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UNIVERSITY OF CALIFORNIA

Radiation Laboratory

BIOLOGY AND MEDICINE SEMIANNUAL REPORT October 1957 through March 1958

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Biology and Medicine

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UNIVERSITY OF CALIFORNIA
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BIOLOGY AND MEDICINE SEMIANNUAL REPORT

October 1957 through March 1958

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BIOLOGY AND MEDICINE SEMIANNUAL REPORT

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Contents

BIOLOGICAL STUDIES OF RADIOACTIVITY AND IRRADIATION

BIOLOGICAL EFFECTS OF INTERNALLY DEPOSITED RADIOISOTOPES

Reports Issued 4

RADIATION CHEMISTRY

Oxygenated Protein Solutions 5
Oxygen-Free Protein Solutions 13
Organic Liquids 14

BIOLOGICAL STUDIES OF RADIATION EFFECTS

RADIATION THERAPY AND RADIOBIOLOGY

Work in Progress: Summary of Selected Studies
Irradiation of the Human Pituitary in Advanced Cancer with High-Energy Particles from the 184-Inch Cyclotron . 23
Hypophyseal and Hypothalamic Irradiation in Animals . . 24
The Influence of Ion Density on Radiobiological Effects . . 24
Acute and Delayed Effects of Radiations on Yeast Cells . . 25
Leukemia Induction by Radiation 26
Effects of Radiation on Fat Metabolism 26
Fallout and Related Subjects 26
Hematological Effects of Low-Level Radiation Doses in Man . 28

PHYSICAL BIOCHEMISTRY AND ITS APPLICATIONS

Work in Progress: Summary of Selected Studies
Electron Microscopy 31
Influence of Diet on Serum Lipoproteins 31
Ultracentrifuge Studies of Serum Lipoproteins 32
Infrared Spectroscopy 32

BLOOD AND BLOOD CIRCULATION

Red Blood Cell Studies 34
Studies on Erythropoietic Substances in Urine 35
Effect of High Altitude on Red Cell Formation 35
Heart Function and Circulation 35
Autoradiographic Studies of Mouse Leukocytes 35

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*Preceding (Quarterly) Reports: UCRL-8031, UCRL-3880

OTHER PHYSIOLOGICAL AND MEDICAL STUDIES

Proliferation of the Liver Reticuloendothelial System 41
The Regulation of the Volume of the Body Fluids 42
Carbohydrate Metabolism 47
Estrogen Determinations in Carcinoma of the Breast Following Pituitary Irradiation 47
The Scintillation Camera 57
Low-Background Human Whole-Body Counter 57
Reports Issued 58
Papers Published 60

RADIATION DETECTION AND PROTECTION

HEALTH CHEMISTRY

Apparatus and Equipment 62
Control of Airborne Activity 63

HEALTH PHYSICS

Statistical Summary of Monitoring Program 64

BIOLOGY AND MEDICINE SEMIANNUAL REPORT

October 1957 through March 1958

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

Muriel E. Johnston, Nylan Jeung, Marilyn H. Williams,
Marshall W. Parrott, George Barr, Ann Henderson, and Justine Burg

REPORTS ISSUED

The work done in this field has been reported during this period as follows:

Parrott, Durbin, and Burg, Serum Calcium Levels in Thyroid-Ablated Rats, submitted April 1958 to Proc. Soc. Exptl. Biol. Med. UCRL-8193, Feb. 1958; also

Parrott, Durbin, and Hamilton, Effects of Thyroidectomy on Breeding in the Rat. I. Comparison of Thyroid Ablation by Surgery, Radioiodine, and Radioastatine, UCRL-8042, Nov. 1957.

Durbin, Asling, Johnston, Parrott, Jeung, Williams, and Hamilton, Induction of Tumors in the Rat by Astatine-211, UCRL-8026, Nov. 1957; also Radiation Research (to be published).

Asling, Johnston, Durbin, and Hamilton, Localization of Ce^{144} in the Skeletal Tissues of Fetal Rats, UCRL-8024, Oct. 1957.

Durbin, Asling, Jeung, Williams, Post, Johnston, and Hamilton, The Metabolism and Toxicity of Radium-223 in Rats, UCRL-8189, Feb. 1958.

Patricia W. Durbin and Hardin B. Jones, Estimation of the Turnover Equation of Strontium-90 for Human Bones, in Proceedings of the Second International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958 (to be published).

Michael E. Jayko and Warren M. Garrison, Formation of >C=O Bonds in the Radiation-Induced Oxidation of Protein in Aqueous Systems, Nature 181, p 413-414 (1958).

