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October 1958 through March 1959

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BIOLOGY AND MEDICINE SEMIANNUAL REPORT

October 1958 through March 1959

BIOLOGICAL STUDIES OF RADIOACTIVITY AND IRRADIATION

Crocker Laboratory
University of California
Berkeley, California

BIOLOGICAL EFFECTS OF INTERNALLY DEPOSITED RADIOISOTOPES

Patricia W. Durbin and C. Willet Asling in charge

Results of long-term experiments now being conducted by the Biology and Medicine Group at Crocker Laboratory will be given in a subsequent report.

RADIATION CHEMISTRY

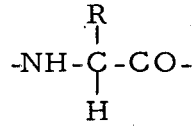
Warren M. Garrison in charge

Winifred Bennett, Sibyl Cole, Mathilde Kland-English, Michael E. Jayko, Evelyn Palmer, and Boyd M. Weeks

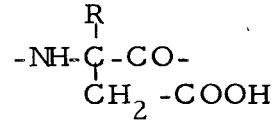
RADICAL-COMBINATION REACTIONS IN THE RADIOLYSIS OF PROTEIN-$CH_3C^{14}OOH$ SOLUTIONS

In the radiolysis of oxygen-free solutions containing a protein species RH and a simple organic solute R'H, there is a certain probability that intermediate free radicals R and R' combine to form the modified protein R-R'. Preliminary studies in which solutions containing both pepsin and $CH_3C^{14}OOH$ were irradiated with 40-Mev helium ions from the Crocker Laboratory cyclotron showed that a nondialyzable C^{14}-labeled product was formed.[1] Conventional acid hydrolysis of this high-molecular-weight material yielded a series of C^{14}-labeled amino acids, one of which has been identified as aspartic acid. The simplest explanation for the formation of C^{14}- labeled aspartic acid is that $CH_2C^{14}OOH$ radicals derived from acetic acid[2] combine with "protein" free radicals containing the -NH-CH-CO- configuration. The possibility that this is representative of a general system of radiation-induced reactions in aqueous protein-CH_3COOH solutions is under study.

Consider the general amino acid residue in the protein molecule,



if primary radical attack occurs at C-H bonds along the peptide chain and combination with CH_2COOH follows, a series of α-substituted aspartic acid residues is formed,



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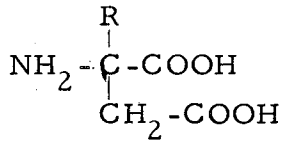
[1] Biology and Medicine Semiannual Reports, UCRL-8265, April 1958, pp 13-14; UCRL-8513, Oct. 1958, pp 37-38. B. M. Weeks and W. M. Garrison, Formation of C^{14}-Labeled Protein Derivatives in the Radiolysis of Aqueous Pepsin-$C^{14}H_3COOH$ Solutions, Radiation Research 9, 202 (1958).

[2] Garrison, Haymond, and Weeks, Some Effects of Heavy-Particle Irradiation of Aqueous Acetic Acid, Radiation Research 1, 97 (1954).

Hydrolysis of the modified protein yields the free aspartic acid derivatives,



Now, except for the case of glycine (R = H), it must of course be assumed that hydrogen abstraction may occur not only at the peptide linkage but also at various sites on the side chain R. In alanine, for example, abstraction at the methyl group would, in terms of the proposed mechanism, yield C^{14}-labeled glutamic acid, whereas abstraction at the α-carbon position would yield C^{14}-labeled α-methyl aspartic acid. It is noted in this regard that in our earlier studies with pepsin, which contains no alanine, neither C^{14}-labeled glutamic acid nor C^{14}-labeled methyl aspartic acid was observed (or indeed expected). Our more recent experiments, however, have been carried out with aqueous $CH_3C^{14}OOH$ solutions containing (a) gelatin, and (b) β-lactoglobulin. The amino acid composition of these two proteins is such that the relative importance of reaction at both glycine and alanine residues can be evaluated. These two loci have been of primary interest in the initial phases of this work because (a) authentic compounds corresponding to the expected C^{14}-labeled products have been more readily available;[3] (b) interpretation of results can be developed most logically on the basis of the effects of increasing complexity in the side chain R. Elution curves for C^{14}-labeled amino acids obtained from gelatin and β-lactoglobulin are shown in Figs. 1 and 2 respectively. In neither case was C^{14} activity found in fractions corresponding to authentic glutamic acid. The C^{14} activity associated with peak A of each curve was found to cochromatograph with aspartic acid. As would be expected, this is a major C^{14}-labeled product from gelatin which contains a large proportion of glycine residues. However, the fraction of alanine residues in gelatin is also high. In attempting to measure the relative contributions of $CH_2C^{14}OOH$ combination reaction at -NH-CH-CO and -NH-C(CH_3)-CO-, we found that the respective products, aspartic acid and methyl aspartic acid, were not resolved under the column conditions employed in obtaining the data of Figs. 1 and 2. We have subsequently found that these two amino acids can be separated on Dowex-50 if a modified elution procedure is employed. Analysis can also be made after conversion to the corresponding hydroxy acids by nitrous acid treatment; partition chromatography on the silicic acid column is then employed to effect separation. Determination of C^{14}-labeled aspartic and methyl aspartic acids from the gelatin and β-lactoglobulin irradiations is now in progress.

---

[3] The authentic α-methyl aspartic acid employed in these studies was prepared by the method of Strecker. (H. D. Dakin, On Methyl-Aspartic Acids and their Methylation, J. Biol. Chem. 141, 995-50 (1941).) This procedure which involves the addition of ammonium cyanide to the appropriate keto acid appears to be applicable as a general method for the preparation of the α-substituted aspartic acids which are encountered as irradiation products in these studies.

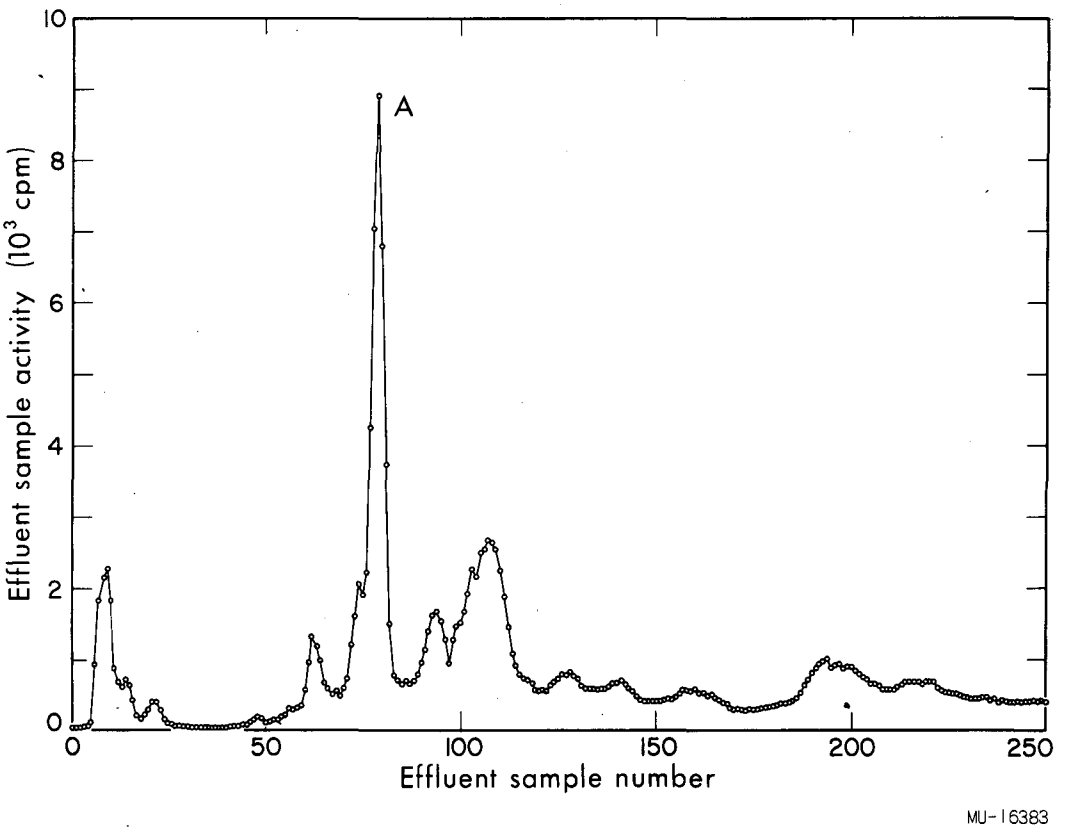


Fig. 1. Typical elution curve for hydrolized gelatin-$CH_3C^{14}OOH$ derivatives. Irradiation: 40-Mev He^{+2} ions, 0.0005675 μah (2.6 x 10^{20}ev) at 10 mμa. Target: 100 ml solution (evac) containing 55.5 mg gelatin (E. K. Calfskin Batch No. 60-9680) and 0.211 meq acetic acid ($CH_3C^{14}OOH$).

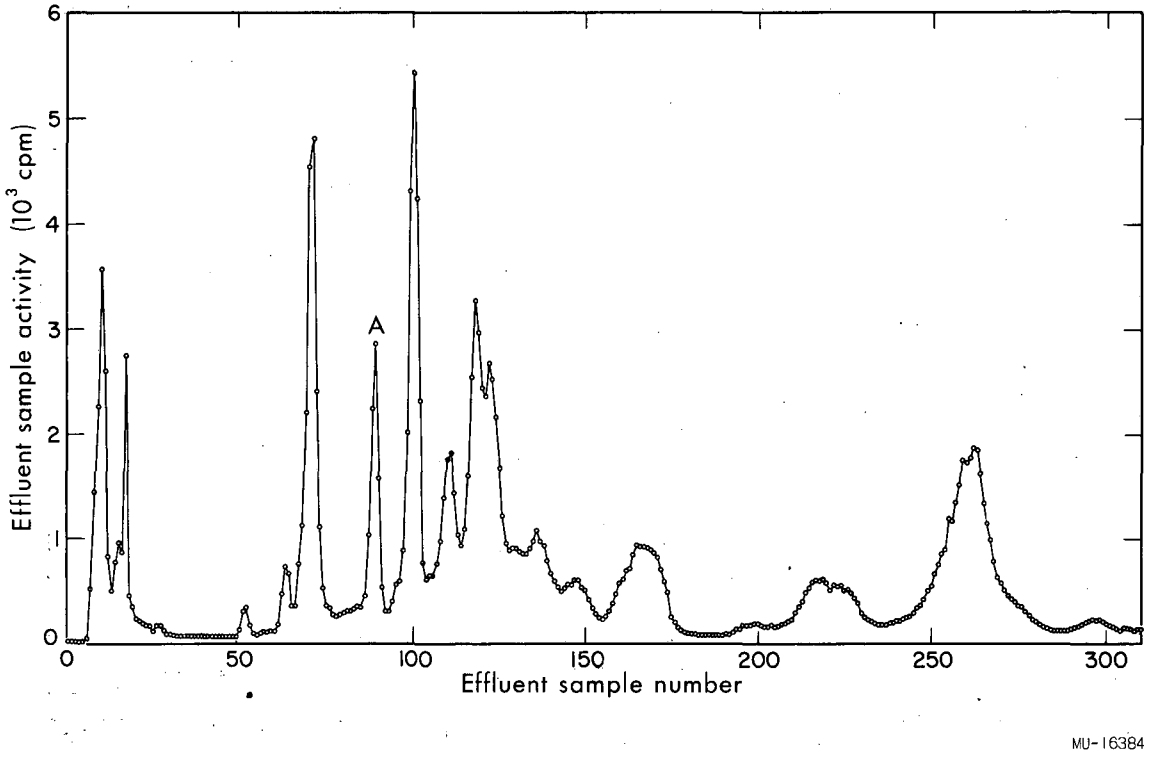


Fig. 2. Typical elution curve for hydrolized β-lactoglobulin-$CH_3C^{14}OOH$ derivatives. Irradiation: 40-Mev He^{+2} ions, 0.0005 μah (2.3×10^{20} ev) at 10 mμa. Target: 100 ml solution (evac) containing 50 mg β-lactoglobulin (Bovine, Nutritional Biochemicals Corp.) and 0.17 meq acetic acid ($CH_2C^{14}OOH$).

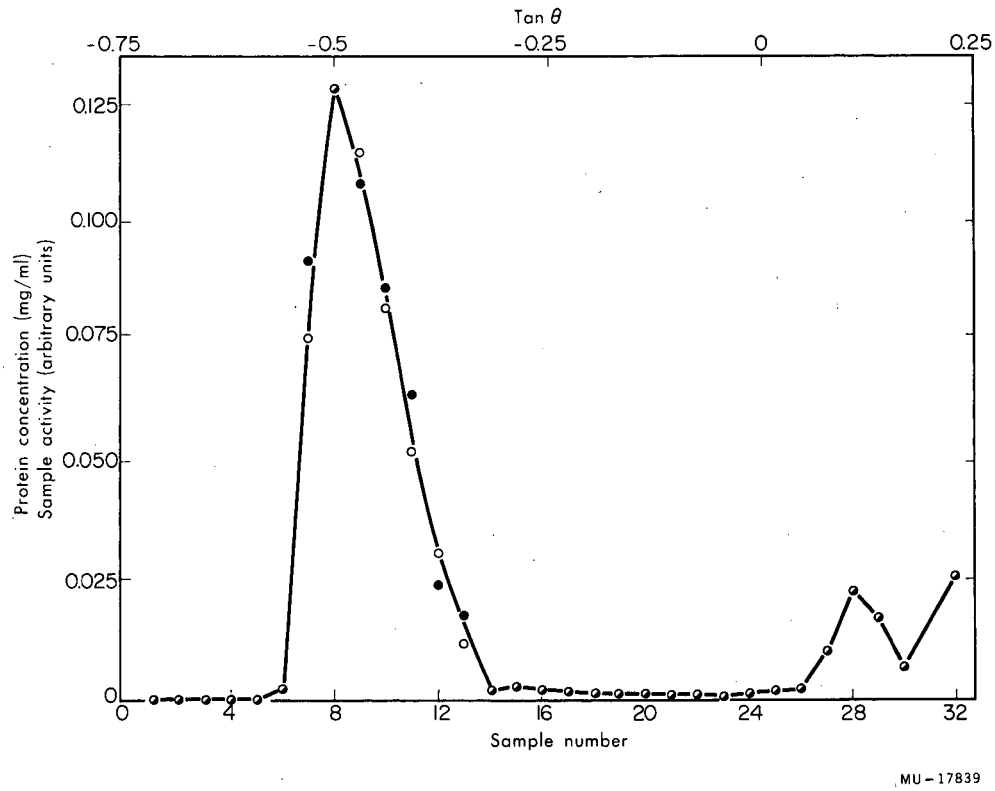


Fig. 3. Typical elution profile showing correspondence between product activity and protein concentration in a 6-hr sample from the Beckman Spinco continuous-flow electrophoresis cell (see text). Irradiation: 40-Mev He^{+2} ions, 0.005 μah (2.3×10^{21} ev) at ~50 mμa. Target: 100 ml solution (evac) containing 50 mg β-lactoglobulin (Nutritional Biochemicals Corp.) and 0.14 meq acetic acid ($CH_3C^{14}OOH$).

