these daughter products, removed by the lungs as particulates from the air, rather than by gaseous radon and the degradation products of the radon molecules decaying in the lungs. Implications of these results in deciding upon maximum permissible levels from radon in the air of mines and industrial plants are discussed.

* * * * *

The existence of radon has been known for more than fifty years. We have realized only recently, however, that the major exposure of human beings to radioactivity today may be from exposure to radon and its degradation products. This exposure is predominantly to miners in unventilated uranium mines or mines that penetrate uranium and radium bearing rocks. Investigations of exposure levels have been conducted jointly by the United States Public Health Service and the Colorado State Department of Public Health.

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* This paper was given December 30, 1952 at the St. Louis meeting of the A.A.A.S as a part of a symposium on radiation hazards.

It should not necessarily be assumed that radon levels in the mines are high enough to be injurious to the health of exposed miners. We do not know of any evidence that injury to American miners has occurred. The levels are high enough, however, if measured in terms of acceptable levels in other parts of the atomic energy industry, to warrant further investigation.

Data on which present Maximum Permissible Concentration Levels are based.

There is undeniable evidence that among miners of the Schneeberg and Joachimsthal regions of central Europe there existed in the early 20th century a very high incidence of cancer of the lung. It was probably one of the major causes of death to miners of this area. These mines were also, in general, regions of high radon concentration. Today this high rate of cancer is generally attributed to radon in excessive amounts. The available data is probably not good enough, however, to exclude completely the importance of other carcinogenic agents or to demonstrate conclusively that radon is the primary cause.

According to Robley D. Evans a summary of published data indicated a value of 2.9×10^{-9} curies radon per liter as an average for measurements made on these mines (1). The measurements are given in Fig. 1. The median value of published measurements was 1.8×10^{-9} curies per liter. It seems to be largely on the recommendation of Dr. Evans that a value of 10^{-11} curies radon per liter has been widely accepted in the radium dial painting industry as the maximum permissible concentration level for radon. This is about 1/100 the concentration Dr. Evans considered common in the mines. Thus this maximum permissible concentration level is based primarily upon human exposure data and depends for its validity upon the accuracy of the

radon levels as reported for these European mines and on the assumption that these lung cancers were in fact radon produced. It may be pointed out that mine radon values were accumulated at the time a high incidence of lung cancer was recognized and may not, even if accurate in themselves, be representative of the effective concentrations encountered by the miners over the many years of exposure presumably necessary to produce the disease.

Figure 1

Radon Content of Air Samples From Mines in Saxony and Bohemia

Mine	No. of Samples	Radon Concentrations per Liter of Air: Range 10^{-9} curies	Average 10^{-9} curies
1	9	0.6 to 3.2	1.9
2	5	0.4 to 18	5.5
3	8	1.2 to 2.2	1.6
4	6	2.0 to 8.7	5.6
5	7	0.7 to 1.8	1.1
6	6	0.3 to 1.8	0.8
7	6	2.3 to 8.8	5.0
		Average	2.9

Data taken from Evans (1).

Calculations of radiation exposure from radon and its degradation products.

Several investigators have in the past made calculations of the dosage delivered to the lung by the alpha particles from radon itself and from the degradation products of the radon molecules decaying in the lung.

On the basis of one type of calculation (1) published by Evans, for example, a concentration of 10^{-9} curies/liter of radon breathed 40 hours per week would lead to a dosage, averaged over the lung, of 5.03 mrep/week or, on the bases of a 1 to 20 ratio of rep to rem, 100.6 mrem. This is 34% of a widely used tolerance figure for human tissues of 300 mrem/week.

Other calculations assume that the important radiation dose is to cells lining the bronchial passageways. On the basis of a calculation of this type (2) the average dosage for a forty hour week at a level of 10^{-9} curies/liter is 172 mrem or 57% of tolerance.

Radiation dosage from the radioactive daughter products of radon.

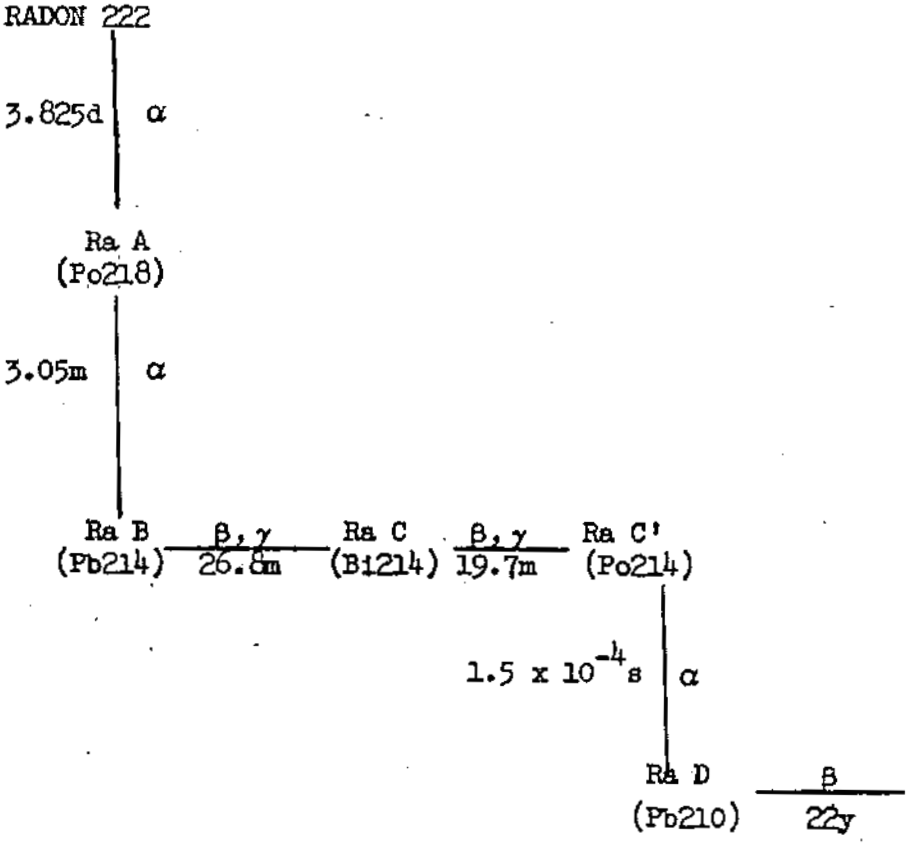
It was recognized recently, perhaps first by one of the authors of this paper, that the radiation dosage to the lung produced by the short lived degradation products of radon may be many times more important than the dosage from radon itself. These degradation products commonly remain in the air in considerable concentrations, usually attached to floating microscopic dust particles and are drawn into the lungs along with radon. The radon is mostly breathed out on the next exhalation, but the dust particles on the contrary can be appreciably retained in the lung. Thus in time the degradation products can reach in the lung levels of radioactivity far exceeding that of radon itself.