Michael E. Jayko and Warren M. Garrison, Further Studies of the Formation of the >C=O Bond in Radiation-Induced Oxidation of Protein, UCRL-8218 Abstract,* March 1958.

Boyd M. Weeks and Warren M. Garrison, Formation of C^{14}-Labeled Protein Derivatives in the Radiolysis of Aqueous Pepsin - $C^{14}H_3COOH$ Solutions, UCRL-8219 Abstract,* March 1958.

CORRECTION

*Submitted to International Congress of Radiation Research, Burlington, Vermont (1958).

RADIATION CHEMISTRY

Warren M. Garrison in charge

Boyd Weeks, Michael Jayko, Winifred Bennett, Sibyl Cole, and Gordon Hughes

OXYGENATED PROTEIN SOLUTIONS

Radiation-induced oxidation of the simpler organic compounds in oxygenated aqueous solution generally involves formation of the >C=O function. Although not necessarily the main products, aldehyde and(or) keto derivatives are formed in reactions initiated by OH attack at--for example--methyl, hydroxy, methylene, phenyl, alkyl phosphate, and amine groups.[1] Oxidation of the $-CH_2-NH_2$ linkage in primary amines has been interpreted in terms of the intermediate formation and subsequent hydrolysis of the imino intermediate -CH=NH.[2] We have also found that the $-CH_2-NH-$ linkage in secondary amines undergoes an analogous reaction to give primary amine and aldehyde. This radiation-induced rupture of the C-N bond was suggested as a possible path for cleavage of the peptide chain.[3] Preliminary observations appear to substantiate this proposal, and indicate that detailed study of >C=O formation in irradiated protein solution would provide information on the locus of OH attack on both main and side-chain bonds.[4]

Information on the indirect action of Co^{60} γ rays on pepsin and gelatin has been obtained. Irradiations were made at a dose rate of $\sim 2 \times 10^{17}$ ev/ml/min. The solutions (3 ml) were exposed under one atmosphere of oxygen in sealed pyrex tubes mounted in a motor-driven reel situated a standard distance from the source.

---

[1] For recent reviews see: E. Collinson and A. J. Swallow, The Radiation Chemistry of Organic Substances, Chem. Revs. 56, 471-568 (1956); W. M. Garrison, "Radiation Chemistry", Ann. Rev. Phys. Chem. 8, 129-154 (1957).

[2] B. M. Weeks and W. M. Garrison, Mechanism in the Radiolysis of Aqueous Glycine Solutions. II, J. Chem. Phys. 25, 585 (1956).

[3] M. E. Jayko and W. M. Garrison, Indirect Action of Radiation on the $-NH-CH_2$ Linkage in Diethylamine (A Mechanism for the Radiation-Induced Decomposition of the Peptide Chain), J. Chem. Phys. 25, 1084 (1956).

[4] M. E. Jayko and W. M. Garrison, Formation of >C=O Bonds in the Radiation-Induced Oxidation of Protein in Aqueous Systems, Nature 181, 413-414 (1958).

Total Carbonyl Production

Immediately after irradiation, the protein solution (5 mg/ml) was treated with platinum black to destroy hydrogen peroxide and then treated with 2, 4-dinitrophenylhydrazine-hydrochloric acid solution to form the hydrazone derivatives.[5] The solution was dialyzed to remove excess reagent and possible reaction products of low molecular weight. An appropriate aliquot of the dialyzed protein was then added to methanol-potassium hydroxide solution for spectrophotometric analysis after the method of Lappin and Clark.[6] A typical absorption curve (filled circles) for irradiated pepsin solution treated in this manner is shown in Fig. 1. Similar curves were obtained with gelatin. The absorption spectrum of a representative monocarbonyl derivative (acetaldehyde 2, 4-dinitrophenylhydrazone) is shown for purposes of comparison. The upper curve (open circles) shows the absorption spectrum of 2, 4-dinitrophenylhydrazine reagent under identical conditions. The absorption curve of irradiated pepsin treated with 2, 4-dinitrophenylhydrazine is seen to be characteristic of the $>C=N-NH-C_6H_4(NO_2)_2$ chromophore. Lappin and Clark have shown for monocarbonyl 2, 4-dinitrophenylhydrazones that the position of the absorption maximum (as well as the value E_{max}) is nearly independent of the structure of the carbonyl compound.[6] If the acetaldehyde derivative is used as a standard, an initial yield of 1.2 carbonyl groups is obtained per 100 ev absorbed in the pepsin solution. Pepsin in unirradiated controls showed negligible retention of 2, 4-dinitrophenylhydrazine.