In the studies on protein-$CH_3C^{14}OOH$ systems described thus far, the irradiated solutions were dialyzed under carefully controlled conditions to insure that the C^{14}-labeled amino acids liberated in the subsequent hydrolysis step were indeed associated with high-molecular-weight C^{14}-labeled products. Recently, with the aid of a newly acquired vertical-curtain continuous-flow electrophoresis unit (Beckman Spinco Model CP), we have been able to undertake various physico-chemical studies of the C^{14}-labeled "protein" formed in the radiolysis of the β-lactoglobulin-$CH_3C^{14}OOH$ system. An aliquot of the irradiated target solution was first vacuum-distilled to recover $CH_3C^{14}OOH$. The residue was redissolved in 0.01 N acetic acid (0.67 mg/ml) and added continuously (at 0.75 ml/hr) to the top of the electrophoresis curtain (Schleicher and Schuell filter paper # 470). Electrolyte (0.01 N acetic acid) flowed down the curtain at 11 ml/hr. The voltage gradient was 15.4 v/cm across the curtain, with a total current of 3 ma. Effluent was collected in test tubes from 32 drip points across the bottom of the curtain. Each sample was assayed for C^{14} activity after protein concentration was measured colorimetrically at 2800 Å. Figure 3 shows that the major part of the activity is associated with the protein fraction. Control runs with a number of different possible contaminants showed trajectories quite distinct from that given by Fig. 3. Protein activity recovered from samples Nos. 7 to 12 yielded, upon conventional acid hydrolysis and ion-exchange chromatographic separation, a spectrum of C^{14}-labeled amino acid products very similar to that obtained with another aliquot (of the same target solution) which had been dialyzed in the usual manner. See Figs. 4 and 5.

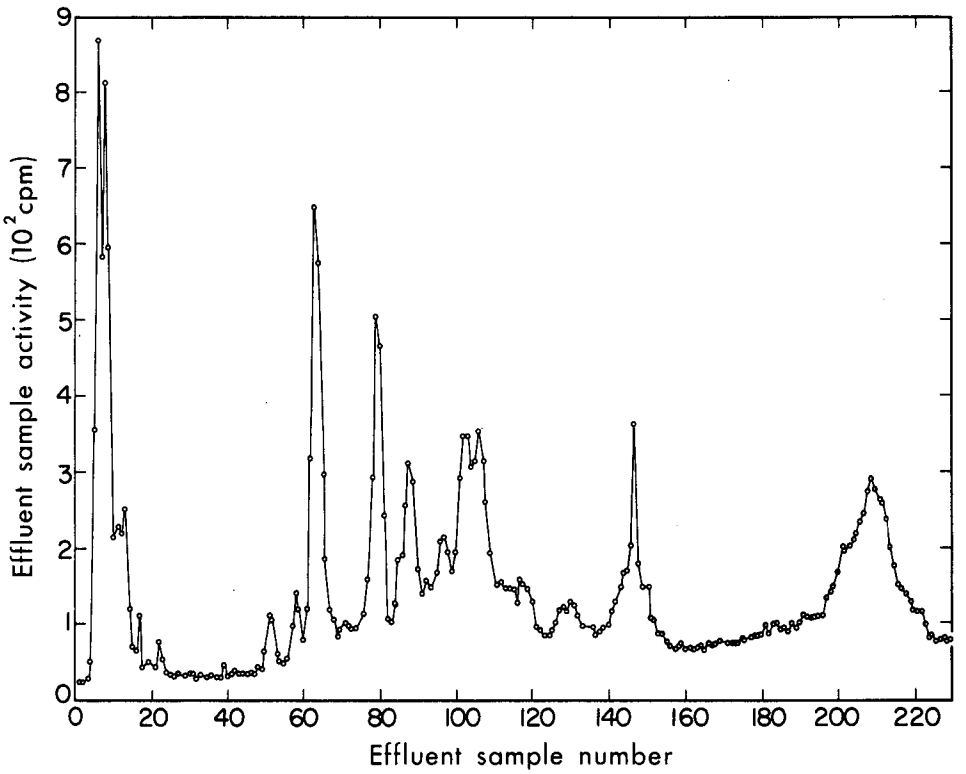


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Fig. 4. Elution curve for hydrolyzed β-lactoglobulin-$CH_3C^{14}OOH$ derivatives from samples Nos. 7 to 12 of Fig. 3.

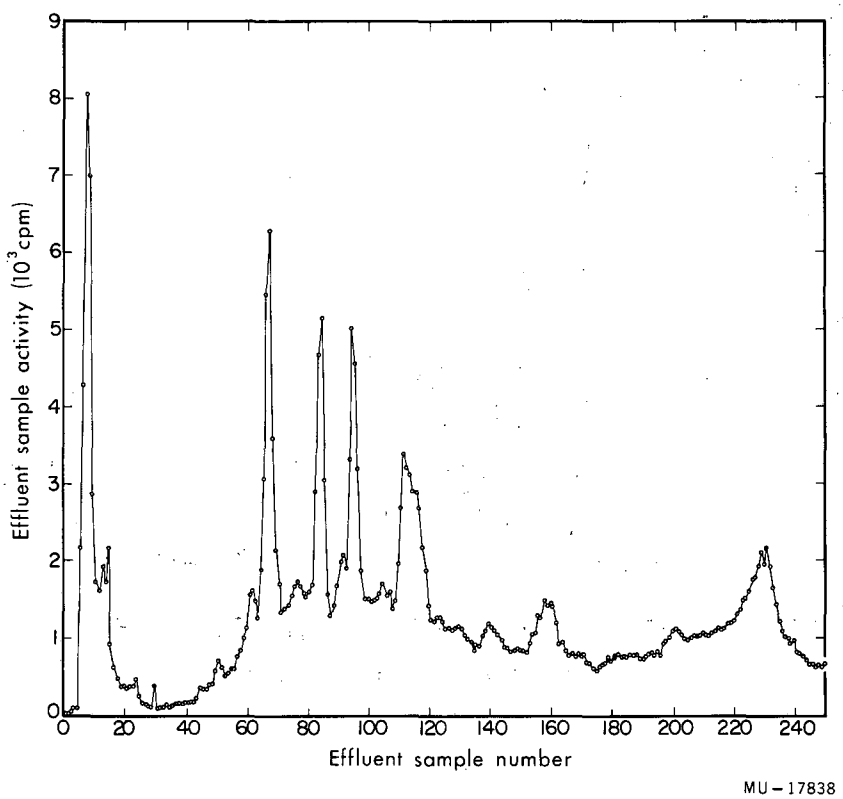

Fig. 5. Elution curve for hydrolized β-lactoglobulin-$CH_3C^{14}OOH$ derivatives obtained from a separate, dialyzed aliquot of the same target used to obtain the curve in Fig. 3.

PRODUCTION OF CARBONYL BONDS IN THE RADIOLYSIS OF AQUEOUS PROTEINS

Studies are being continued of the processes of indirect action that lead to the introduction of >C=O bonds in high-molecular-weight oxidation products derived from protein. Earlier reports have described studies of (a) the total yield of >C=O function; (b) specific loci of carbonyl-forming reactions.

Total carbonyl yield has been measured in the following way: Immediately after irradiation, the protein solution is treated with platinum black to destroy hydrogen peroxide, and then is treated with 2, 4-dinitrophenylhydrazine (2, 4-DNPH) to form the hydrazone derivatives of all carbonyl groups. The solution is dialyzed to remove excess reagent and any products of low molecular weight. The hydrazone chromophores of the high-molecular-weight material are then measured spectrophotometrically in alkaline solution at 450 mμ. Uncertainties involved in the use of this assay procedure for total >C=O yield have been discussed. To obtain increased precision, a method has been developed to measure G(>C=O) directly in terms of -G(2, 4-DNPH). The analysis is based on the fact that the equilibrium constant for the reaction of carbonyl with 2, 4-DNPH in dilute acid is such that the reaction is essentially complete in the presence of a stoichiometric amount of reagent even at carbonyl concentration as low as 10^{-7} M. The irradiated protein solution is treated with 2, 4-DNPH, and after reaction is complete any excess reagent is removed for assay by successive extractions with equal volumes of ether. (Time required for this reaction under various concentration conditions has been established in separate kinetic studies.) Figure 6 shows the optical densities of control and irradiated gelatin solutions as a function of initial 2, 4-DNPH concentration. The initial steep segment of the curve for the irradiated system corresponds to the condition in which the number of molecules of 2, 4-DNPH added is less than the number of >C=O bonds associated with the oxidized protein. In this region no reagent is found in the either extract. As the initial 2, 4-DNPH concentration is increased, the optical density of the protein solution reaches a limiting value, and excess reagent begins to appear in the ether phase. The data of Fig. 6 give -G(2,4-DNPH)=1.5. This value is in essential agreement with results obtained in earlier studies, the difference being due to the loss of low-molecular-weight hydrazones during the dialysis. The present method, however, involves no assumptions regarding the extinction coefficient of the "protein hydrazone." It is to be noted, too, that Fig. 6 shows a measurable "dark" reaction between protein and 2, 4-DNPH. However, under appropriate conditions, this phenomenon introduces but a negligibly small error in the measurement of irradiation products.

One of the processes involved in the formation of carbonyl bonds in protein radiolysis has been shown to correspond to the over-all reaction

$$\sim CO\text{-}NH\text{-}CH(R) + O_2 + H_2O \rightsquigarrow -CONH_2 + RCO\sim + H_2O \qquad (1)$$

Evidence for this radiation-induced reaction at the peptide bond has been derived largely from the observation that part of the carbonyl function associated with the high-molecular-weight products is found as a series of α-keto acids.

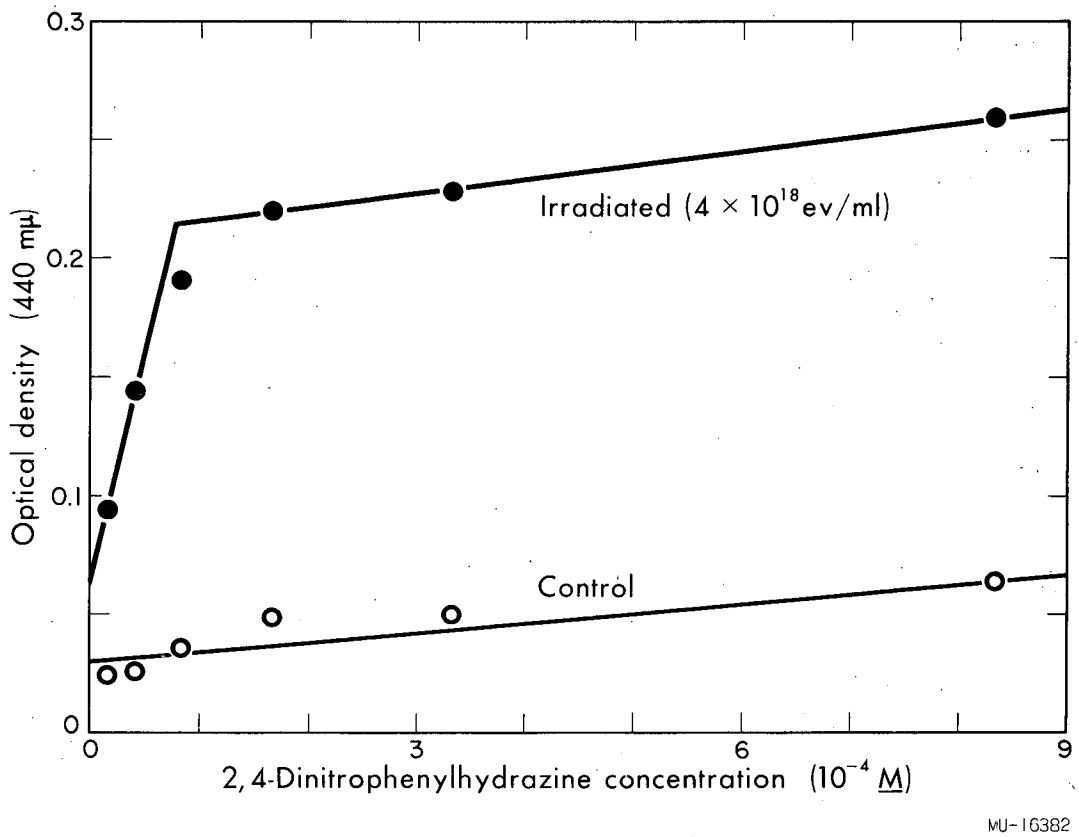

Fig. 6. Effect of irradiation on the reaction of 2, 4-dinitrophenylhydrazine with gelatin.

After conventional (in vacuo) acid hydrolysis of the irradiated protein system, excess 2, 4-DNPH was added and the hydrazone derivatives were extracted with diethyl ether and examined chromatographically. A typical chromatogram of α-keto acid hydrazones from an irradiated gelatin solution and an identical control solution (of gelatin) are shown in Fig. 7. [Origin of small amounts of α-keto acid (pyruvic) in the control has been discussed previously.]

To further substantiate the identification of the α-keto acid products from gelatin, we have employed the following procedures. The irradiated solution is hydrolyzed in 2 N hydrochloric acid for 20 hours in an evacuated tube. The target is neutralized with sodium hydroxide and passed through a column of Dowex-50 ion-exchange resin (acid form). The α-keto acids (amino-acid free) are recovered in the breakthrough volume. This fraction is treated with 2, 4-DNPH reagent, and the resultant hydrazone mixture is then chromatographed on filter paper with a butanol-ammonia solvent.[4] The separated hydrazones are eluted and then hydrogenated (in methanol over PtO_2) at 40 pounds pressure for 16 hours.[5] The amino acids formed are in turn co-chromatographed[5] with authentic compounds. In each case the original α-keto acid identification has been confirmed.