Figure 2 shows the decay scheme of radon and its daughters. Other radiations are negligible in their effect as compared with the alpha rays. We note that up to the 22 year half life radio-lead there are three alpha emitters, radon itself, its immediate daughter Radium A with a 3 minute half life, and finally Ra C'. Radium A decays to Ra B, a beta-gamma emitter with a half life of 26.8 minutes, Ra B to Ra C with half life of

19.7 minutes which in turn finally decays to Ra C'. Ra C' emits an alpha ray almost instantaneously; its half life is about 1.5×10^{-4} seconds.

Figure 2

Decay Scheme of Radon



These daughter substances, no longer chemically inert gases like their mother substance, radon, seem normally to be deposited very largely upon dusts and other matter present in the atmosphere where they are produced.

John Harley of the New York Operations Office of the Atomic Energy Commission, reports in a yet unpublished thesis (3) that in an exposure chamber, essentially a small airtight room, the concentration of these degradation products in the air varies in the range of 10 to 75 percent of

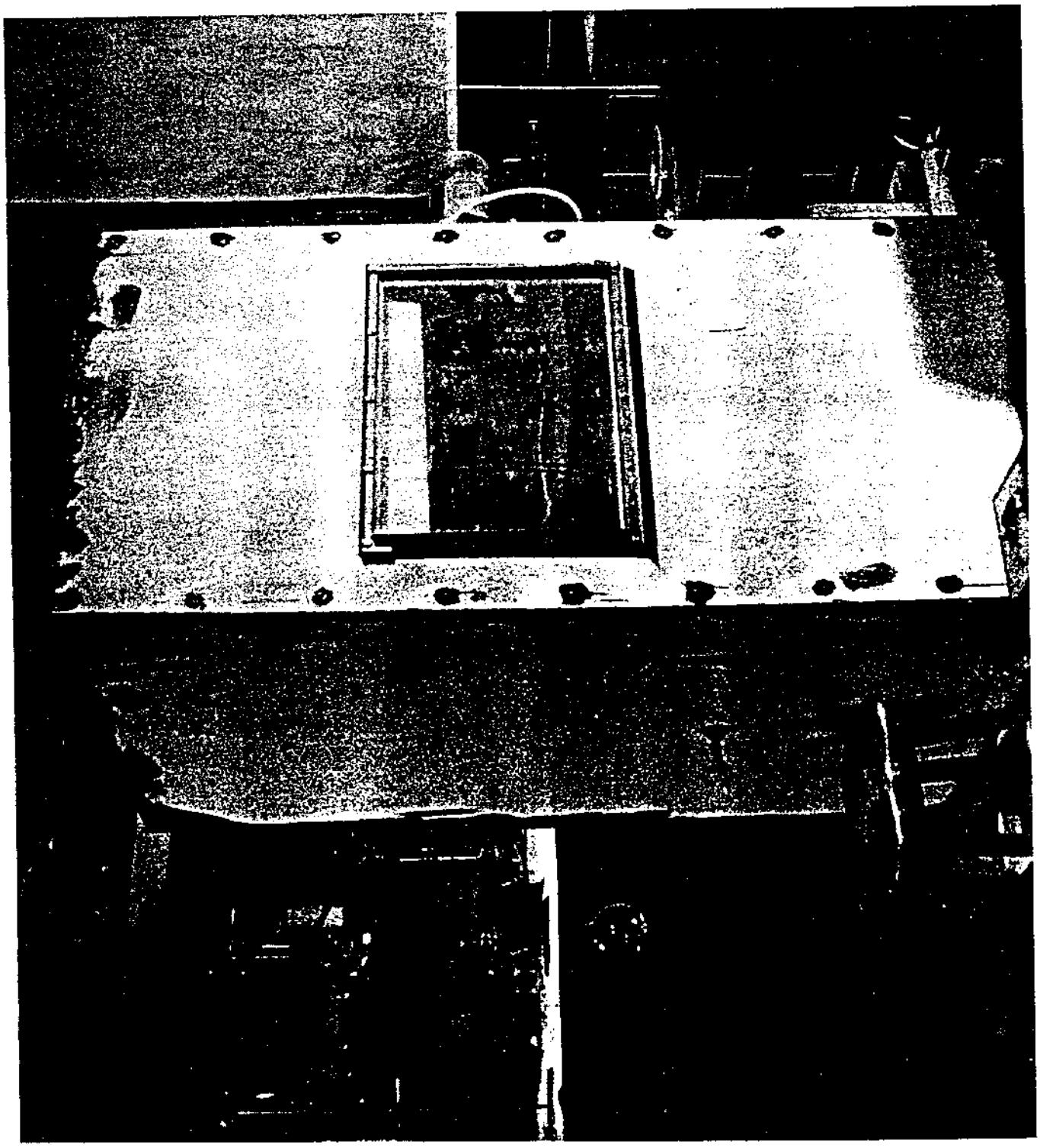
the equilibrium value. Experiments he reports indicate to him that the human respiratory tract retains about 50% of such air-borne degradation products.