The effects of a number of different variables on the carbonyl yield in irradiated pepsin solution are summarized in Table I and Figs. 2-4. Included are data on the effects of (a) dose, (b) oxygen concentration, (c) pH, and (d) added solutes including chloride ion, sulfate ion, and a typical protective agent for indirect action, viz: the sulfhydryl derivative cysteine.

Locus of Carbonyl Production

Preliminary information on one of the processes of carbonyl formation has been obtained. The evidence is derived from studies of the carbonyl products found to be liberated on hydrolysis of the irradiated (and dialyzed) protein. The irradiated protein solutions were hydrolyzed in 4 N HCl (in vacuo) for 24 hours prior to treatment with 2, 4-dinitrophenylhydrazine reagent. (In the identification studies we found it necessary to add the hydrazine reagent after hydrolysis, since hydrazone derivatives are generally rather rapidly destroyed by heating in mineral acids.) The hydrazone derivatives were extracted with chloroform and examined chromatographically.[7] It was found that the irradiated samples yield a series of α-keto acids. It is of interest to note that control runs with unirradiated pepsin showed the presence of measurable amounts of pyruvic acid.

---

[5] H. A. Iddles and C. E. Jackson, Determination of Carbonyl Compounds by Means of 2, 4-Dinitrophenylhydrazine, Ind. Eng. Chem., Anal. Ed. 6, 454-456 (1934).

[6] G. R. Lappin and L. C. Clark, Colorimetric Method for Determination of Traces of Carbonyl Compounds, Anal. Chem. 23, 541-542 (1951).

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

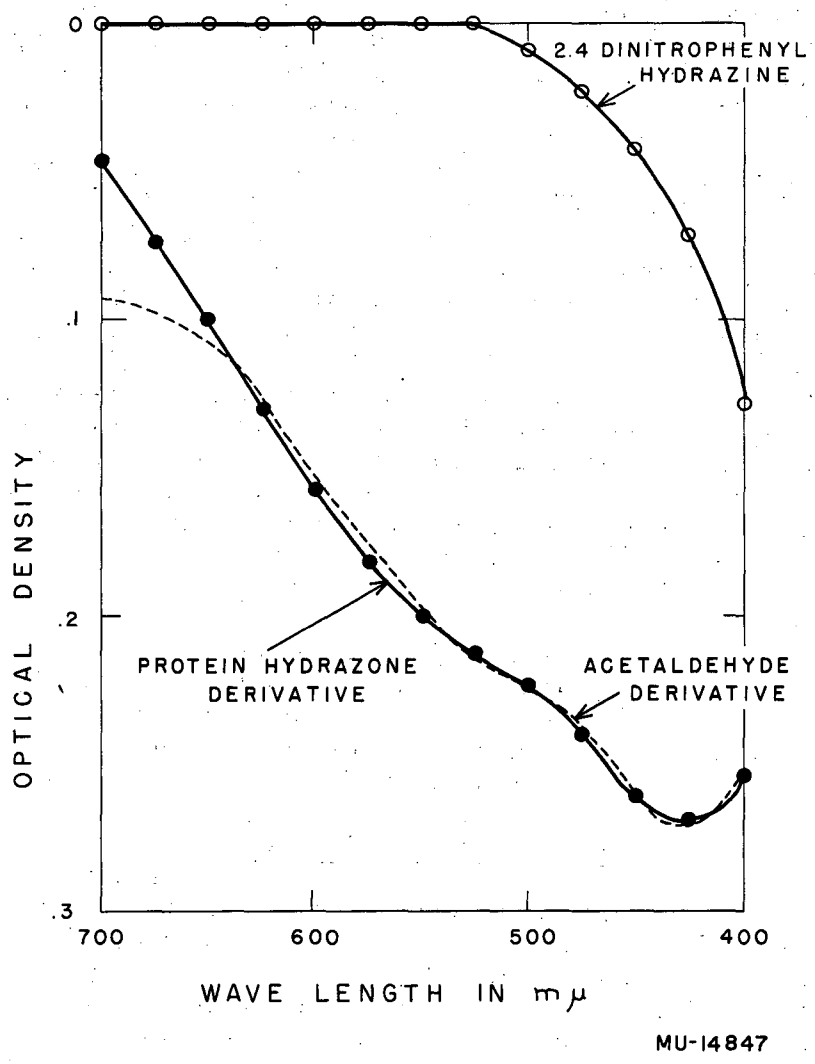

Fig. 1. Absorption spectra of hydrazone derivatives.