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[4] Cavallini, Frontali, and Toschi, Determination of Keto-Acids by Partition Chromatography on Filter-Paper, Nature 163, 568-569 (1949).

[5] Alton Meister and Patricia A. Abendschein, Chromatography of Alpha-Keto Acid 2, 4-Dinitrophenylhydrazones and their Hydrogenation Products, Anal. Chem. 28, 171 (1956).

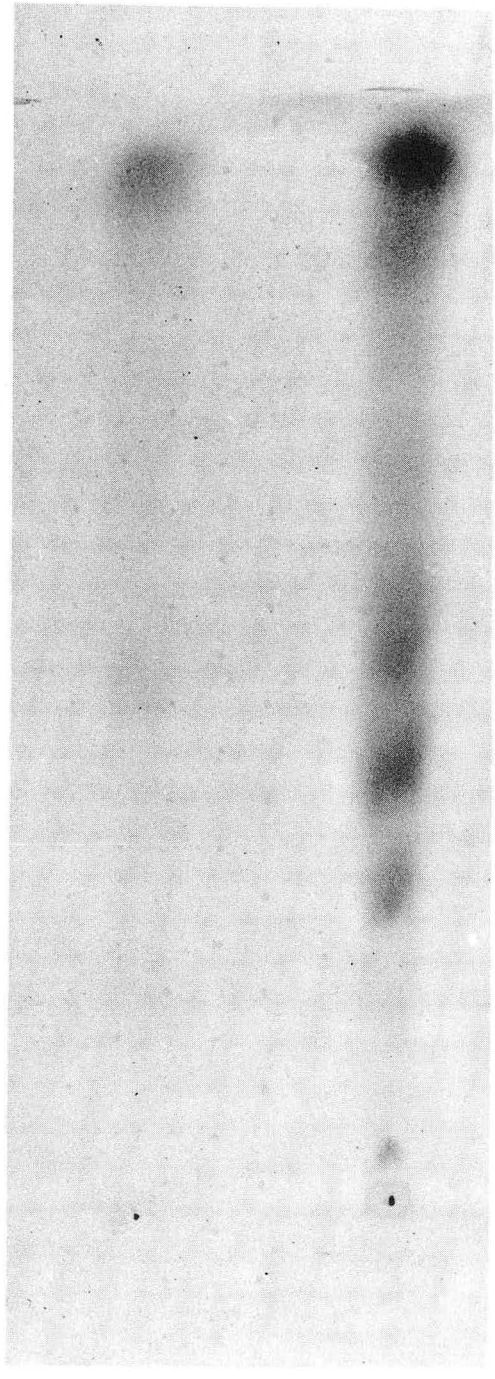

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Fig. 7. Typical chromatograph of α-keto acid hydrazones recovered from an irradiated gelatin solution and an identical control solution.

PRODUCTION OF AMIDE GROUPS AND AMMONIA IN THE RADIOLYSIS OF AQUEOUS PROTEIN

Substantiating evidence for Reaction (1) as written above has been obtained from studies of the amide content of reaction products formed by γ-ray-induced oxidation of aqueous gelatin. Preliminary data were given in the preceding semiannual report. Subsequent and more extensive work has confirmed the earlier observations.

Commercially available lime-process gelatin was found to be particularly suitable for this study, since most of the amide groups of the glutamine and asparagine residues of the parent collagen are removed through hydrolysis during the manufacturing process. Unmodified proteins, in general, contain amide groups in sufficient number to mask Reaction (1) at the lower radiation dosages necessarily invoked in studies of initial reaction products.

Analyses were made for "free" ammonia and for amide groups, both before and after irradiation. The stock solutions (3.5% gelatin, purified calfskin, Eastman Lot 60-9680) has been previously dialyzed to reduce the ammonia content to a minimum value. Analytical procedures were, in brief, as follows: An appropriate aliquot of the solution was made alkaline to phenolphthalein and distilled at room temperature *in vacuo* into a receiver which contained 1 ml of 0.1 N sulfuric acid at the temperature of liquid nitrogen. The distillate was isolated, thawed, and then assayed for ammonia by means of the Nessler reaction. A second aliquot of each solution was made 1 N in hydrochloric acid and heated for 1 hour at 95°C to liberate ammonia from amide linkages.[6] Subsequent treatment was then as described for ammonia. A control series of hydrolysis studies showed for the conditions described that (a) the liberation of amide ammonia is essentially quantitative, and (b) hydrogen peroxide in amounts equivalent to a G value of ~4 does not interfere in the determinations. Prolonged hydrolysis leads to the gradual breakdown of various labile amino acids with the formation of what in this study would be "artifact" ammonia. Figure 8 shows typical hydrolysis data for control and irradiated solutions. An increase of 25% in the number of amide groups in an irradiated solution over that in the corresponding control solution could easily be observed with the method outlined above. Data shown in Table I were obtained on solutions that had been irradiated until the amide and ammonia content had reached values at least three times those of the control.

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[6] H. B. Vickery, The Rate of Hydrolysis of Wheat Gliadin, J. Biol. Chem. 53, 495 (1922).

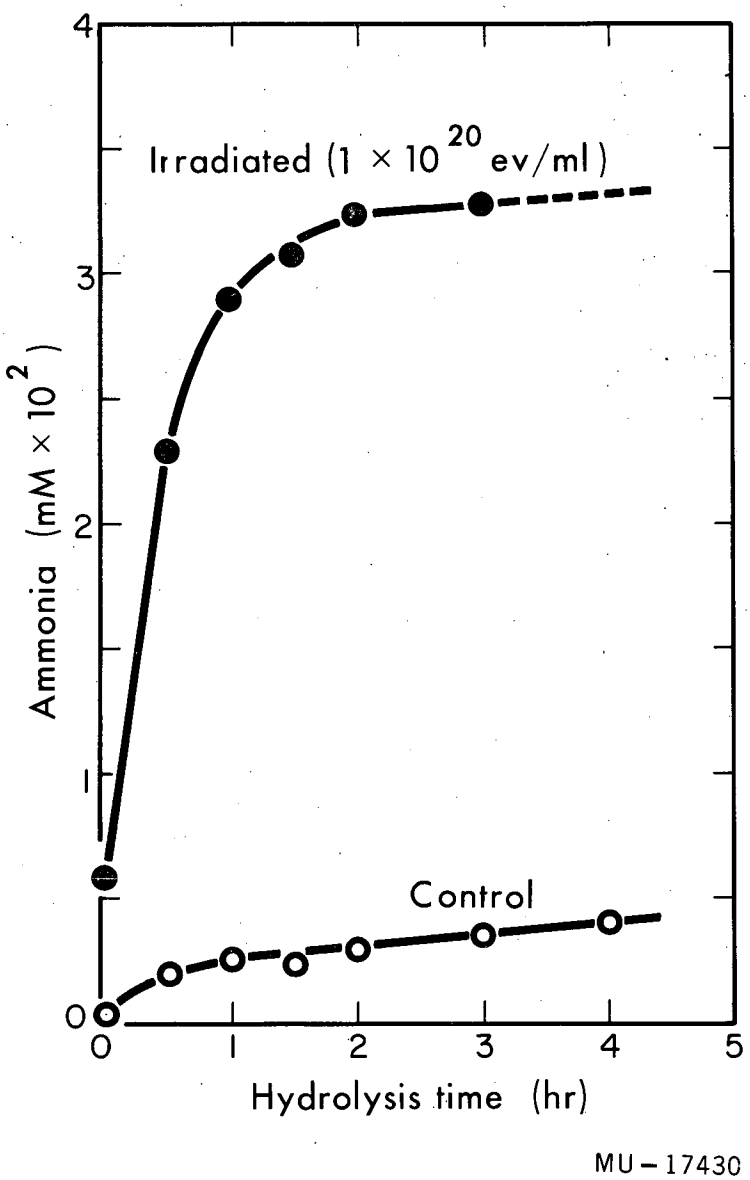

Fig 8. Typical hydrolysis data for control and irradiated solutions.

Table I

Production of amide groups and ammonia in the γ-ray radiolysis of aqueous gelatin solution

	Yield, G (per 100 ev absorbed energy)	
	Series 1	Series 2
Amide groups	1.0±.1	0.9±.1
Amonia	0.25±.05	0.30±.05

A 2000-curies Co^{60} γ-ray source was used in the irradiations. Solutions (10ml) were exposed under oxygen (5 ml) at 1 atmosphere in sealed pyrex tubes. Contents were shaken at intervals to prevent depletion of oxygen in the solutions. The dose rate was 2.5×10^{18} ev/ml/min.

The data of Table I indicate that the formation of amide groups ($G \approx 1$) corresponds to a principal reaction in the radiolysis of aqueous solutions of gelatin. Further, the G value for this product group is very close to that obtained for total carbonyl formation under equivalent irradiation conditions. The simplest explanation for these results would seem to involve the postulated Reaction (1). Ammonia formation ($G \approx 0.3$) may be attributed to a radiation-induced oxidation of terminal and (or) side-chain amino groups.

IRRADIATION OF PEPTIDES IN THE SOLID STATE

As discussed earlier, the simplest mechanism for indirect action of radiation at the peptide bond involves the concept that observed carbonyl products arise through hydrolysis of imino-type intermediates,

$$\sim\!\sim CO\text{-}N{=}C(R)\sim\!\sim + H_2O \longrightarrow \sim\!\sim CONH_2 + RCO\sim\!\sim. \qquad (2)$$

A corollary of this hypothesis appears to be of significance in the interpretation of the mechanism involved in the direct action of radiation on peptides in the solid state. It is well known that unsaturation is introduced in solid (and liquid) aliphatic hydrocarbons by irradiation. The overall reaction is represented simply by

$$R\text{-}CH_2\text{-}CH_2\text{-}R \rightsquigarrow R\text{-}CH{=}CH\text{-}R + H_2. \qquad (3)$$

An analogous reaction for nitrogenous compounds may be written

$$R\text{-}NH\text{-}CH_2\text{-}R \rightsquigarrow R\text{-}N{=}CH\text{-}R + H_2, \qquad (4)$$

and for peptides, more specifically,

$$\sim\!\sim CO\text{-}NH\text{-}CH(R)\sim\!\sim \longrightarrow \sim\!\sim CO\text{-}N{=}C(R)\sim\!\sim + H_2, \quad (5)$$

$$\sim\!\sim CO\text{-}N{=}C(R)\sim\!\sim + H_2O \longrightarrow \sim\!\sim CONH_2 + RCO\sim\!\sim. \quad (5a)$$

Proteins

Some time ago we observed that irradiated solid pepsin (evacuated) on dissolution in water yields high-molecular-weight products containing carbonyl groups,[7] and it was suggested that reaction (5) followed by the hydrolysis reaction, (5a), could be involved. Total carbonyl function in protein solutions prepared in this manner has been measured with 2, 4-dinitrophenylhydrazine reagent by the application of methods developed for the study of indirect action. Yield calculations based on energy absorption in the solid state give $G(>C{=}O) \simeq 0.78$ for the Co^{60} γ-ray irradiation of pepsin and gelatin.

The magnitude of this value would indicate that a principal effect of direct action is being measured. Evidence that part of the total carbonyl yield is indeed derived from radiation-induced reaction at the peptide bond, possibly via Reaction (5), has been obtained. Samples of solid gelatin after irradiation (in vacuo) with Co^{60} γ rays have been found to yield on hydrolysis a series of α-keto acids. Here again, the procedures were those developed in studies of α-keto acid formation by indirect action. The α-keto acid spectrum obtained from the solid gelatin is qualitatively similar to that observed in the indirect-action studies. However, the relative α-keto acid yields appear to be quite different. This work is being continued.

Glycine Anhydride

In view of the above results, we have undertaken a detailed study of radiation-induced reactions of a "simple" peptide model under solid-state conditions. Glycine anhydride was selected because of the relative structural simplicity and because of the obvious relationship to our recent studies on glycine radiolysis.[8]

In preliminary experiments a 5-gram sample of solid glycine anhydride [Nutritional Biochemicals Co. Lot #5025, m.p. (sinters) 267°C, decomp. 272°C. (lit. 275°C)] was evacuated under high vacuum in a pyrex cell fitted with a break seal and irradiated for 2 hours with Co^{60} γ rays at a dose rate

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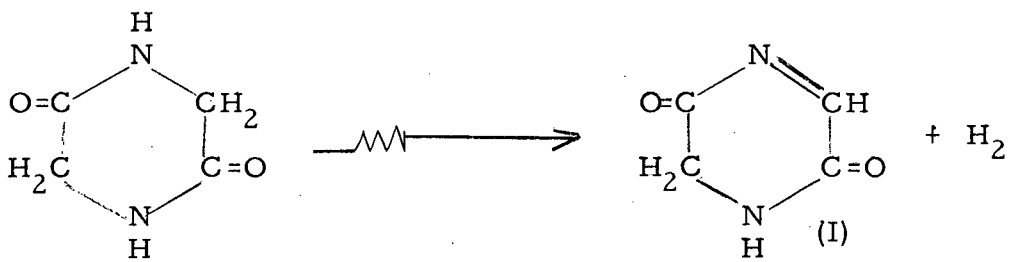
[7] M. E. Jayko and W. M. Garrison, Formation of >C-O Bonds in the Radiation-Induced Oxidation of Protein, Nature 181, 413 (1958).

[8] Boyd M. Weeks and Warren M. Garrison, Radiolysis of Aqueous Solutions of Glycine, Radiation Research 9, 291 (1958).

of 2.5×10^{18} ev/g/min.