We have recently carried out experiments in which rats have been exposed to radon in an exposure chamber about 4 by 3 by 6 feet. Our data indicates that at a given radon concentration the radiation exposure of the rat lungs was dependent upon how thoroughly the air had been cleaned of dust.

Figure 3 is a photograph of our exposure chamber. In one set of experiments radon was introduced with no precautions to remove dust normally present in the air in the chamber. Rats were exposed for 2-1/2 hour periods at intervals from a few hours up to several days after the radon was put in. The chamber atmosphere was not artificially agitated. At the end of the exposure period the radon concentration and concentration of degradation products that were removable by a millipore filter were determined. Radon was measured with a vibrating reed electrometer, disintegration products by an alpha scintillation counter and a well type gamma scintillation counter following the design of Anger (4).

On removal from the exposure chamber the rats were anesthetized with ether and the lungs rapidly removed. Gamma counts were made using the well type counter starting about 20 minutes after the animal was removed from the chamber.

Earlier calibration experiments using radium, Fe^{59} and I^{131} indicated a counter efficiency of about 10% for the geometry of these experiments for a gamma ray spectrum similar to that from Radium B and C in equilibrium with radon. Decay curves indicated an effective half life of about 35 minutes for this gamma activity in reasonable agreement with theoretical calculations. Based on these assumptions concerning counter efficiency and radio-



active decay rate, the experimental data in gamma quanta per minute is given in the second column of Figure 4, corrected to the time of removal of the rat from the chamber.

Figure 4

Results of Rat Radon Inhalation Experiments

Atmospheric Dust Not Removed

	Rat Wt. gms.	Activity of lungs, gamma quanta/min.	Radon Conc. curies/liter	Ra C' Conc. curies/liter
1	188.6	20,650	24.0×10^{-9}	13.6×10^{-9}
2	233.2	66,500	23.3	10.6
3	196.9	20,600	18.7	8.7
4	209.6	15,150	18.3	7.9
5	189.7	13,020	15.6	4.4
6	177.0	11,030	14.7	4.4

Note: Experiments performed over a period of three days.

The third column gives the radon concentration in the chamber air as measured with a vibrating reed electrometer. The fourth column gives the concentrations of Ra C' in the chamber just before the rat was removed as obtained by an alpha count on the dust removed by a millipore paper as a known volume of chamber air was pulled through it.

From calibration experiments we know with reasonable accuracy the relation between lung gamma activity and the total dose in ergs to the lung from Ra C' alpha particles derived from retained degradation products. We can also estimate the alpha exposure produced by radon itself plus the products of the radon actually decaying in the lungs on the assumption

of complete retention of these degradation products. These results are shown in Figure 5. We can also compute the average dose to the lung tissue from both these sources of radiation providing we know the weight and volume of our rats' lungs.

Figure 5

Ergs Per Hour Imparted to Rat Lungs

Atmospheric Dust Not Removed

Rat	Dosage (1) from Radon	Dosage from (2) Airborne Daughters	Ratio
1	0.785	8.22	10.48
2	0.945	26.50	28.10
3	0.638	8.20	12.85
4	0.666	6.03	9.06
5	0.514	5.18	10.10
6	0.452	4.38	9.72

(1) This includes the dosage from daughters of radon atoms decaying in the lung on the assumption that they are retained and undergo radioactive decay there.

(2) This dosage is computed on the assumption that the gamma activity of column 2 Figure 4 is due to airborne daughters of radon retained by the rat lung.

Following Stokinger et al (5) we assume this weight to be 0.0043 times the body weight. The rat lung volume was computed assuming it to be six times the tidal volume calculated in cc as 0.0074 times the rat weight in grams by the formula of Guyton (6). Figure 6 shows the results of such computations for animals of the first series. It is interesting to note that if we extrapolate these results to a rat exposed

40 hours per week to a concentration of 10^{-9} curies radon per liter the disintegration product dose is 4657 mrem. At a level of 10^{-11} for 168 hours, it is 196 mrem per week.

Figure 6

Dosage to Rat Lungs For Radon Concentration of 10^{-9} C/L

Atmospheric Dust Not Removed

Rat	Dosage from Radon (1) MREP/hr x 20	Dosage from Daughters (2) MREP/hr x 20
1	8.68	90.8
2	8.75	245.0
3	8.65	111.2
4	8.74	79.1
5	8.71	88.0
6	8.68	84.5

(1) See note 1, Fig. 5
(2) See note 2, Fig. 5

We have also conducted experiments in which we endeavored to make our exposure chamber as dust free as possible before animals were exposed. This was carried out by lining the chamber with glycerine coated glossy paper and blowing in air through a millipore filter. Figure 7 shows the results of this experiment. You will note that the radioactivity of rat lungs was dramatically reduced. The rat which was receiving 4657 mrem per week in a normal atmosphere now receives at the most, 86 mrem/ week from the air-borne daughters.

Figure 7

Dosage to Rat Lungs For Radon Concentration of 10^{-9} C/L

Cleaned Air Used

(a) Air Still

Rat	Dosage from Radon (1) MREP/hr x 20	Dosage from Daughters (2) MREP/hr x 20
7	8.65	16.1
8	8.60	15.1
9	8.62	15.4
(b) Air Stirred with Fan		
10	8.72	2.38
11	8.74	1.73
12	8.70	2.45

(1). See note 1, Figure 5.
(2) See note 2, Figure 5. The measured activity in rats 10, 11, and 12 may in fact be due almost entirely to the daughters of radon decaying in the rat lungs.

The results of these rat experiments do tend to confirm the general idea that it may well be air-borne degradation products of radon, rather than radon itself, that represent the main hazard to humans associated with radon in the air.

It is interesting to see what, if anything at all, we can deduce from the results of these rat experiments as to the maximum permissible level of radon and its daughters in air breathed by human subjects. We note that figures obtained on rats in the first group breathing atmospheric air which has not been cleaned show that at a level of 1.53×10^{-11} curies/liter of radon, the average dose to the lungs would be 300 mrem per week with continuous exposure.

There are several important qualifications that must be made in considering this value as a tolerance figure, even for rats. Two of these are:

1. The actual pattern of deposition of radioactive material may be such that the dosage delivered is very non-uniform. In particular this means that some areas or tissues of the lungs may receive far higher doses than the average calculated above.

2. In our experiments, about 10% of the suspended daughter products were deposited in the lungs. This may be characteristic of our experimental setup rather than typical of all conditions that might commonly exist. One can easily suppose that retention might run as high as 50% under some conditions.

When we endeavor to extrapolate to human subjects additional uncertainties become evident. The percentage retention in the human respiratory system may be higher than in the rat lung. As noted earlier, Dr. Harley found a value of about 50% on human subjects. A difference may conceivably exist between rats and men for small particles deposited largely by Brownian movement caused by the higher respiratory rate in the rat allowing a shorter time for lung deposition. The ratio of lung weight to ventilation rate may also be different in rat and man. Some of these points are the subject of further investigation in our laboratory.

It will probably be possible by means of radioautography and other techniques to map out the distribution of deposited radioactivity in rats and larger animals. Work is also starting at Rochester on the determination of the lethal dose of radon in animals with a proper evaluation of the contribution of airborne degradation products to this effect. Surveys and evaluations of actual human exposures in mines and manufacturing plants

are being carried forward by the United States Public Health Service and the New York Operations Office of the Atomic Energy Commission. The Naval Radiological Defense Laboratory in San Francisco is also conducting interesting work in this general field.

Finally we may note that radon eventually decays to a 22 year half life radio-lead, which in turn decays to 120 day half life polonium. Since lead can be stored in bones for long periods while polonium is excreted, it may turn out that polonium excretion in an individual's urine may be a useful measure of his overall integrated radon exposure. Preliminary measurements carried out by Miss Mary Sulzer and Dr. J. B. Hursh in our laboratory on urine specimens of uranium miners furnished by Dr. Duncan Holaday of United States Public Health Service indicate that in fact such polonium excretion is measurable and far higher than from unexposed individuals.

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PREPARATION OF CHROMIUM(VI) SOLUTION HAVING LOW IODINE BLANK FOR PBI DETERMINATION

by
W. B. Mason and S. Coco

ABSTRACT

Chromium(VI) solutions which are essentially free of iodine (less than 1×10^{-9} g. I per g. Cr) can be prepared by converting CrO_3 to CrO_2Cl_2. The crude chromyl chloride can be mixed with water and used directly with concentrated sulfuric acid for wet combustion of serum in the protein-bound iodine determination.

* * * * *

One of the annoying problems encountered in determination of serum protein-bound iodine (PBI) lies in obtaining reagents which are themselves sufficiently free of iodine. Three ml. of serum normally contain roughly 150×10^{-9} grams of protein-bound iodine. Reagents necessary to determine the PBI in this amount of serum, using a procedure employing dialysis, wet combustion with H_2SO_4 and Cr(VI), liberation of I with H_3PO_3, and estimation of I by its catalytic effect on the reaction between Ce(IV) and As(III), usually contain 30-50 $\times 10^{-9}$ grams of iodine. Most of this occurs in the chromium, of which 3-4 grams are required.

Both CrO_3 and $Na_2Cr_2O_7$ can be obtained having as little as 5×10^{-9} g. I per g. Cr, but the value is often much higher. Of various procedures tried for preparing Cr(VI) solutions which are essentially free of iodine, conversion to chromyl chloride has proved most satisfactory. Using the method recommended by Fernelius[1] for preparing crude chromyl chloride,

1. Inorganic synthesis, Vol. II, p 205. W. C. Fernelius, editor. McGraw Hill Book Co., Inc., 1946.

heavily contaminated CrO_3 containing 88 x 10^{-9} g. I per g. Cr. for example, was converted to CrO_2Cl_2 containing 1 x 10^{-9} g. I per g. Cr, with a yield of 66%. Studies utilizing I-131 (as NaI) added to the initial CrO_3 solution confirmed the efficiency of the separation by indicating at least a 50 fold purification.

The crude chromyl chloride can be mixed with water and used directly with concentrated sulfuric acid for wet combustion of serum in the protein-bound iodine determination.

VARIABLES AFFECTING DEGREE OF I^{131} BINDING IN IN VITRO IODINATION OF RABBIT SERUM PROTEINS

by

Irving Spar and William F. Bale

ABSTRACT

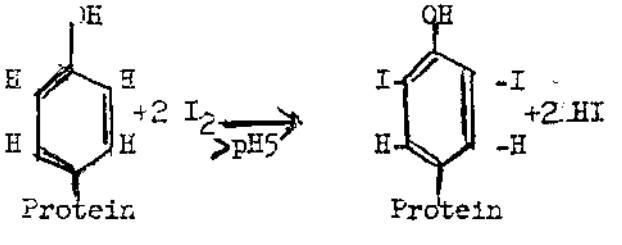
An experimental study was carried out in which the degree of iodination with I^{131} of rabbit serum proteins and gamma globulin was measured as a function of various experimental variables. The general procedure was to add the I^{131} as received from Oak Ridge to KI solution, oxidize to free I with $NaNO_2$ and HCl, and add this mixture to the protein in borate or phosphate buffer. Additional $NaNO_2$ is necessary to oxidize the $NaHSO_3$ added at Oak Ridge to keep I^{131} as iodide during shipment. Too great an excess of $NaNO_2$ greatly reduces I^{131} binding to proteins. Other variables studied were the amounts of other reactants and the effects of temperature, pH, and duration of reaction.