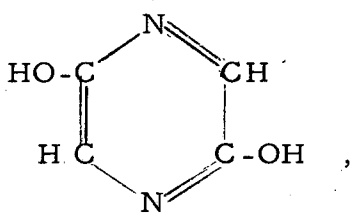
Gaseous products were transferred by appropriate methods to a calibrated bulb-manometer system, and from there to a sampling bulb for mass spectrometric analysis. The product gas was more than 85% hydrogen. Calculations gave $G(H_2) > 0.5$.[9]

Samples of the solid residue (a light yellow which turned buff if the material was heated in air) were hydrolyzed in 1 N hydrochloric acid (in vacuo) for 90 minutes and then treated with 2, 4-dinitrophenylhydrazine in excess. Carbonyl products were extracted as the respective hydrazone derivatives with chloroform and examined chromatrographically with previously described solvent systems, (a) butanol-ammonia, (b) methanol-heptane. R_f and spectral measurements indicate that glyoxylic acid, formaldehyde, and small amounts of glyoxal are formed. Rough estimates of carbonyl yields indicate that in the irradiation of solid glycine anhydride only a relatively small fraction of the observed hydrogen production can be accounted for in terms of a process analogous to Steps (5) and (5a):



$$I + H_2O \longrightarrow NH_2CH_2COOH + CHOCOOH\,(CH_2O + CO_2) + NH_3.$$

However, the possibility that I is relatively stable under the hydrolysis conditions employed cannot be dismissed. The imino intermediate (I) can also be written as the tautomer



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[9] This is probably a lower value. Considerable water vapor was present in the irradiated sample. It is likely that this arose from reaction of H atoms with residual oxygen that had not been removed in the de-gassing operation. We are indebted to Dr. Amos Newton for the mass spectrometry.

which is related to the dihydro-1, 4-pyrazines and could be a relatively stable species. An alternate explanation for the apparent discrepancy between the observed hydrogen and carbonyl yields may be that appreciable cross-linking occurs in the radiolysis of solid glycine anhydride.

Experiments now in progress will provide information on the relative importance of these various processes in the radiolysis of this "simple" peptide system.

REPORTS ISSUED

During this period the following reports have been prepared at Crocker Laboratory:

Michael E. Jayko and Warren M. Garrison, Formation of >C=O Bonds in the Radiation-Induced Oxidation of Protein, Nature 181, 413 (1958).

Winifred Bennett and Warren M. Garrison, Production of Amide Groups and Ammonia in the Radiolysis of Aqueous Solutions of Protein, Nature 183, 889 (1959).

Warren M. Garrison, Winifred Bennett, and Sibyl Cole, Synthesis of Products of Higher Molecular Weight in the Radiolysis of Aqueous Solutions of Formic Acid, Radiation Research 9, 647 (1958).

Winifred Bennett, Sibyl Cole, Warren M. Garrison, and Herman R. Haymond, Radiation-Induced Oxidation of Aqueous Acetic Acid Solutions, Radiation Research 10, 273 (1959).

Warren M. Garrison, Indirect Action of Ionizing Radiation on Some Organic Compounds containing the C-N Bond. To be presented at Sixth International Congress on Nuclear Energy, Rome, Italy, June 18, 1959.

Muriel E. Johnston, A Bibliography of Biological Applications of Autoradiography, 1954 through 1957, UCRL-8400, July 1958.

Muriel E. Johnston, and Patricia W. Durbin, Autoradiography, Medical Physics, Vol. 3, 1959.

BIOLOGICAL STUDIES OF RADIATION EFFECTS

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RADIATION THERAPY AND RADIATION BIOLOGY

CLINICAL AND METABOLIC STUDIES IN PATIENTS AFTER ALPHA-PARTICLE IRRADIATION TO THE PITUITARY

John H. Lawrence, James L. Born, Richard A. Carlson, and Franco F. Sangalli

Pituitary irradiation of patients with advanced metastatic mammary carcinoma, pituitary tumors, and endocrine-controlled diseases has continued. By April 1959 103 patients have received pituitary irradiation, and 27 of these have been irradiated during the past six months. The principal modifications that have been made in irradiation techniques during the past six months are: (a) the size of the aperture shaping the beam has been increased in size so that a larger volume of irradiation is delivered to the pituitary gland; (b) pituitary irradiation has been done on all twelve planes at each of the six irradiation sessions in an effort to distribute the irradiation equally in time and space to all planes treated, and (c) the time interval over which the irradiation dosage is fractionated has been shortened to 11 days.

In view of the changes made it is desirable to review the results of the clinical and metabolic studies of all patients so as to evaluate the effectiveness of pituitary irradiation at the higher dose levels.

The largest group of patients irradiated were those with mammary carcinoma. All of these had progressive metastatic disease and in most instances were in an advanced stage, previously having been subjected to all conventional forms of therapy and in some instances, bilateral adrenalectomies. Of the total number of patients receiving pituitary irradiation with high-energy particles from the 184-inch cyclotron at the Lawrence Radiation Laboratory of the University of California, the type and distribution of cases is listed below.

	Patients Irradiated
Cancer of the breast	82
Acromegaly	7
Diabetes mellitus	8
Chronic lymphatic leukemia	1
Acute leukemia	1
Malignant exophthalmus	2
Dysgerminoma	1
Cancer of the adrenals	1

Selection of patients for irradiation was done after careful clinical and laboratory investigation. All patients were in a progressive state of disease and were no longer considered responsive to the usual conventional forms of treatment. The patients with diabetes mellitus had progressive retinopathy and in some instances nephropathy. They were typical long-term diabetics difficult to control. The acromegalics were all symptomatic and two of them had diabetes associated with their acromegaly. The majority of tese had received one or more courses of conventional 200-kv x-ray therapy.

The early schedule of irradition was based on prior experience gained from animal studies. Since the effect of high-energy particles in suppressing the function of the human pituitary gland was not known, it was necessary to investigate its effects by starting conservatively with low dosages. These were gradually increased towards an optimal level as histological studies, patient response, and sequelae indicated. On the present schedule the patients receive pituitary irradiation on three times weekly for 2 weeks. The highest total amount given has been 30,000 rad for the patients with advanced metastatic mammary carcinoma. At a dose range of 26,000 to 30,000 rad it is believed that complete radiation hypophysectomy can be obtained. The patients with diabetes mellitus received up to 24,000 rad and those with acromegaly up to 7,200. These doses were calculated to the center of the pituitary, the average hypophyseal dose integrated over the isodose surfaces being between 55% and 75% of the peak dose at the center of the gland.

Changes in pituitary function were measured before and after irradiation by determination of the urinary excretion of pituitary gonadotropins and by effects on the target end organs, namely the thyroid, adrenals, and ovaries. The iodine^{131} uptake of the thyroid gland, the protein-bound iodine, the 24-hour urinary steroid excretion, and the 24-hour urinary estrogen excretion were considered as important indicia of the effects of pituitary irradiation on the target and organs.

Laboratory and clinical evidence of decreased pituitary and target-end-organ function followed a dose-time relationship. The onset of a decline in the hormone excretion of the above endocrine organs occurred sooner with the higher amounts of irradiation. In addition, when the total amount of irradiation was fractionated over a shorter time the biological effectiveness was further enhanced, as evidenced by a still earlier decline. The degree of histological destruction on necropsy examination also showed that both the total amount of irradiation and the period over which it was given were essential in determining the biological effectiveness of the dose given.

The endocrine assessments done on these patients after high doses of irradiation indicate a pronounced decrease in pituitary and end-organ function. They are of further interest in determining the time of onset of this decrease following irradiation and the order in which definitive falls in the various measurements were noted.

The earliest changes observed were the decrease of the pituitary gonadotropins excreted in the urine. This change occurred in all patients who received the higher dosages of alpha-particle irradiation except those whose initial gonadotropin excretion was in the extremely low range. This decrease was evident in the majority of patients as early as the first week following the completion of irradiation, and seemed to be the most sensitive of all indications, as it occurred even in those patients receiving low doses of irradiation.

In determining adrenocortical function, measurements of the 17-ketosteroids and--more recently--of the 20, 21-ketols have been of value. The 17-ketosteroids in more than half the high-dose group have shown a marked drop by the end of the first month. By the end of the second month 80% of this patient group have shown an average decrease of 40% in the urinary excretion of 17-ketosteroids.

There was no definitive fall of the iodine^{131} uptake of the thyroid gland at the end of the first week in the same high-dose group receiving irradiation with alpha particles, but by the end of 2 months two-thirds showed an average fall of 40%. This showed a close correspondence to results obtained in the same dose range with proton particles. The protein-bound iodine showed a concomitant decrease.

Total estrogens and the estrone, estradiol, and estriol fractions were determined on all patients by the revised method of Brown (Edinborough). Serial determinations, however, are available on only a relatively small number of the patients receiving high dosages of alpha particles. On all eight of these patients the total estrogens averaged a 60% fall from pre-irradiation levels within the first 3 to 4 months. An equivalent fall was found in estrone, estradiol, and estriol levels.

Clinical Management

Since there was no morbidity as a result of pituitary irradiation, those patients who were ambulatory could be treated on an outpatient basis and did not require hospitalization. Follow-up studies were done on all patients at intervals of 4 to 6 weeks in order to evaluate response to irradiation as manifested by a decrease in pituitary function, and to follow the clinical course of their disease.

Mammary Carcinoma

Replacement therapy did not constitute a problem with this group of patients. It was commenced when the measurement of urinary steroids and iodine uptake declined to low levels. On hypophysectomizing doses of irradiation, early signs of adrenocortical insufficiency were observed as manifested by anorexia, fatigability, nausea, vomiting, and in some cases by fall in blood pressure and by low-grade fever. These signs of adrenocortical insufficiency were well correlated with a fall in the levels of the 17-ketosteroids and the 20, 21-ketols. At high dose levels the signs of

adrenocortical insufficiency were almost predictable in their onset at the end of 3 to 4 weeks following irradiation. The patients in this category responded immediately to corticoid replacement therapy with disappearance of their signs of adrenocortical insufficiency. Diminished thyroid function was reflected in decreased I^{131} uptake, with a somewhat less marked fall in the level of the protein-bound iodine. The only complications following irradiation were encountered in those receiving the higher doses of irradiation. Most of these developed a mild transitory diabetes insipidus several weeks after irradiation. In those few cases with daily urine volumes in excess of 5 liters, satisfactory control was achieved with the use of pitressin tannate in oil. Many in this group also developed a transitory diplopia, and in two instances ptosis of one eyelid. These eye signs gradually disappeared over a period of several months.

Decrease in pituitary and target-end-organ function could not always be correlated with improvement in those patients with advanced metastatic mammary carcinoma. Some showed arrest or remissions, while in others the metastatic disease continued to progress despite similar evidence of depression of pituitary activity. Objective evidence of arrest or remissions was obtained in both osseous and soft-tissue metastases except in those with marked liver or brain invasion. Twenty-nine patients of the group of 82 with metastatic carcinoma irradiatied with high-energy particles are now living. Ten of these are living for more than 1 year following irradiation, the longest now being 4 years postirradiation. Eight of these are in an excellent state of remission. The largest and most beneficial response was obtained in the group of patients receiving the highest amounts of irradiation, which ranged from 26,000 to 30,000 rad.

Acromegaly.

Seven patients with acromegaly received irradiation to the pituitary with alpha particles in dosages ranging from 4,000 to 7,200 rad. Those receiving the lower amounts of irradiation were those who had previously received one or more courses of conventional 200-kv x-ray. Two of the group who had, associated with their disease, a diabetes necessitating management with insulin no longer required insulin following irradiation. In all these patients subjective symptioms of headache, loss of potency, and ease of fatgability were present and were difficult to evalutate. All but one patient claimed marked relief following irradiation. There was a fall in serum phosphorus in all patients receiving 7,200 rad.

Diabetes Mellitus

Eight patients with diabetes mellitus received alpha-particle irradiation in the dose range 18,000 to 24,000 rad. These patients had marked diabetic retinopathy and in some instances nephropathy. While downward adjustments in insulin dosage were necessitated in some patients following alpha-particle irradiation in the 18 — 22,000-rad dose range, no definite pattern of decreased insulin requirements could be ascertained. Marked change, however, occurred in those patients receiving the higher dosages. One patient who received the higher dose of 24,000 rad over an 18-day period, became hypersensitative to insulin and by the end of the fifth week postirradiation no longer required any insulin. Two patients who received the same high dose over a shorter period of time had a rapid onset of adrenocortical

insufficiency, which complicated the clinical management of their diabetes. With the institution of replacement therapy their diabetes was brought under control with less insulin. Since these latter patients were irradiated within the past few months it is still too early to determine whether their retinopathy and nephropathy will show beneficial responses.

Post-mortem examination have been made of 53 of the 60 deceased patients. Varying degrees of destruction of the pituitary gland were seen, depending on the dose of irradiation, the time over which it was given, and the length of survival. On dosages of more than 20,000 rad cellular necrosis was seen. With still higher doses damage to the pituitary without evidence of injury to the surrounding structures and cranial nerves became more marked, and necrosis of approximately 90% of the cellular elements of the pituitary was seen as early as the fifth day following the completion of irradiation. On all patients, even those receiving lesser amounts of irradiation, the degree of destruction was progressive with the length of survival so that on some of those surviving for many months necrosis of 95 to 98% of the entire gland was observed. In no case was there histological evidence of pituitary regeneration.

Direct injury to the cells of the pituitary as well as vascular damage within the gland was observed. No detectable injury, either grossly or histologically, was found in the brain or surrounding cranial nerve.

SUMMARY

High-energy protons and alpha particles from the 184-inch cyclotron at the Lawrence Radiation Laboratory of the University of California have been used for pituitary irradiation. One hundred and three patients have been irradiated. Most of these patients had metastatic mammary carcinoma, and promising effects have been shown on patients with this and various pituitary and hormonally dependent diseases. Peak doses of 14,000 to 30,000 rad have been delivered to the center of the pituitary. At the higher hypophysectomizing dose level, decreased pituitary function and marked gross and microscopic damage to the pituitary have been demonstrated. Decrease in pituitary function and the consequent effects on the target end organs were manifested in depression of the thyroid iodine^{131} uptake and decrease in 24-hour pituitary gonadotropins, urinary steroids, and estrogen excretion. These correlated well with clinical evidence of hypopituitarism.

BIOLOGICAL EFFECTS OF HEAVY-ION BOMBARDMENT

Tor Brustad

The heavy-ion linear accelerator (hilac) and the 60-inch cyclotron have been used to study radiobiological effects of beams of stripped nuclei of hydrogen, helium, carbon, oxygen, and neon. With this variety of particles an extremeley wide range of linear energy transfer (LET) is obtained.

The problem studied thus far are as follows.

a. Effects on Enzymes Irradiated in Vaccum

It has been shown that the efficiency per heavy particle in inactivating enzymes (lysozyme, trypsin, and DN-ase) increase with increasing LET, whereas the sensitivity per unit dose absorbed decreases with increasing LET.