A procedure was developed for iodination at room temperature of 2 cc rabbit gamma globulin present in borate buffer, pH8, at a concentration of 10 mg protein per cc. To one cc of a 1 to 5 dilution of Oak Ridge I^{131} is added 1 drop 0.01 M KI, 5 drops 0.071 N $NaNO_2$, and 3 drops

Iodination of proteins with I^{131} is now a widely used technique for labelling proteins for biological experiments. Chemical iodination of proteins has been studied extensively (1,2). Numerous investigators have described methods they used to secure the degree of iodination with I^{131} necessary for their experiments. They have however reported little in the way of experimental studies as to conditions under which iodination does, or does not, take place, or as to variations in the degree of utilization of I^{131} with variations in experimental conditions. This is particularly true for the case in which a high concentration of I^{131} per unit weight of protein is a desirable objective. We are therefore reporting here experimental studies on this subject, not because they are exhaustive or complete, but simply because we hope other investigators may find our data a useful guide in setting up their own iodination procedures.

For tracer purposes a minimum denaturation or other alteration of the protein that would cause the iodinated protein to differ in its chemical and biological behavior from the uniodinated native protein is usually desirable. Various authors have reported that alterations of this type are minimal when the iodination does not exceed four atoms iodine per molecule protein, but that such alterations become significant as the amount of attached iodine approaches 8 to 10 atoms per molecule protein (3,4,5).

It is generally assumed that useful mild iodination of protein involves coupling of iodine to tyrosine residues of the protein to form di-iodo-tyrosine. The reaction equation is usually written (1):



The most commonly reported method for using I^{131} as received from Oak Ridge for iodination involves as a first step the addition of the I^{131} received as iodide to a solution of iodine and potassium iodide in water. Exchange reactions convert a portion of the radioactive iodide to free iodine. This solution is then in turn added to the protein, present usually in a buffered, slightly alkali, water solution (6, 7, 8, 9, 10).

Under proper conditions this method is widely reported as satisfactory. A limitation to its general applicability depends upon the fact that the I^{131} as received from Oak Ridge is present as iodide maintained in the reduced state by excess sodium bisulphite in basic solution. In the reaction mentioned above the excess sodium bisulphite will reduce an equivalent amount of the iodine of the I_2-KI solution to iodide. This is of no great consequence if the I_2-KI is present in such large amounts that the percentage of free iodine in solution is not greatly reduced by this reaction. If, however, it is desirable to keep the amount of chemical iodine small, as in making a small amount of protein highly radioactive, then this reducing substance may destroy all, or nearly all, the free iodine and reduce the resulting percentage of iodination to a very low value. Pressman has described and used a different method in which the Oak Ridge I^{131} solution, after the addition of some non-radioactive KI as carrier, is largely oxidized to free iodine by the addition of sodium nitrite followed by acidification (11). After adjusting this solution to pH8 it is added to the protein solution in borate buffer.

Fine and Seligman (4) have described a method in which the radioactive iodine was distilled as free iodine into CCl_4 and this solution mixed with the protein solution that was to be iodinated. This method was particularly useful when the starting material was a cyclotron target,

but would also be useful as a means of getting rid of excess reducing substances in an iodide solution.

Iodine^{131} from Oak Ridge is currently shipped (1952), according to the shipping memo that accompany thsse shipments, as in chemical form carrier free iodide in $NaHSO_3$ solution. Total solids are reported as about 4.5 mg/cc. The pH varies between 11 and 12. Radioactivity at time of shipment is about 10 millicuries per cc.

Assuming that $NaHSO_3$ accounts for the major portion of total solids, the amount of this material at time of shipment would approach 0.45 mg per millicurie. As the I^{131} decays the ratio will of course increase. At our laboratories typical values when iodinations are carried out are up to twice these values. In order to get reasonable iodination values probably the sodium sulphite ought not to reduce more than half the free iodine of an I_2-KI solution. Therefore, on the assumption that one molecule of $NaHSO_3$ can reduce one molecule I_2, the I_2-KI solution used ought to contain 2.2 mg I per millicurie I^{131} at time of shipment, of which 50% might be expected to remain free iodine and take part in iodination. Assuming that one-half of this iodine is reduced to iodide as iodination of tyrosine residues take place, the iodine incorporated in protein would be 0.55 mg. The amount of protein necessary so that only four atoms of iodine will be attached to one protein molecule of molecular weight 160,000 is 0.176 grams.

There are many potential experiments in which this maximum degree of labelling, 0.95 mc I per gram protein, is an undesirably low activity. Experiments carried out in which we reduced the amount of I_2-KI about ten fold from those given above confirmed that under these conditions the

amount of iodination was small; around one to two percent of the iodine was firmly bound to the protein.

In principle, a more attractive method for achieving a high degree of specific radioactivity seemed to be that of Pressman and Eisen (11), mentioned earlier. The experimental work reported here is a study of iodination achieved using various variations of their basic method.

Experimental:

The test system utilized. In order to do numerous experiments a procedure was sought that could be performed in test tubes and without the necessity for dialysis. As a substitute for dialysis, on completion of the iodination, proteins were precipitated by bringing the solution to a 6% trichloroacetic acid (TCA) concentration by adding 100 grams percent TCA. The general procedure finally developed was as follows: pyrex culture tubes 13 x 100 mm were used. An iodine ^{131} preparation was used, made by a series of dilutions of the stock solution from Oak Ridge to a final concentration of about 0.1 microcurie per cc, using as a dilutent distilled water to which had been added $NaHSO_3$ in an appropriate amount.

One cc of the above solution was pipetted into each test tube of an experimental series. Solutions of other reagents, i.e. KI, $NaNO_2$ and HCl, were made up in dropping bottles in concentrations so that the desired amount of a reagent would be contained in a few drops. Immediately after adding the HCl, a rubber stopper was put in the tube and the contents mixed by inversion. After a waiting period, usually two minutes, the protein solution in buffer was added. After another period, usually 30 minutes, sufficient 100 grams percent TCA was added to give a 6% TCA concentration in the solution; this precipitated the protein. In most experiments the addition of TCA was preceded by the addition of excess $NaHSO_3$ to convert

the free iodine to iodide.