Preliminary studies on lysozyme and its derivative, lysozyme methyl ester, seem to indicate that the inactivation of the enzyme is the result of inactivation either of those structural features necessary for the catalytic properties of the enzyme or those responsible for the combination of the enzyme with its substrate. Studies with the heavy-particle seem to indicate that the latter structural features represent about 10% of the entire lysozyme molecule.

b. Effects on Unicellular Organisms Irradiated Aerobicaly and Anaerobicaly

The organisms studied in greatest detail are *Saccharomyces cerevisiae* of different ploidy(from haploid to tetraploid) (Laskowski - Brustad) and *Shigella flexnerei*.

From the studies of radiation effects on yeast it appears that there are some recessive mutable loci which are especially sensitive towards the most densely ionizing radiations.

The effects observed on *Shigella flexnerei* show that the relative biological efficiency (RBE) of densely ionizing radiation, for this particular organism, under anoxic conditions exhibits a pronounced peak at about 175 Mev g^{-1} cm^2 and decreases rapidly for radiations having higher LET. Under aerobic conditions, the RBE however, decreases constantly with increasing LET.

The information supplied by these studies, in addition to its basic radiobiological interest, also will bear on the consequences of effects of recoil nuclei produced in an organism, and on the hazards of heavy primary cosmic-ray particles encountered in space travel.

MAMMALIAN RESPONSE TO X-IRRADIATION

John Hewitt

An attempt has been made to evaluate, in the rat, the radiosensitivity of individual animals by the depression of Fe^{59}uptake following x-irradiation. Two groups of 20 animals each were used. Following an initial dose of 100 r, the animals were arranged in order according to their ability to incorporate Fe^{59}into the red blood cells. They were subsequently given an LD^{-50} dose, but no relationship was found between survival following this latter dose and the depression of Fe^{59}-incorporating ability following 100 r.

In view of the ability of bone-marrow suspensions to increase survival of irradiated animals, a preliminary experiment was performed to examine the relationship between the injection of such suspensions and the postirradiation lipemia that is correlated with short-term death in the rabbit. A small group of rabbits was irradiated and the animals injected with filtered bone-marrow suspensions from donor rabbits at 15 to 30 minutes postirradiation. Of the group, two became lipemic at 30 hours postirradiation. This was the number expected at this dose (800 r). Thus there is no reason to believe that bone-marrow suspensions will inhibit postirradiation lipemia. It seems reasonable to believe, then, that the early deaths, which are a characteristic of the survival pattern in the rabbit, will not be prevented by bone-marrow injections. A larger number of animals would be needed to test this point.

WHOLE-BODY HUMAN COUNTER AND LOW-BACKGROUND COUNTING FACILITY

Thornton W. Sargent

Architectural plans for new construction within Donner Laboratory to provide temporary space for the Human Whole-Body Counter and other low-background counting have reached the final design stage. The location is the most favorable possible within Donner for shielding from the beam of the 60-inch Crocker cyclotron and other high-level sources.

Preliminary planning is under way on a permanent solution to the increasingly difficult problem of obtaining a location free from the variable influence of man-made radiation and the constant level of activity from the earth and cosmic rays. The plan consists of a complex of counting rooms, shielded by high-density low-background concrete, at the end of a 100-ft deep shaft in the ground.

The 100-channel analyzer has arrived and is in operation with its associated amplifier, data-readout equipment, and a small NaI crystal. The large NaI crystal and low-background photomultiplier will be installed in a low-background assembly under construction in our machine shop. Most efficient use of the entire gamma-ray spectrometer for humans must await construction of a steel room, now also in final design stage, which will be assembled in the temporary location mentioned above.

The Whole-Body Human Counter will be used for quantitative and qualitative diagnosis of accident cases involving radioactive materials, either occupational or resulting from a disaster in the area. On a routine basis it will be used for studies of natural and fallout activities in humans and animals, body composition by K^{40} activity and other isotopes, and many other medical researches which will be made possible for the first time because this device will detact extremely low levels of isotopic tracers in humans.

Because of the vast amount of data that can be accumulated in a short time with this device, plans are under way for rapid automatic data storage and computing machines.

Preliminary plans are under way for a 2π human counter, probably using a plastic scintillator, which will make possible very short counting times. The plastic scintillator is expected to produce better gamma-ray energy resolution and hence better isotope identification than liquid scintillators, although still inferior to NaI in this respect. Such a device will be extremely useful in studying large populations for body composition or fallout activity(as is currently being done at Los Alamos), or in case of large-scale disasters involving radioactivity.

EFFECTS OF pH AND ANOXIA ON GROWTH AND X-RAY SENSITIVITY OF E. COLI, B/r

Thornton W. Sargent

In spite of the wide variety of radiochemical reactions postulated as responsible for radiobiological damage, and the probable dependence of the rates of some of these reactions on hydrogen-ion concentration, very little work has been done on the effects of pH, during and after irradiation, on the radiosensitivity of microorganisms.

Investigations of such effects with Escherichia coli, B/r, in this laboratory have shown that there is little or no effect of pH on survival either during x-irradiation or postirradiation incubation. This is evidence against the theory of intercellular indirect action, but gives no information about radiochemical reactions inside the cell, where control of pH is not possible at least by the methods used here. However, growth of this organism at different pH values produced interesting information on another aspect of radiobiology.

Previous workers had found not only that E. coli, B/r, was protected by anoxia during irradiation, as were other organisms, but also that anoxic conditions during growth before irradiation conferred protection independently of the gas present during irradiation. The latter effect was attributed to an increased anoxia produced inside the cell by anaerobic growth, although no similar effect was found in any other organism.

E. coli when grown anaerobically with glucose normally causes the medium to become acid. It was found here that if this growth is forced to occur under alkaline conditions the protection of anaerobic growth disappears, independently of the gas present during irradiation. It thus seemed probable that protection by anaerobic growth was independent of the "oxygen effect" known to occur during irradiation. Aerobic growth at different pH values caused no differences in radiosensitivity.

Cytological and nucleic acid determinations demonstrated that anaerobic-acid conditions produce multinucleate cells of E. coli, while anaerobic-alkaline-grown cells have fewer nuclei per cell. Thus the resistance conferred by anaerobic growth of this organism can be attributed to a multiple-hit requirement (according to classical target theory), rather than to intracellular oxygen depletion as assumed by previous workers.

PARAVENTRICULAR NUCLEI STUDIES

Orland K. Anderson, Julian P. Henry, and
Gilles LaRoche

Four adult male dogs (beagles) were irradiated to study the possibility of aiming the collimated beam of accelerated alpha particles on the parventricular nuclei of the hypothalamus.

Three dogs were autopsied at various times after irradiation in order to determine the nature and site of the lesion. Histological observations show that severe damage of nervous tissue occurs within 1 month after the animal has received 20,000 rads per nucleus.

From this work, it appears that dogs can survive at least 3 months after this treatment.

Addiational work on the fractionation of the above-mentioned dose is being carried out in two other dogs. Extensive clinical blood and urine evaluations are carried out in order to correlate hypothalamic damages with functional disorders.

HEMATOLOGY, CIRCULATION, AND BLOOD BIOCHEMISTRY

RADIOSOTOPES IN CLINICAL RESEARCH

Myron Pollycove

Iron and Red-Cell Kinetics in Man

During this 6-month period Fe^{59} was used to study 35 patients with a wide variety of blood disorders. Red cell volume was determined with P^{32}. The production and destruction of red blood cells was localized by simultaneous serial measurements, for two weeks of radioiron in plasma, red cells, spleen, liver, and bone marrow. In some patients abnormal iron deposition in storage form was detected and quantitated. These measurements were used to select appropriate therapy, which was successful in many cases.

Survival of Human Red Cells, White Cells and Platelets in Blood

Ten patients with anemia, leukopenia, or thrombycytopenia were studied with P^{32} diisopropylfluorophosphonate. This compound is particularly useful in the dosage used for it quickly labels all three elements of circulating blood without elution, relabelling, or detactable damage to these elements. The results of these studies were correlated with the clinical course, routine hematologic studies, and iron kinetic studies.

Intermediary Metabolism of Glucose and Uric Acid

C^{14}-glucose and C^{14}-uric acid were given intravenously in tracer amounts to nine normal subjects and patients. Following injection serial measurements were made to determine specific activities of glucose of uric acid in the body and the specific activity of $C^{14}O_2$ in the expired breath. In this way the size of the body pool, turnover rate, and the rate of oxidation to carbon dioxide were determined. Intermediary glucose metabolism was studied in normal subjects and diabetic patients. These subjects were studied under control conditions without administration of any drug. Subsequently they were restudied after the administration of insulin or one of the new oral compounds that lower blood sugar such as orinase and DBI. One patient was studied before and after irradiation hypophysectomy. Preliminary results indicate that:

(a) Uric acid is not entirely and end product of metabolism, but approximately 10 to 15% of the compound is oxidized within the body to carbon dioxide.

(b) Orinase and DBI, like insulin, decrease endogenous hepatic release of glucose from glycogen but, unlike insulin, do no appreciably increase glucose oxidation.

Heart Function

Cardiac output and blood volume were measured with I^{131}-albumin in four patients with polycythemia or anemia. A new method involving external measurements of heart radioactivity was compared with the established method of measuring radioactivity of blood obtained by arterial

cannulation. The heart outputs determined simultaneously and independently in this way were found to be in good agreement. Preliminary results indicate that cardiac output may be accurately determined by external measurement over the heart without arterial puncture. This new procedure may eventually become adapted for routine clinical use in hospitals as a bedside procedure.

Miscellaneous Clinical Research Studies

As a semiroutine aid to our clinical research in hematology, Co^{60}-vitamin B_{12} absorption tests were made in eight patients to aid in the differential diagnosis of pernicious anemia. Gastrointestinal bleeding was detected and quantitated in five patients by collection of stool specimens for a period of 4 weeks and measuring the amount of Fe^{59}-labeled red blood cells present. I^{131} thyroid uptake tests and body scans have been continued as a semiroutine study in 25 patients. This has complemented our other clinical research in these patients with thyroid and hematologic disorders, and in patients in whom radiation hypophysectomy is done (metastatic breast carcinoma, diabetic retinitis, etc.) Improvement of the scanning device has continued concurrently with these studies.

ACTIVATION-ANALYSIS STUDIES OF THE ELEMENTAL COMPOSITION OF BLOOD

John W. Gofman

In the past interval quantitative consideration has been given to the possibilities for development of an instrumental method for activation analysis for studies of the elemental composition of blood. Yield data in disintegrations per second have been calculated per gram of element for all the isotopes produced by the (n, α) reaction and for which cross-section data are available. Such yields are calculated for activation times of 5 minutes, 10 minutes, 1/2 hour, 1 hour, 2, 4, 8, 16, and 24 hours, and 3, 5, and 10 days. The extent to which chemical processing, with its attendant tedious steps, can be avoided is being evaluated currently. Major obvious limitations reside in Compton effects from constituents with high gamma ray energy and high abundance chemically in the blood. Exploration of Compton suppression and other alternative techniques are in progress.

The medical and biological applications possible with such a system would be very extensive, especially if chemical processing could be minimized.

It is planned to continue the quantitative evaluation of this problem in the next period.

ANALOG MODEL TO ILLUSTRATE A HOMEOSTATIC MECHANISM ACCOUNTING FOR THE PHYSIOLOGICAL REGULATION OF BODY FLUID VOLUME

Howard Yanof and Ernest L. Dobson

That animals are able to maintain both the composition and the volume of the body fluids at a relatively constant level is well known. In 1935 Verney[1] postulated the existence of osmoreceptors in the brain which control the output of antidiuretic hormone (ADH) from the posterior pituitary gland in response to changes in tonicity of the blood. Dobson[2] has postulated a homeostatic mechanism to account for readjustment in body fluid volume in response to purely quantitative volume changes. This hypothesis explains the homeostatic response to addition of isotonic saline, blood plasma, etc.

Under isotonic conditions ADH is released from the posterior pituitary at a relatively constant rate. A constant fraction of the ADH in the blood passing through the liver is inactivated. One might say that the concentration of ADH entering the liver is reduced logarithmically, and therefore a steady-state concentration of ADH is reached. If a volume of fluid is added to the body the concentration of ADH is reduced, i.e., an increase in body fluid volume is accompanied by an increase in the liver blood flow,[3] for when the liver blood flow is increased a larger amount of ADH is destroyed in a given time for a given concentration. Thus a new lower steady-state concentration of ADH is reached. The smaller the concentration of ADH in the blood the more urine is excreted by the kidney. Therefore by addition of a volume of isotonic body fluid an increase in the excretion of urine by the kidney is achieved. Thus the body tends to maintain a constant volume of body fluid.

An hydraulic model of this system is being disigned. This analog will operate as follows:

A cylindrical tank holding approximately 4 liters will represent the body fluid space. A dye, neutral red, dripped into a tank at a constant rate will simulate the controlling hormone. There are two separate outlets at the bottom of the tank—one recirculates the fluid back to the top of the tank, and serves to represent the liver blood flow; the other does not recirculate the fluid and represents excretion by the kidney. A supply of fresh water is provided at the top to correspond to water intake by the animal.

The first outlet flows into a large funnel. The flow rate is proportional to the height of the fluid in the cylinder. Thus an increase in the volume of the fluid in the cylinder, which increases the height of the fluid in the tank,

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[1] E. B. Verney Proc. Roy. Soc., London, Series B 135: 25 (1947).

[2] E. L. Dobson, Homeostatic Mechanisms, Brookhaven Symposia, No. 10 (1957).

[3] E. L. Dobson, et al., The Regulation of the Body Fluids, UCRL-8265, April 1958.

causes an increase in "liver blood flow." At the bottom of the funnel a pump recirculates the fluid through an ion-exchange resin back into the top of the tank. The ion-exchange resin removes the dye from the fluid. This corresponds to the removal of ADH from the blood by the liver. Thus an increase in the height of fluid in the tank has a net effect of increasing the flow of fluid through the resin and thus reducing the concentration of dye in the tank. This is analogous to an increase in body fluid volume, having a net effect of reducing the steady-state concentration of ADH by increasing the liver blood flow.

The other outlet represents the kidney. A matering valve opens the outlet in proportion to dilution of the dye in the tank, corresponding to the response of the kidney to a decrease in ADH concentration. In the analog a photocell transduces the dilution of the dye to a signal which causes the metering valve to open in proportion to the signal. Thus an increase in the volume of the fluid in the tank has the effect of increasing the flow from this outlet in the same way as increasing the body fluid volume has the effect of increasing excretion by the kidney. This tends to stabilize not only the volume but also the flow through the liver of the animal--or its analog, the resin column in the model.