After centrifugation the supernatant was removed by aspiration and the I^{131} in the precipitate determined by gamma ray counting in a well-type scintillation counter similar to that described by Anger (12). The precipitate was washed again in 5 cc 6% TCA, centrifuged, and the precipitate again counted. This washing cycle was repeated two or three times until the loss of activity was small. Iodination was computed as the percent of the original I^{131} added remaining in the final precipitate.

GROUP I EXPERIMENTS. Effects of varying amount of KI, $NaNO_2$ and HCl on percent of iodination.

To each tube 1 cc of carrier free I^{131} in 0.715 g/l $NaHSO_3$ was added first. This is 6.87μ equivalents of sodium sulfite. KI was added from a 0.01 molar solution, $NaNO_2$ from a 0.071 N solution, and HCl from a 0.178N solution. Two minutes later two cc of a serum protein solution was added. This solution was prepared by dialyzing rabbit serum against a borate buffer of pH8 made by adding 0.16N NaOH to a solution 0.2M in H_3BO_3 and 0.16M in NaCl (11), and then diluting the dialyzed protein with an additional six parts of the borate buffer. The final concentration was 8.6 mg protein per cc. If for purposes of calculation we made the crude assumption that the protein was all of molecular weight 160,000, the two cc would contain 0.107 micromols of protein. After rapid mixing each solution sat thirty minutes at room temperature to allow iodination to take place. Then $NaHSO_3$ was added from a solution containing 5.01 μe per drop, in amounts equivalent to 1.5 times the $NaNO_2$ originally added. Precipitation, washing, and radioactive measurement of the protein was then carried out according to the general procedure outlined previously. The results of these experiments are shown in Table I. Column 5 gives the radioactivity in the precipitate

after centrifugation and removal of the first supernatant; column 6 after the first wash with 6% TCA. Column 7 gives the radioactivity after the second wash with TCA, and probably represents the percentage iodination achieved.

The data of Table I shows that too great an excess of $NaNO_2$ can greatly reduce the percentage iodination with I^{131} as compared with more optimal amounts. Other experiments (See Exp. 3, Table IV) show that little or no iodination occurs if $NaNO_2$ is completely omitted. Increasing the amount of chemical iodine seems to give a higher percent binding of I^{131}. Too little HCl can reduce the iodination achieved.

In order to check that the I^{131} remaining in the precipitate after the second washing was a true measure of iodination as measured by other methods, Experiments 8 and 16 were repeated except that double portions of all reagents were used for the reaction and that after the half hour wait for iodination no $NaHSO_3$ was added, but instead the protein solution was emptied into a cellophane dialyzing sack and diluted with an equal volume of borate buffer used as a test tube wash making a total volume of 12 cc. In the repeat of Experiment 8 the dialysis against two liters of 0.85 of saline was carried out for 19 hours at 4° C. An aliquot portion of the protein solution was precipitated by bringing the solution to a 6% TCA concentration and the I^{131} content determined. The iodination achieved by this measure was 27.3%. In the repeat of Experiment 16, the dialysis was carried on for 15 hours, a portion removed for assay, and the remainder dialyzed against a new portion of saline for an additional 8 hours. No measurements or corrections for possible changes in volume of dialyzed protein solution were made. The 15 hour dialyzed sample had an I^{131} content indicating 16.8% iodination, the 23 hour sample 18.2%.

Table I

1	2	3	4	5	6	7
				% added I^{131} in precipitate		
Exp. No.	Drops 0.01MKI (1 drop = 0.53 μe)	Drops 0.071 N $NaNO_2$ (1 drop = 3.77 μe)	Drops 0.178 N HCl (1 drop = 9.45 μe)	Supernate removed	1st wash	2nd wash
1	1	14	2	15.1	3.5	1.6
2	3	14	2	21.9	12.1	10.9
3	5	14	2	25.3	16.6	14.3
4	1	10	2	14.6	4.5	2.5
5	3	10	2	27.8	17.1	15.8
6	5	10	2	30.7	21.7	20.7
7	3	5	2	30.7	19.1	18.2
8	5	5	2	40.5	31.3	31.5
9	5	5	1	29.8	18.8	16.5
10	5	5	3	44.2	38.1	35.7
11	5	3	2	40.2	32.1	32.7
12	7	5	2	37.3	29.1	26.7
13	7	3	2	39.0	31.5	30.3
14	1	3	2	35.6	25.8	22.8
15	2	6	2	39.9	33.0	32.4
16	1	5	2	28.0	17.1	15.5
17	1	5	2	36.4	27.0	25.3
18	1	5	2	32.5	24.3	24.3
19	1	5	2	34.4	26.9	25.2
20	1	5	2	34.8	25.9	24.8

The figure of 27.3% is considered in reasonably good agreement with 31.5%, and 16.8% and 18.2% in reasonable agreement with 15.5%.

Experiments 17, 18, and 19 were run later with a new preparation of dialyzed rabbit serum. The reason for the higher I^{131} binding is not known.

GROUP II EXPERIMENTS. Relation between amount of $NaHSO_3$ added before protein precipitation and pseudo-binding of I^{131} to protein. In early experiments similar to those described in Group I, but in which no $NaHSO_3$ was added just prior to protein precipitation, there was much more I^{131} present in the protein precipitated and washed with 6% TCA than in similar protein preparations dialyzed against saline. Neither adding NaI to the TCA washing solution, dissolving the protein precipitate with NaOH, and then re-precipitating, or combinations of the two procedures consistantly brought the test tube iodination values close to those obtained with dialysis. Addition of $NaHSO_3$ just before protein precipitation turned out to be useful for this purpose. In Table II experiments are reported in which the conditions of Experiment 16 of Table I are repeated but with variations in the amount of sulfide added before protein precipitation. In Experiments 2 through 11 a $NaHSO_3$ solution of 4.5 grams per liter was used, and in Experiments 12 through 19, 10 grams per liter.

GROUP III EXPERIMENTS. Relation between percentage incorporation I^{131} in protein, and variations in temperature, time of reaction, buffer composition and pH. These experiments, except where noted otherwise, were repetitions of Experiment 17 of Table I, excepting that the reaction time for Experiments 10 through 15 is fifteen rather than thirty minutes. Double values for iodination indicate duplicate experiments.