This analog model serves as a visual display of the proposed homeostatic mechanism. In addition it allows a study of the effect of variation of parameters on the stability of the constant volume in the tank, which should lead to the design of physiological experiments to test their validity *in vivo*.

VENO-ARTERIAL PERFUSION AS THERAPY FOR THE FAILING HEART

Marvin Bacaner

with

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Fifty-four experiments were performed to quantitate the influence of coronary blood flow (CBF) upon performance of the in-situ failing dog heart. Heart failure was induced by progressive pulmonary artery constriction (PAC) until systemic blood pressure, cardiac output, CBF, and left ventricular stroke work (LVSW) were significantly reduced.

After establishment of failure, the coronary arteries were perfused (PAC unaltered) by shunting venous blood to a femoral artery by pump, or by constricting the thoracic aorta.

By external counting over the exposed heart

(a) relative CBF was determined by disappearance rate, $T_{1/2}$ of 0.1 ml Na^{22} injected into the left ventricular myocardium,

(b) cardiac output was measured by the arterial-dilution curve of I^{131} rose bengal. Data were programmed for the IBM 650 digital computer to calculate LVSW and myocardial power.

During failure, LVSW decreased, often to ventricular standstill, with concomitant decrease in CBF. Coronary perfusion by either method was invariably accompanied by dramatic changes

(a) the markedly dilated, feebly contracting, occasionally asystolic heart rapidly decreased in size and began contracting vigorously,

(b) the Na^{22} $T_{1/2}$ indicated a marked increase in CBF associated with a striking increase in LVSW which could be maintained by perfusion (when perfusion was stopped the heart rapidly failed, but improved when perfusion was restarted),

(c) about one time in five, cardiac performance remained well sustained after cessation of perfusion (despite unchanged PAC), indicating an acquired capacity to adjust to work loads that were disabling when CBF was inadequate,

(d) rarely (5%), when PAC was extreme, LVSW increased transiently, then fell again despit perfusion.

These results demonstrate a crucial relationship between CBF and cardiac performance, and indicate that contractile power, diastolic volume, and adaptive mechanisms to increased loads are critically influenced by CBF. The veno-arterial shunt procedure is under clinical trial in myocardial failure and shock where increasing CBF might improve cardiac performance.

STUDIES ON THE HOMEOSTASIS OF CARDIOVASCULAR FUNCTIONS

Marvin Bacaner and Ernest Dobson

The quantitative demonstration in this laoboratory that the ability of the heart to perform external work is critically related to the adequacy of its coronary blood supply has prompted a study of the mechanism of this relationship. In addition to providing oxygen and metabolic substrate to the myocardium, the coronary blood flow washes out the products of cardiac metabolism in its venous drainage. These metabolities are of interest because they may represent a means of conveying information about the "state" of cardiac metabolism to other body structures involved in regulating and maintaining cardiovascular homeostasis (i.e., autonomic nervous system, adrenals, kidneys).

Visscher has shown that the cardiovascular system is designed to give highest priority to the blood-flow requirements of the heart (and brain). It seems likely that the concentration and biochemical nature of cardiac metabolities would carry information to reflect, among other things, coronary blood flow that is inadequate in relation to the work demands upon the heart. When coronary blood flow is inadequate, the coronary venous blood may contain substances capable of eliciting appropriate responses from the cardiovascular system in defense of optional cardiac metabolism through an increase in coronary blood flow. This could be accomplished by increasing coronary perfusing pressure (aortic mean pressure) and (or) coronary artery dilatation. An increase in coronary perfusing pressure may be accomplished by increasing myocardial contractility and (or) peripheral vasoconstriction. Thus it is pertinent to consider whether cardiac metabolites contain pressor factors, how they function, and their composition.

In previous experiments we have observed that plasma obtained from the coronary sinus blood of a failing hypoxic dog heart with inadequate coronary blood flow contained a substance, not present in unmixed venous blood, which elevated the blood pressure of an anesthetized recipient dog. This would be analogous to the pressor substance derived by Goldblatt from the hypoxic kidney, and may have a role in hypertension related to coronary heart disease.

The pressor effect on the whole dog requires large quantities of the test material. In order to further characterize and study the pressor material, we required a much more sensitive assay. Consequently we adapted a technique which utilizes the effect on the contractility of driven rat ventricle strips as an assay for this pressor substance. The ventricle strips are immersed in a nutrient bath to which is added the test plasma from the coronary sinus of the failing dog heart preparation as well as suitable control plasma. Progress to date has been largely limited to working out the assay technique, which is now fairly reliable.

DETERMINATION OF SERUM PROTEIN-BOUND IODINE

Ann Coxworth and Gilles LaRoche

Serum protein-bound iodine is now being determined routinely on Donner Pavilion patients.

The first procedure tried was a modification of the original Chaney method, in which the serum is digested with chromic acid. All iodine compounds present are thus converted to iodate. This is then reduced to iodine, which is immediately steam-distilled into basic solution. The trapped iodide is determined by its catalytic effect on the oxidation of arsenious acid by ceric ion, which is followed colorimetrically.

Difficulties were encountered both in the design of the distillation apparatus and in the nature, concentration, and purity of the reducing agents. It is intended to return to this problem in the near future, but meanwhile a simplified and less sensitive method is being used. This is essentially a procedure devised by Bodansky et al.[1]

Digestion is carried out with chloric acid in the presence of a small amount of dichromate, which maintains iodine in the iodate form. There is no distillation stage, and the colour reaction is carried out in the digestion vessel.

The details of the Bodansky method are being followed except in one respect. The recommended ratio of H_3AsO_3 to Ce^{4+} appears to be much lower than would be desirable. A relatively large excess of the reducing agent is necessary if the reaction is to follow simple pseudo first-order kinetics, which it must be assumed to do if the reaction rate is considered proportional only to the amount of catalyst present. Therefore, this ratio (on a molar basis) has been increased in our procedure from 1.5:1 to 20:1.

Both protein-bound and butanol-extractable iodine are being determined on patients and on a number of normal subjects.

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[1] Bodansky and co-workers, Am. J. Clin. Path 30, 375-83 (1958).

FRACTIONATION OF PLASMA PROTEINS FOR DETERMINATION OF PROTEIN-BOUND IODINE: STUDIES IN THYROID METABOLISM

Charles Oakes and Gilles LaRoche

The experiments of the past six months have attempted to further explicate information on the sites of iodine binding in rat plasma. The general guiding assumption for these experiments has been that iodine be bound not only to proteins but also to other substances in the plasma, notably free amino acids. The studies are being made to determine which proteins are involved and which other plasma components.

The experiments have followed two general paths. The first has entailed fractionation of I^{131}-labeled rat plasma of normal and thyroidectomized rats by methods of dialysis and ion-exchange resins. The second and current series of experiments is of an immunological nature in which rat-plasma-sensitized rabbit plasma is used to identify rat plasma components through diffusion-precipitin reactions.

The primary rationale for use of ion-exchange resins is fractionation of plasma proteins in the least "harmful" way; namely, the employment of that method which will least alter the natural configuration of proteins and their "bound" components. Several resins have been used, such as Dowex-50, Amberlite CG-50, Amberlite CG-45, and Cellex-CM. The last, a cellulose cation type, has proved the most successful in ease of use and binding capacity.

The general procedure comparing the I^{131}-labeled plasma of normal and surgically thyroidectomized adult male rats is as follows. The labeled plasma is first dialyzed to separate the proteins from plasma salts and amino acids. The activity of the dialyzate was usually 15 to 25%, depending on which of a variety of solutions was used in dialysis; this seems to corroborate the initial assumption that thyroxine may be free in the plasma or that substances other than proteins may be involved in thyroxine binding. The dialyzed plasma was then fractionated over the ion-exchange resin using, successively, various eluting agents of gradient pH and molarity, and the radioactivity in each eluted fraction was computed.

The employment of immunological techniques of plasma protein identification was desired as an alternative method to corroborate the findings by ion-exchange fractionation. The early experiments in this direction are patterned after the Oudin and Ouchterlony diffusion-precipitin reactions using rat-plasma-sensitized rabbit serum as the antibody reactant and rat serum as the antigen. Germ-free rats were used to obtain precipitation bands descriptive of plasma proteins. Current studies involve the fractionation of rat sera by "salting-out" techniques to obtain single purified proteins which are examined by the immunological, electrophoresis, and ultracentrifugation methods.

URINARY ESTROGENS

Anne Cleveland

Studies are being continued on urinary estrogen excretion in patients with advanced metastatic mammary carcinoma, to note any change in levels and fractions following irradiation hypophysectomy. We have elected to use the revised procedure of Brown (Edinburgh) for the determination of estrone, estradiol-17B, and estriol because it is one of the best available chemical procedures and because it is being used by various groups to assay urinary estrogens after surgical hypophysectomy. The method involves hydrolysis of the estrogen conjugates, ether extraction, partition between benzend-petroleum ether and water and alkali, saponification, preparation of the methyl ethers, fractionation by adsorption chromatography on alumina, development of the Kober chromogens, measurement of their optical densities at three wave lengths, and use of the Allen correction, which is dependent on the assumption that the interfering urinary contaminants gave a linear absorption curve in the critical range of 480 to 560 u, the region of the absorption maximum of the Kober chromogens of the estrogen methyl ethers.

The following data give the urinary estrogens of a woman with metastatic mammary carcinoma (Patient "M," age 34), and is a rather typical example of our results on various patients.

			Estrogens (in γ/24 hr)				
State	Time	Date	Estrone	Estradiol	Estriol	Total	Proportion, one: diol: triol
post-L-ooph	2 yr						
post-R-ooph	1 yr	2-3-58	10.24	7.00	2.33	19.57	52:36:12
Post-conclusion of irradiation	0 days	2-21	9.74	2.58	7.22	19.54	50:13:37
26,850 rads	41	4-3	6.58	2.74	7.20	16.52	40:16:44
2/3-2/21/58	74	5-6	6.40	5.40	4.20	16.00	40:34:26
(18 days)	111	6-10	3.14	2.20	3.74	9.08	35:24:41
	238	10-15	5.90	6.50	1.80	14.20	41:46:13
	329	1-14-59	2.66	1.33	2.94	6.93	38:20:42
	376	3-4	2.76	1.68	2.40	6.84	40:25:35

The estrogens were quite high post-oophorectomy, and the decrease in levels has been gradual over the period of a year after the completion of irradiation. A quantity of 6.84 γ of estrogens/24 hr is still present in the urine. The proportions of estrone, estradiol, and estriol are variable.

Evaluation of the above data on Patient "M" necessitates a consideration of the work of other responsible groups using the same method. Pertinent studies have to do with expected recovery by this method, recovery and metabolism of administered estrogens, relationship between estrogen values and remission of disease, and the excretion of estrogens post-oophorectomy-adrenalectomy and post-oophorectomy-hypophysectomy.

The following data of Brown indicate the recovery and metabolism of estrogens administered to a postmenopausal woman. Similar data were obtained from premenopausal women and from men.

One intramuscular injection	Total estrogens recovered in urine (% administered dose)	Total estrogens corrected for method loss (% administered dose)	Proportion of separate estrogens in the 15% recovered (% of the 15%)
1 mg estrone	12	15	Estrone 45 Estradiol 13 Estriol 42
1 mg estradiol	13	17	Estrone 41 Estradiol 18 Estriol 41
1 mg estriol	58	73	Estrone 0 Estradiol 0 Estriol 100%

Levels of estrone and estradiol in the urine reach a maximum the first day after injection of either one, so that it may be assumed that they are easily interconverted, but the peak value of the estriol into which they are partially and more slowly converted is not reached until the second day. During both the ovulatory and luteal phases of the menstrual cycle, estrone, estradiol, and estriol from endogenous sources are present in the urine in a 40:15:45 relationship, roughly the same as that following the injection of estrone or estradiol. The primary hormone is perhaps either estradiol or estrone or a mixture of the two.

Burke, Diczfalusy, et al, (Stockholm) find about the same mean urinary estrogen values in oophorectomized-adrenalectomzed patients whether or not they have a remission of their disease.

		Mean value (in γ/24 hr)			
	No.	Estrone	Estradiol	Estriol	Total
Patients with remission	10	5.3 (0-9.3)	1.7 (0-4.6)	5.5 (0-22.5)	12.5
Patients with remission	9	5.2 (0-27.3)	1.2 (0-7.0)	11.1 (2.4-33.9)	17.5

They suggest that remission when achieved may be due as much to nonspecific change of the hormone milieu as to the specific elimination of certain hormones, and speculate whether a specific hypophyseal hormone such as growth hormone may be involved.

Scowen (London) reports a study of fourteen patients with mammary carcinoma. Posthypophysectomy, the protein-bound iodine of the blood

fell to values of less than 1.5 γ/100 cc, and the patients had no 17-oxysteroid output greater than that derived from the replacement cortisone. Five of these patients showed continued estrogen excretion posthypophysectomy. The values ranged from 3 to 5 γ/24 hr in one patient and over 20 γ/24 hr in another. Dr. Scowen found the persistence or disappearance of estrogen no indicator of the course of the disease.

Much thought has been given by all workers using the Brown method to the nature of the small amounts of estrogen excreted by oophorectomized-adrenalectomized patients and by oophorectomized-hypophysectomized patients.

The fractions of estrone, estradiol, and estriol obtained by Brown's method were first proven to be indeed these fractions by Diczfalusy, using, counter-current distribution studies. Diczfalusy has recently undertaken similar studies on large pools of urine which had persistent low estrogen values post-oophorectomy-adrenalectomy. The usual fractions gave the Kober chromogens, but estrogen derivatives could not be prepared from them. An aliquot equivalent to 750 cc of the urine (which had a Kober chromogen value of 3 γ of estrone and 3 γ of estradiol /l) gave also a negative bioassay.

In some cases, however, estrogens that persist in small amounts post-oophorectomy-hypophysectomy have been biologically active. Bulbrook has raised the question of diet as an estrogen source. Crooke has suggested that the liver may secrete a limited amount of estrogens. Zander has observed that progesterone administered to postmenopausal women is retained in the body fat. Perhaps estrogens are also retained.