Table II

1	2	3	4	5
		% added I^{131} in precipitate		
Exp. No.	μ equiv. added $NaHSO_3$	Supernate removed	1st wash	2nd wash
1	0.0	83.2	73.4	68.1
2	2.3	79.1	69.7	64.9
3	6.8	74.0	61.9	56.1
4	11.4	66.4	53.6	48.3
5	13.7	67.3	50.8	44.1
6	16.0	62.8	43.0	35.3
7	18.2	62.8	44.4	37.6
8	20.5	35.2	25.1	22.4
9	16.0	67.6	52.6	43.0
10	18.2	68.8	44.7	35.4
11	20.5	33.2	25.0	21.7
12	15.0	66.1	50.6	40.6
13	20.1	63.8	47.7	39.2
14	25.1	28.0	17.1	15.5
15	30.1	29.0	17.1	15.5
16	35.1	29.4	18.5	17.4
17	40.2	29.4	18.0	17.4
18	45.2	26.6	16.1	14.5
19	75.0	33.2	26.2	22.8

Table III

Exp. No.	Deviation from Exp. 17, Table I	% I^{131} in final washed precipitate
1	Reaction time 5 minutes instead of 30 min.	21.0
2	Reaction time 2 hours instead of 30 min.	25.3
3	Reaction time 5 minutes 5° C	29.0
4	Reaction time 30 minutes 5°C	22.2
5	Reaction time 2 hours 5° C	22.2
6	Reaction time 5 minutes	23.8
7	Reaction time 10 minutes	23.1
8	Reaction time 15 minutes	26.2
9	Reaction time 20 minutes	26.9
10	.1 molar phosphate buffer pH 7.5	29.5, 28.6
11	.1 molar phosphate buffer pH 6.55	22.8, 22.8
12	.1 molar phosphate buffer pH 7.0	21.8, 22.8
13	.1 molar phosphate buffer pH 7.75	28.6, 26.6
14	.1 molar phosphate buffer pH 7.95	31.5, 27.4
15	.1 molar phosphate buffer pH 8.15	32.5, 28.6
16	.1 molar phosphate buffer diluted with 6 parts .1 molar KH_2PO_4	15.0, 18.9
17	.3 cc serum added at 7 times usual protein concentration, and 1.7 cc borate buffer added 1/2 hour later	5.9
18	1 cc borate buffer added before adding 1 cc serum at twice usual protein concentration	23.5

The general conclusion from experiments reported in Table III is that the percentage iodination achieved is not very dependent upon the nature of the buffer, borate or phosphate; the temperature, room to 5°C; or reaction time, from 5 minutes upwards to 2 hours. Iodination is probably somewhat increased with increasing pH in the range 6.55 to 8.15 and significantly reduced at lower pH values as shown by Experiments 16 and 17. Experiment 18 seems to indicate that the iodinating solution can first be brought to a pH of 8 before the addition of protein without greatly affecting the extent of iodination achieved.

GROUP IV EXPERIMENTS. Iodination of Rabbit Gamma Globulin. Rabbit gamma globulin was prepared from normal rabbit serum using on a larger scale the method of Jager and Nickerson (13). It involved precipitation by adding saturated ammonium sulphate in an amount equal to half the volume of serum, and later washing the precipitate with one-third saturated ammonium sulphate solution. This precipitate was dissolved in borate buffer and dialyzed against borate buffer. Finally it was diluted with borate buffer to the same concentration as the serum protein used previously.

The iodination experiments reported in Table IV were conducted under the same conditions as Experiment 17 of Table I, except for the use of gamma globulin and variations noted in column 2 of Table IV.

A dialysis experiment using the procedure described for the dialysis experiments with serum protein was carried out on a gamma globulin preparation iodinated by the procedure of Experiment 1, Table IV. The binding of I^{131} achieved was 34.4% after a single dialysis for 16.5 hours against 0.85% saline. This result, when compared with Experiment 2,

Table IV

Iodination of Rabbit Gamma Globulin

Exp. No.	Deviation from Exp. 17, Table I	% I^{131} in final washed precipitate
1	No deviation	32.2, 33.9, 35
2	No $NaHSO_3$ added before protein precipitation	78.
3	No $NaNO_2$ added	1.3
4	3 drops 0.07I $NaNO_2$	35.
5	2 drops 0.01 M KI	39.
6	2 cc of protein at 1/4 usual concentration	40.

again indicates that addition of $NaHSO_3$ before TCA precipitation is necessary to bring the results of this type of experiment into conformity with a measurement of I^{131} binding as determined by dialysis. Experiment 4 indicates that iodination is not sharply increased by further reduction in $NaNO_2$ used. Experiment 3 shows some $NaNO_2$ is necessary. Experiment 6 indicates the degree of I^{131} binding achieved is probably not greatly dependent upon the amount of protein used. We do not know whether or not the somewhat greater apparent iodine binding in this Experiment 6 is real and reproducible.

Procedure for Iodination of Rabbit Gamma Globulin Based Upon These Experimental Results. The method for iodination of rabbit gamma globulin in current use in our experimental program is essentially that of Experiment 1, Table IV. Carrier free iodine as received from Oak Ridge is diluted with about 4 parts distilled water, bringing the I^{131} to a concentration of about 1.7 mc per cc. In a typical shipment the solids were 3.25 mg/cc, the pH 11.0. One cc of the diluted material containing 0.65 mg/cc solid was pipetted into a pyrex culture tube. To this was added 1 drop 0.01 molar KI (.53μe), 5 drops 0.071 N $NaNO_2$ (18.85μe), and 3 drops 0.178 N HCl (27.35μe). Following each addition the fluids were mixed by gentle shaking. After addition of the first two drops of HCl the fluid remained colorless. On addition of the third drop a slight iodine color was shown by visual comparison with a water control against a white background. A rubber stopper was put into the tube to minimize loss of iodine during a two minute waiting period.