Dr. Marrian concluded the most recent Edinburgh Hormone Conference with the statement, "The problem of the source of urinary estrogen excreted by ovariectomized patients after adrenalectomy or hypophysectomy is clearly one that needs further careful study."

ATHEROSCLEROSIS STUDIES

Wei Young

General Metabolic Factors: Lipoproteins

Rabbits fed with cholesterol (2%) and oil (6%) develop a measurable atherosclerosis within 2 months. In the course of the experiment blood samples were taken at the beginning of the experiment, and after 1 month and 2 months. Serum lipoproteins were analyzed by ultracentrifuge. The results are shown in Table I.

Table I

The relationship between atherosclerosis and lipoproteins in 73 rabbits with median aortic grading 4.7

Lipoproteins S_f Class	Lipoproteins mg%	Significance of correlation between individual aortic grading and lipoprotein values
0—15	455	0.05
15—30	943	0.001
30—100	1320	0.01
100—400	840	N. S.

From the table one can see that there is a very high correlation between atherosclerosis and the S_f15—30 class of lepoproteins, while there is little correlation with the S_f100—400 class. The relationship between atherosclerosis and S_f0—15 and S_f30—100 classes are at 5% and 1% levels respectively.

Local Factors

a. In order to learn more about the local factors, the major effort has been translating vast amounts of quantitative measurements of human coronary and cerebral arteries into meaningful parameters. Thus one can investigate the local factors on a firm basis. Analysis of the interrelationship among various sections has been carried out to evaluate the role played by the local factors. The data will be published soon.

In the analysis of quantitative measurements of different layers of the arterial wall, we have found a strong inverse relationship between the amount of intima tissue and the amount of media in the artery (Table II). It appears that as the intima increases it encroaches upon the media. This relationship drops off sharply as the artery becomes smaller (radius less than 1 mm). With further drop in size this relationship becomes nil.

This interrelationship in various sizes of arteries could be explained

Table II

The interrelationship between area of intima and area of media layer in arteries

Number of Arteries	Mean size (cm^2)	Intima (cm^2)	Media (cm^2)	Pearson r	P
78	524	133	103	-.625	< .001
118	473	107	99	-.479	< .001
66	436	110	83	-.593	< .001
74	412	103	86	-.567	< .001
85	387	108	73	-.727	< .001
80	362	89	71	-.551	< .001
89	337	85	70	-.564	< .001
132	313	68	66	-.559	< .001
139	286	59	63	-.409	< .001
164	261	47	59	-.457	< .001
182	235	41	53	-.315	< .001
197	210	34	48	-.234	< .01
79	194	34	45	-.226	~ .05
150	182	25	41	-.036	N. S.
206	138	17	33	-.017	N. S.
187	112	14	26	-.059	N. S.
123	88	10	21	-.123	N. S.
54	63	7	15	.005	N. S.

by the fact that there is an atherosclerosis gradient, i.e., the smaller the artery, the less the degree of atherosclerosis. The inverse intima-medial relationship breaks at about I/E = 20. At such a value the artery still can handle the flow demands without much stress on the arterial wall. However, once the I/E value is above 25, because the luminal size must be maintained to meet the flow demands, the intravascular pressure presses the intimal tissue into the realm of muscle layer.

b. An experimental model has been set up in order to verify hydrodynamically the pathological as well as experimental findings on the stragetic anatomic spots of the artery.

PHYSICAL CHEMISTRY OF LIPOPROTEINS

Frank T. Lindgren and Alexander V. Nichols

In connection with a continuing basic program of the physical chemistry of the serum lipoproteins in states of health and various metabolic abnormalities a gas-phase chromatographic apparatus has been developed. This unit, similar to the type recently described by Lovelock, is designed primarily for long-chain fatty acid methyl ester analysis. Chromatography at approximately 200°C is achieved by using silicic acid or firebrick as a support media in which is impregnated a suitable liquid phase, such as acid polyesters of diethylene glycol. Argon of high purity is used as the carrier gas. Detection of the fatty acid methyk esters is accomplished by a strontium-90 radiological detector of extremely high sensitivity. For instance, samples containing mixtures of various fatty acid methyl esters of the order of 50 micrograms may be completely analyzed quantitatively for each component fatty acid methyl ester by this appartus.

At present this unit is undergoing calibration studies and shortly will be in full operation. It is planned to study the chemical characterics of the fatty acids in each fatty-acid -containing chemical fraction (cholesteryl esters, glyceryl esters, phosphatides, and unesterified fatty acids) present as part of the structure of the serum lipoproteins. Serum lipoproteins isolated ultracentrifugally from the sera of normal individuals and of patients with various disease states will be studied. Further, the effects of diet, known to influence the levels of the circulating serum lipoproteins, will be investigated to determine what chemical changes in the fatty acids may be involved.

SERUM PHOSPHOLIPIDE STUDIES

Gary Nelson

A study of the serum phospholipides of humans with respect to the serum lipoprotein class has been begun. The serum lipoproteins are divided into three groups by density in the ultracentrifuge. The lipoprotein classes correspond to Gofman nomenclature as follows: Class I, density less than 1.007 g/ml, S_f^o 20—400; Class II, density greater than 1.007 and less than 1.063, S_f^o 0—20; Class III, density greater than 1.0075 and less than 1.21, HDL-2 and HDL-3. The phospholipides are separated from the total lipid extract of each class by silicic acid column chromatography and quantitatively determined by infrared spectrophotometry. Preliminary results have indicated the following phospholipide distributions (in percent):

Phospholipide	Class		
	I	II	III
Caphalin	7	5	6
Lecithin	79	73	83
Sphingomyelin	13	21	11

INFRARED SPECTROMETRY AND LIPID CHEMISTRY

Keith Freeman

Statistical evaluation of serum lipid measurements for the group of 43 multiple sclerosis patients has been completed. The results have been described and will be submitted in a report. The measurements were made by the novel infrared spectrophotometric method developed in this laboratory. Similar measurements on the sera of 37 healthy persons provided determinations of total lipids, total cholesterol, total phospholipids, and esterified fatty acids as a basis for comparison. The over-all average value for each of these lipid components was slightly higher in multiple sclerosis than in normals. However, these differences were largely eliminated when the difference in the average ages of the two groups was taken into account. For age-matched subgroups (30–39 years) the largest remaining difference was in the phospholipids, the average for multiple sclerosis being 243 mg% and the average for normals 224 mg%. This could not be shown to be statistically significant at the 5% probability level. Although there are conflicts in the literature regarding serum lipid abernations in multiple sclerosis, our finding of no significant difference from normality is in agreement with the majority of recent evidence.

Data are still being accumulated on serum lipids in Hodgkin's disease.

An experiment with cholesterol-fed rabbits has been repeated. It had been carried out earlier with the intent of comparing the effects of various added dietary fats on the extent of aortic plaque development that results from cholesterol feeding. Owing to the small number of animals used, and the variability of the results, no difference could be shown between olive oil and a catalytically isomerized olive oil. (In the isomerized oil most of the double bonds have been converted from the natural cis configuration to the trans configuration.) In repeating this experiment we have found this time a sharp distinction between these two oils with respect to their apparent influence on aortic deposition. When the results of three different experiments are pooled, the distributions of aorta grades (extent of plaque formation) associated with the two oils are skewed in opposite directions, but application of the χ^2 test shows that the difference is not statistically significant. Serum lipid and lipoprotein measurements are also being correlated with these results.

ELECTRON-MICROSCOPE STUDIES

Thomas Hayes and James Koehler

The electron microscope has been used in a continuing study of the physical properties of the serum lipoprotein macromolecules and also in the study of the ultrastructure of tissue culture cells.

A determination of lipoprotein molecular weight was carried out by using the direct particle-counting technique. For this purpose, a very narrow $S_f 6-8$ lipoprotein band was isolated by using a sedimentation equilibrium salt gradient. The lipoprotein macromolecules were fixed in 1% buffered osmium tetroxide solution and sprayed along with polystyrene latex marker molecules onto a Formvar membrane. After shadowing, entire drops were photographed in the electron microscope and the numbers of polystyrene and lipoprotein particles in each drop were determined.

To determine the lipoprotein molecular weight, the volume of a drop is calculated by counting the number of polystyrene latex spheres present in the drop. This volume is used to determine the weight of lipoprotein present in the drop from the known lipoprotein w/v concentration. The wight of the lipoprotein present is then divided by the number of lipoprotein particles present to give the weight per particle, and this is converted to molecular weight.

The average value for the molecular weight as determined by this method is 6.9×10^6 molecular weight units. This value would tend to support the hypothesis that the particle seen in the electron microscope is a dimer or higher multiple unit of the macromolecule observed in the ultracentrifuge and by light scattering, where the molecular weight has been set at about 3×10^6 molecular weight units.

Tissue culture cells of the stable cell line HeLa have been examined in the electron microscope. Work has been done primarily on normal cells in order to establish a base line for further experiments. Some experiments have been carried out with cells incubated in lipoprotein media, and work is in progress on x-irradiated cells. No clear-cut results have yet been obtained on these experimental modifications of the cells. The cultures were grown on glass slides and imbedded in methacrylate in such a way that sections could be cut parallel to the plane of the original glass culture slide. This method is of advantage in investigating intercellular processes and in locating dividing cells with chromosomes displayed in the plane of the slide.

AUTORADIOGRAPHIC STUDIES OF CELLULAR PROLIFERATION IN BLOOD-FORMING TISSUES

John C. Schooley, Lola S. Kelly, and B. J. Bryant

Autoradiographic experiments are being extended, using tritiated thymidine as a specific DNA precursor to obtain information on the proliferating cells of the blood-forming tissues of rats and mice. The temporal pattern of cell labeling in lymphoid and myeloid tissues of mice and the significance of these observations with regard to the kinetics, generation times, and ultimate fate of the cells comprising these tissues are subjects of work in press. Various other experiments are in progress to supplement these previous observations. The *in vitro* incorporation of tritiated thymidine into cellular suspensions of lymph nodes, thymus, lymph, blood, and bone marrow are being determined and compared with the *in vitro* findings. A method of perfusing the isolated hind limb of the rat has been developed, and experiments are in progress to obtain information about the action of various erythropoietins on different stages of maturation in the erythroid series of bone marrow. In addition to these experiments, studies are in progress on the fate of the lymphocyte using parabiotic rats and a transfusion technique whereby labeled thoracic-duct lymphocytes are transfered from a donor to a nonlabeled recipient rat over an extended period. Examination of autoradiographs of tissues obtained from recipients at various times after transfusion may give information on the ultimate fate of thoracic-duct lymphocytes.

ERYTHROPOIETIN STUDIES

Donald C. Van Dyke

When animals or human beings become severly anemic, a hormone (erythropoietin) which stimulates red blood cell production appears in the blood and urine.

The development of methods of assay for erythropoietic activity utilizing radioactive isotopes has greatly stimulated investigation into the possible sources of erythropoietins and the extent to which they are responsible for the normal regulation of erythropoiesis. Elevated titers of erythropoietin occur frequently when erythropoiesis is either greatly depressed or greatly accelerated, such as in severe cases of aplastic or hemolytic anemia. It is from the urine of such patients that potent erythropoietic extracts have been obtained. The clinical, biological, and chemical characteristics of human urinary erythropoietin have been studied. This glycoprotein material has been used to produce polycythemia in a variety of mammals. Normal monkeys have been made to increase their circulating red cell mass by 60 to 80%. However, these red cells appear to have a greatly reduced survival in the circulation.

New sources for erythropoietin, its possible therapeutic usefulness, and the relationship of erythropoietin to human disease are under investigation.

CONTINUOUS FREE-BOUNDARY ELECTROPHORESIS

Howard C. Mel

In biophysical separation processes one often has need for rapid processing of large liquid volumes under mild handling conditions, and some sort of continuous-flow procedure frequently appears to offer the most promise in this respect. To make this spearation electrophoretically requires a method for stabilizing the system against mixing of the components, and most methods advanced thus far have involved the use of a medium such as paper, glass powder, or gel. Such methods are generally relatively slow and they also may add difficulties from adsorption on the surface. A new compact apparatus has been developed in this laboratory which can effect such continuous separations in the free liquid state. In the conductivity-density gradient conditions used thus far, dye separations have been effected at sample flow rates varying from 0 to 5 cc per minute. A new collection system in combination with other factors permits processing without thermostating for periods of at least up to 1 hour (the longest tried so far) with stabilized flow patterns maintained during this time. Further possibilities of the method are being explored.

CELLULAR BIOLOGY

SOME FACTORS THAT MODIFY THE EFFECTS OF DENSELY IONIZING RADIATIONS ON YEAST

Thomas Manney

The heavy-ion linear accelerator (hilac), which produces a variety of radiations having a broad spectrum of values of linear energy transfer (LET) is well suited to the study of radiation damage in yeast and other organisms. Certain chemical and physical conditions, such as dehydration and anoxia, are known to modify the effects of lower-LET radiations on living cells. Observations of variations in these modifications at higher LET values may yield information about chemical and physical mechanisms through which these radiations exert their various effects.

Haploid yeast (Saccharomyces cerevisiae), partially dehydrated with 6.9 M glycerol, and untreated cells were irradiated in atmospheres of air, O_2, and N_2. The radiation sources (hilac and x rays) represented a range of LET from 40 to 5000 Mev/g cm^{-2}. Exponential survival curves were shown in all experiments, permitting results to be expressed as dose-reduction factors (DRF).

Both dehydration and anoxia led to increased survival over the entire range of LET studied. The DRF for both effects, however, was significantly smaller with the more densely ionizing heavy ions. Anoxia gave less protection for a given radiation than did 6.9 M glycerol, the two DRF-LET curves being approximately parallel, but apparently not proportional.

There is some evidence that these two protective effects are entirely independent of each other, although this has not been demonstrated conclusively at high values of LET.

With additional data, including survival curves at other glycerol concentrations and with other dehydrating agents, these results could permit calculation of the importance of cellular water in the radiobiological action in yeast of densely ionizing radiation. In addition to their immediate fundamental interest, such results could relate to the problem of protecting organisms from effects of heavy primary cosmic-ray particles.

POSITRON CAMERA

Hal O. Anger

The scintillation-camera principle has been applied to a new instrument, called the positron camera, which produces an image of the distribution of positron-emitting isotopes in a human or other subject. The positron camera will first be applied to the detection and location of brain tumors. The tumors will be treated by irradiating them with the alpha-particle beam of the 184-inch cyclotron. Attempts will also be made to show pancreatic tumors, liver tumors, and possibly the thyroid gland by using a positron-emitting isotope, iodine-124.

The instrument employs a new principle for obtaining images. It does not require a pinhole aperture or other collimator between the subject and the scintillation-camera crystal. It utilizes a gamma-ray counter which is placed on the side of the subject opposite from the scintillation-camera crystal and is connected to a coincidence circuit which detects the decay of positrons in the subject. An image of improved clarity is produced.

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RADIATION DETECTION AND PROTECTION

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HEALTH CHEMISTRY

Nelson B. Garden in charge

Reported by Rosemary J. Barrett

CONSULTATIONS AND SERVICES

In order to enable Livermore operations to be completed before the nuclear weapons testing moratorium, which commenced November 1, 1958, some two dozen Berkeley Health Chemists were made available to Livermore for an intensive 5-to-6-week period of intensive work prior to this date.

The relationship between the Lawrence Radiation Laboratory Fire Department, the Berkeley City Fire Department, and Health Chemistry has been revitalized; there has been reaffirmation of the principle that fires in radioactive areas must be fought with a Health Chemist present. An administrator of the Berkeley Fire Department made an inspection of the radioactive areas and observed the presence of the symbol so marking them.

During this period requests for drawings of Health Chemistry equipment and descriptions of Health Chemistry philosophies and techniques for confinement and control of radioactive materials were received from individuals and institutions in foreign countries and from domestic institutions as follows: France, Switzerland, West Germany, Japan, Israel, Australia, Yugoslavia, Italy, Phillips Petroleum Company, Brookhaven National Laboratory, Princeton University, Argonne National Laboratory, the University of California at Los Angeles, General Electric Company (San Jose), University of Colorado, Westinghouse Electric Corporation (Pittsburgh), Oregon State College, Babcock and Wilcox (Lynchburg, Virginia), Montana State Board of Health, Stanford Department of Physics, John Hopkins University. Many of the requests were preceded by visitors from the requesting institutions.

CONSTRUCTION AND RENOVATION OF HOUSING

Health Chemistry began during this period to participate in the planning of (a) the housing and facilities for the 88-inch cyclotron and (b) the addition to Chemistry Building 70. Recommendations have been made for such features as cave systems, ventilation, waste tanks, target-handling facilities, and pneumatic tubes for transportation of irradiated targets to laboratories.

Old Radiation Laboratory, on the Campus, was made ready for demolition by an outside contractor; Health Chemistry supervised the dismantling of the possibly radioactivity-contaminated rooms 106, 107, 108, and 209, used originally during the Manhattan Project period and subsequently in project research.

A hot laboratory on the mezzanine of Crocker Laboratory has been set up for use in processing bombarded material at this site; heretofore the material had to be taken to laboratory rooms in other buildings. Health Chemistry worked out specifications for the design of this facility.

Health Chemistry was engaged in the planning for and installation of additional laboratory facilities for the atomic-beam group in the basement of Le Conte Hall. The equipment installed required the addition of 2-in. lead shields.

Several rooms in the basement of Life Sciences Building were equipped for radioisotope work for the Bio-Organic Group, evicted from Old Radiation Laboratory prior to its demolition.

EQUIPMENT DEVELOPMENT

The major effort of the Equipment Development Group has been toward the provision of equipment in which four MTR-irradiated slugs will be processed for their Cf, Bk, E, and Fm content and for repackaging the residual Pu, Am, and Cm for re-insertion in the pile. In addition to the normal problems encountered with this material, this run will be further complicated by the presence of rather high neutron fluxes originating from the spontaneous fission of Cf^{252} and Cm^{244}--fluxes of the order of 10^8 neutrons per second. An empirical approach for the evaluation of neutron shielding materials for this work was made: high-density metal halide solutions were compared with lead glass and with rare earth glass for use as shielding windows. Laminated shields of lead-paraffin and uranium-paraffin were compared with water and with paraffin for shielding walls. Measurement of samples of the pure shielding materials compared favorably with published data, where available. Some interesting results were achieved when the materials tested were used in combination. On the basis of the figures obtained, dose-attenuation factors were computed for various thicknesses of the materials tested and recommendations for windows were made.

Health Chemistry collaborated with the 60-in. cyclotron crew in testing various television sets for use in 60-in cyclotron operations; a set has been purchased and is being installed. Extension of this investigation is under way to apply television in conjunction with remote handling of 6-in. -cave microchemical operations.

The 60-in. cyclotron "cat's-eye" target has been improved so that it can accommodate a larger area of target material. An additional feature is the installation of a helium cooling system, which operates on the front cover foil of the target as well as the back. This feature increases safety and makes more efficient use of the intense high-energy beams. This feature will be important in the design of targets for the 88-in. cyclotron.

A hydraulic system for remote-control automatic column loading for use in microchemistry of californium has been developed; the presence of spontaneous fission neutrons in hazardous levels in some of the large samples handled made this system necessary.

The atomic-beam group has continued to increase the levels of activity with which it works, necessitating the development of new capsule-opening and foil-slicing devices, to reduce exposure to personnel during these operations.

An improved technique for removing high-level waste from 2-in. lead caves through a sleeve and thence to a 30-gallon drum which has already been cemented into a 55-gallon drum has been introduced, minimizing possible exposure in this operation.

A mockup of the 6-in. lead caves, accurately reproducing a typical setup for processing pile-irradiated materials, has been created in Bldg. 5 for use in training and giving practice to personnel who will operate in future cave runs.

Maintaining the policy of keeping all radioactive work in enclosures required the fabrication of 50 units during this period. Many were standard units involving gloves or 2-in. lead shielding, and some were special items to provide atmosphere control or to furnish unusual mechanical processes.

Two reports have been or are being published on techniques developed by Health Chemistry: "Isolating Seal for Pressure Gages," (UCRL-8582) and "Adsorption of Xenon on Activated Carbon," both by H. P. Cantelow.

AIRBORNE-ACTIVITY CONTROL

Two special reports covering fallout data collected by this group were submitted at the request of the Division of Biology and Medicine of the U. S. Atomic Energy Commission, one report covering the period October 15 to November 10, 1958, and one for the period April 5 to November 15, 1958 (Hardtack I and II). The combined reports consumed about 16 man-weeks in data collation, drafting, and typing.

A standardized program of stack-sampling, employing locally designed and fabricated equipment, became operational; data gathering at 22 exhaust points of Chemistry Building 70 for both alpha and beta-gamma airborne contaminants has thus become routine.

Five general-purpose, 20-cfm gas scrubbers (for cave and allied operations) were completed and tested and are being installed for the forthcoming cave run, wherein the four MTR-irradiated slugs mentioned above will be processed. Performance data have been gathered.

In anticipation of possible trouble from the higher beam values at the 88-in. cyclotron, a program of air sampling in the environs of accelerators was initiated, employing the 184-in. cyclotron area as the first study point. (Preliminary findings indicate neutron activation of both air and dust constituents to "considerable" but variable magnitudes, depending on location, beam strength, particle, and location, as well as on target parameters.)

Improvement in the checking-and -switching circuit for Berkeley-box manifold controls resulted from the continued search for components with longevity and silence as criteria. The latter factor has permitted the installation within laboratory rooms of units formerly roof-mounted because of objectionable noise. The new location permits efficient access for inspection and servicing.

INSTRUMENT DEVELOPMENT

A 3/8-in.-diameter Geiger-Mueller probe, with a 1.4-mg/cm^2 window, has been developed for use in checking crevices and other small volumes. The probe may be used on any type of meter.

A portable neutron counter whose measurements are independent of size and shape of the source (point source to 40-mil cones) has been created. The counter is shielded so that 0.1% of a sample may be counted in the presence of an entire sample (samples will give on the order of 10^8 neutrons per second in the forthcoming 6-in. lead cave runs, where MTR-irradiated transuranic slugs will be processed).

A high-level alpha-counting ion chamber has been developed, especially for use in the above-mentioned 6-in. cave runs; it is capable of counting up to 10^9 counts per minute.

Aural indicators, considered advantageous by all monitors, have been made available for adaptation to all kinds of meters.

The Instrument Group has converted the obsolete log-ion meter (the Abacus) into a form suitable for use with any meter having transistorized circuitry; the conversion has been inexpensive and thus profitable, inasmuch as the original Abacus was itself not inexpensive.

There has been developed a new low-level-counting portable scaler, employing transistorized circuitry, adaptable for use with any portable meter. Counting rates as low as two counts per hour can be read.

A Geiger-Mueller probe for C^{14} detection that can be used on a number of standard rate meters has been developed.

WASTES AND WASTE DISPOSAL

Some 40-odd liters of plutonium-containing waste (approximately 250 grams of plutonium were present) were prepared for shipment to a recovery center. This material represented various waste streams from the processing of plutonium metal and Hanford waste some years ago for the americium content.

During the period 270 gallons of aqueous liquid waste was solidified with cement-vermiculite and 43 gallons of oil with Napalm. About 45 gallons of high-level alpha-active waste was solidified with cement-vermiculite; new equipment and methods were used for the addition of the solid material to the waste.

The Chemistry Building 70 acid-waste monitoring system is undergoing extensive repairs and redesign. The Bldg. 71 (hilac) acid-waste monitoring system operated satisfactorily during this period. None of the samples processed showed contamination above the acceptable level.

Two reports were written on waste-disposal practices and considerations at this Laboratory and were submitted to the Technical Information Division:

Elmer Nielsen, Comparative Costs of Sea Disposal and Land Burial for the Radioactive Waste of the Lawrence Radiation Laboratory, UCRL-8609, Jan. 1959, and

John A. Kaufmann and Nelson B. Garden, A Method for the Solidification of Radioactive Aqueous Liquid Wastes, UCRL-8684, March 1959.

Unpublished reports to the U. S. Atomic Energy Commission have been submitted by N. B. Garden on the annual quantity and types of radioactive waste from Berkeley and Livermore; some of these data were requested for use at a hearing before the Joint Committee on Atomic Energy of the Congress of the United States.

SPECIAL PROBLEMS GROUP

A report by A. E. Salo and N. B. Garden, Spot Tests and Radiological Corrosions, has been prepared for publication in Chemical Processing.

During this period 49 special radioactive sources were fabricated according to specifications (quantity and energy) by the researchers. Fifty-five sources already in existence were inspected, adjusted, and (or) repaired. Thirty-four commercial materials were tested for their suitability for laboratory purposes. Seven standard solutions were prepared on request. In the course of detecting and identifying radioactive contamination, 142 assays were made.

HEALTH PHYSICS

Burton J. Moyer in charge

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

Beta-gamma meters	22
I D L meters	18
Juno ion chamber	20
Abacus logarithmic ion chamber	24
Recording-intensity meters	8
Victoreen proteximeter	3
Slow-neutron proportional counters	15
Fast-neutron proportional counters (portable)	11
Slow-neutron portable unit	4
Balanced chamber--fast neutron--portable	3
Special tissue wall survey instrument	1

Survey Instruments in Storage

Beta-gamma meters	7

Personnel Meters in Use (Berkeley only)

Total personnel covered with film badges	2,100
Total man-days' coverage with electroscopes	18,900
Total man-days' coverage with dosimeters	6,300
Total man-days' coverage with slow-neutron chambers	6,300

Summary of instrument use:

Instruments	Buildings	Number per day (approx.) for 6 months	Man days of coverage	
Electroscopes	51	40	7200	
	53 and 80	20	3600	
	71	15	2700	
	70	15	2700	
	2	15	2700	18, 900 total

Instruments	Buildings	Number per day (approx.) for 6 months	Man days of coverage	
Dosimeters	53 and 80	20	3600	
	2	15	2700	6,300 total
Slow-neutron	53 and 80	20	3600	
	2	15	2700	6,300 total

Cases of Monthly Exposure above 0.5 r (excluding Livermore) (beta-gamma and neutron film exposures, in rem)

Period	Individual exposure above	60-inch area	Bldg. 51	Chem	Other	Total
9/10/58-2/10/59	0.4/month	22	76	13	47	158
12/10/58-2/10/59	3.0/quarter	0	0	0	0	0
2/10/58-2/10/59	5.0/year	2	6	2	6	16

RADIATION SURVEY WORK AND RESEARCH PROJECTS

88-inch Cyclotron Shielding

A series of shielding and neutron-activation calculations has been completed for the 88-inch cyclotron, which is designed to accelerate deuterons to 60 Mev. It was found that 12 feet of ordinary concrete will be necessary for the main shield, and that copper rather than aluminum is the material of choice for the magnet coils.

LET Measurements of Bevatron Radiation

The LET (linear energy transfer) of the Bevatron radiation was measured at three points near the control room. Measurements were performed by Herald Rossi, Walter Rosenzweig, and Leigh Phillips, on a consultant basis. (Rosenzweig and Rossi are from Columbia Medical Center and Phillips is from Brookhaven National Laboratory.) Although their final data have not been calculated, the preliminary estimate of the intensity is 5 millirads per hour at 3×10^{10} protons per pulse.

Radiation Survey Work at the Accelerators

Radiation survey work at the cyclotron, the hilac, and the synchrotron has continued on an "as-needed" basis, but at the Bevatron considerable effect has been expended in determing the sources within the tangent

tanks and the quadrants from which the Bevatron radiation field arises. As a result of these Bevatron surveys, it has been decided to install beam clippers near the entrance to each quadrant. The purpose of these beam clippers is to cause the high-energy protons in the beam to stop in a quadrant rather than in a tangent tank.

Theromometric Dosimetry at the 1500-Curie Cobalt-60 Facility

Temperature rise during irradiation of ~1-g samples of water and various metals was followed while the samples were being exposed to a gamma field of 10^6 r/hr from Co^{60}. Temperature rise was in all cases exponential, and the total change in temperature of about 4° C took about 20 minutes' exposure time. The use of small metal samples as reliable high-dose dosimeters is being examined.

Nuclear Emulsion Neutron Spectrometry

The use of 600-micron Ilford G5 nuclear emulsion as a neutron spectrometer is being examined. The distribution of recoil proton lengths gives some notion of the stray neutron spectrum from the Bevatron in the 1-to 20-Mev region.

Bismuth Fission Chamber Work

The mean effective fission-fragment range in bismuth has been measured in order that thickness-efficiency corrections may be computed for bismuth fission chambers.

Information Division
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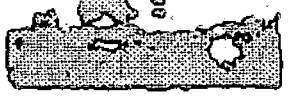
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