Previously the rabbit gamma globulin solution to be iodinated had been dialyzed against borate buffer and brought to a concentration of 10 mg per cc. At the end of the two minute waiting period 2 cc of this protein

again indicates that addition of $NaHSO_3$ before TCA precipitation is necessary to bring the results of this type of experiment into conformity with a measurement of I^{131} binding as determined by dialysis. Experiment 4 indicates that iodination is not sharply increased by further reduction in $NaNO_2$ used. Experiment 3 shows some $NaNO_2$ is necessary. Experiment 6 indicates the degree of I^{131} binding achieved is probably not greatly dependent upon the amount of protein used. We do not know whether or not the somewhat greater apparent iodine binding in this Experiment 6 is real and reproducible.

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Previously the rabbit gamma globulin solution to be iodinated had been dialyzed against borate buffer and brought to a concentration of 10 mg per cc. At the end of the two minute waiting period 2 cc of this protein

solution (0.126 μmoles on the assumption of a molecular weight of 160,000) was pipetted into the culture tube, stoppered, and rapidly mixed by inversion. After 15 minutes the protein solution was poured into a cellophane tubing dialysis bag, and 3 cc of borate buffer, first used as a reaction tube rinse, was also poured into the bag. Dialysis, with gentle stirring, was carried out for 18 hours at 4° C against two liters of 0.85% NaCl and then for an additional 8 hours against a fresh portion of saline. Analysis of the final product indicated 20.1% of the I^{131} was bound to protein. On the basis of these results 0.108 μc of chemical iodine was bound to 0.126 μmoles of protein, or on an average 0.86 atoms iodine per molecule protein. The radioactivity of the protein is the equivalent of 17.1 mc I^{131} per gram protein.

A second iodination of rabbit gamma globulin with a different I^{131} preparation gave 33.7% incorporation of I^{131} under similar conditions to those described above.

In another iodination attempt, conditions were the same except that two drops, rather than three of HCl were added, and no perceptible iodine color appeared. The percent of I^{131} binding to protein was 6%.

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Symposium on Radiobiology. The Basic Aspects of Radiation Effects on Living Systems by James J. Nickson (Editor). Oberlin College - June 14-18, 1950. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, New York. 1952. xii 465 pp. 15.5 x 23.5 cm. Price - $7.50

Reviewed by H. A. Blair

This symposium was organized under the auspices of the National Research Council to review the more fundamental aspects of radiobiology, beginning with the processes of absorption of radiation by matter and proceeding through the consequent chemical, biochemical and cytological changes to a consideration of certain physiological, genetic and lethal effects in the mammal. The ultimate objective of relating all these phenomena satisfactorily is probably a distant one, but a definite advance was made by critical summarization of those factors which appear to be established and by designation of those gaps in present knowledge which might be filled in readily.

Of twenty-three papers the first eight deal about equally with physical and chemical interactions of radiation, including particles, with matter. This is followed by a long chapter by Platzman on that topic of especial pertinence to biological systems, the absorption of radiation by water. The succeeding five chapters review the findings in biochemical systems including studies of factors altering radiosensitivity in organisms. The papers of the three European contributors Dale, Hevesy and Latarjet are in this group. Three of the final nine chapters deal with hereditary problems, chromosome aberrations, gene mutations and mammalian genetics. One of these is a full expression of Müller's views on gene mutations. The remaining papers are on separate topics including factors modifying the sensitivity of cells to high energy and ultraviolet radiation, discussions of the effect of the rate of energy loss along the path of ionization, the development of a diffusion theory by Tobias which accounts for the different sensitivities of different ploidys in yeast, the influence of quantity and quality of radiation on the biologic effect, some physiological effects on

mammals and finally, a concluding chapter by Brues and Sacher giving an analysis of mammalian radiation injury and lethality using, in part, the Gompertz equation and in part, a function developed previously by Sacher.

Owing to the arrangement, the editing and the discussions following each subject, the continuity of the story is much better than is usual in an effort of this kind. For this reason students will find it useful for orientation although it is not sufficiently detailed for a text. However, there are over 700 references leading to original sources.

Teachers and researchers in this field will find the volume very useful for reference and review.

Abstract of Paper Presented at the 12th Annual Meeting
of the Institute of Food Technology - Grand Rapids, Michigan - June 9, 1952

TOXICITY TESTING OF CHEMICAL ADDITIVES

by
Elliott A. Maynard

The steps involved in testing the toxicity of chemical additives are described and illustrated. The need for more rapid testing methods is reported and a possible solution for some cases is described.

TECHNICAL REPORTS ISSUED FOR DISTRIBUTION

October 1, 1952 thru December 31, 1952

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UR-216	The Relation of Protein Synthesis to Nucleic Acid Phosphorus Turnover in the Rat Liver (UNCLASSIFIED) Issued: October 31, 1952	Frankel	Health and Biology
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UR-223	The Metabolism of Glutaric Acid-1,5-C^{14}: I. In Normal and Phlorizinized Rats (UNCLASSIFIED) Issued: November 26, 1952	Rothstein Miller	Health and Biology

Report No.	Title	Authors	Subject Category
UR-225	Selected Blood and Urine Changes in Experimental Beryllium Poisoning (UNCLASSIFIED) Issued: November 26, 1952	Spiegl LaFrance Ashworth	Health and Biology
UR-227	Variation of X-radiation-Induced Lipemia in Rabbits with Dosage, Administration of Insulin or Ammonium Chloride, and the Incidence of Tetany (UNCLASSIFIED) Issued: December 17, 1952	Steadman Grimaldi	Health and Biology
UR-228	An Improved Determination of Exchangeable Ions in Bone Preparation (UNCLASSIFIED) Issued: December 31, 1952	Weikel Neuman	Health and Biology
UR-230	The Surface Chemistry of Bone. VII. The Hydration Layer (UNCLASSIFIED) Issued: December 31, 1952	Neuman Toribara Mulryan	Health and Biology