Table I

Carbonyl production in the Co^{60} γ-ray radiolysis of aqueous pepsin solution. Pepsin concentration = 5 mg/ml. irradiated volume = 3 ml.

Solution	Dose (ev/ml)	Optical density of product hydrazone solution at 430 mμ[a]
O_2 sat., pH 5	1.35×10^{18}	0.068
O_2 sat., pH 5	4.05×10^{18}	0.197
O_2 sat., pH 5	8.10×10^{18}	0.296
O_2 sat., pH 5	8.10×10^{18}	0.311
O_2 sat., pH 5	13.50×10^{18}	0.485
evac pH 5	8.10×10^{18}	0.108
evac pH 5	8.10×10^{18}	0.116
O_2 sat., pH 5, 4.8×10^{-5} M cysteine	8.10×10^{18}	0.287
O_2 sat., pH 5, 4.8×10^{-4} M cysteine	8.10×10^{18}	0.224
O_2 sat., pH 5, 4.8×10^{-3} M cysteine	8.10×10^{18}	0.101
O_2 sat., pH 5, 0.1 M NaCl	8.10×10^{18}	0.288
O_2 sat., pH 5, 0.1 M Na_2SO_4	8.10×10^{18}	0.300
O_2 sat., pH 1.3	8.10×10^{18}	0.292
O_2 sat., pH 3.6	8.10×10^{18}	0.269
O_2 sat., pH 5.2	8.10×10^{18}	0.301
O_2 sat., pH 6.4	8.10×10^{18}	0.312
O_2 sat., pH 7.5	8.10×10^{18}	0.342
O_2 sat., pH 9.4	8.10×10^{18}	0.526

[a]As obtained by using the analytical procedure of Lappin and Clark (Ref. 6). Irradiated volumes and dilution factors were uniform throughout the several series of runs.

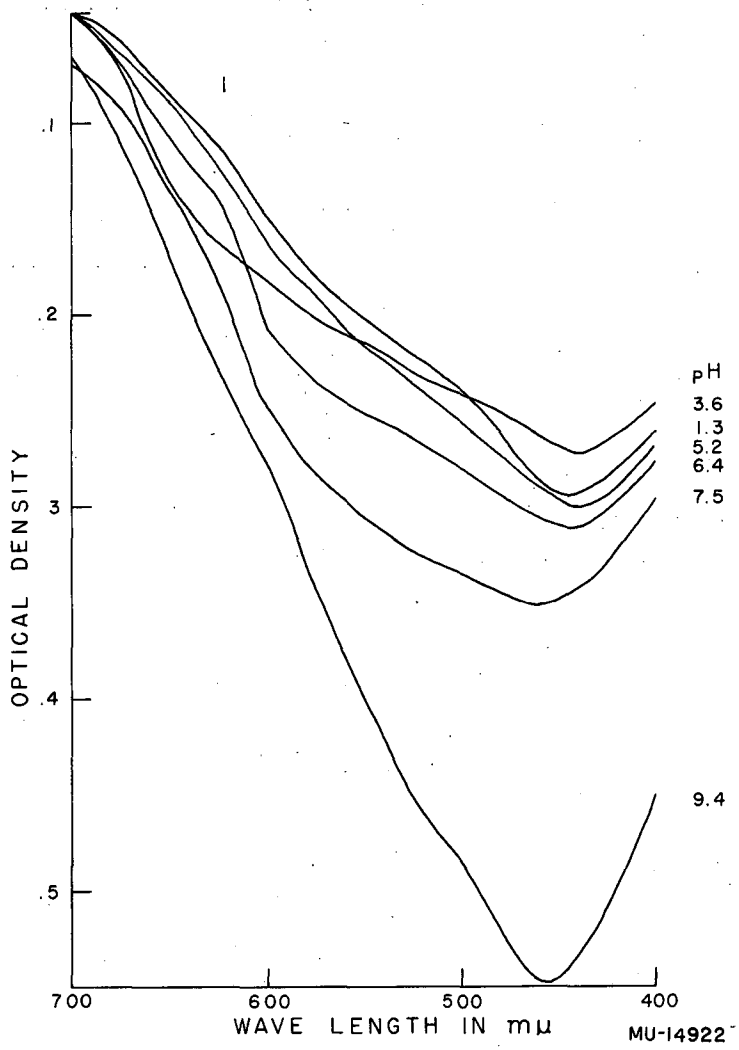

Fig. 2. Effect of pH on formation of carbonyl groups in irradiated 10^{-4} M pepsin solutions.

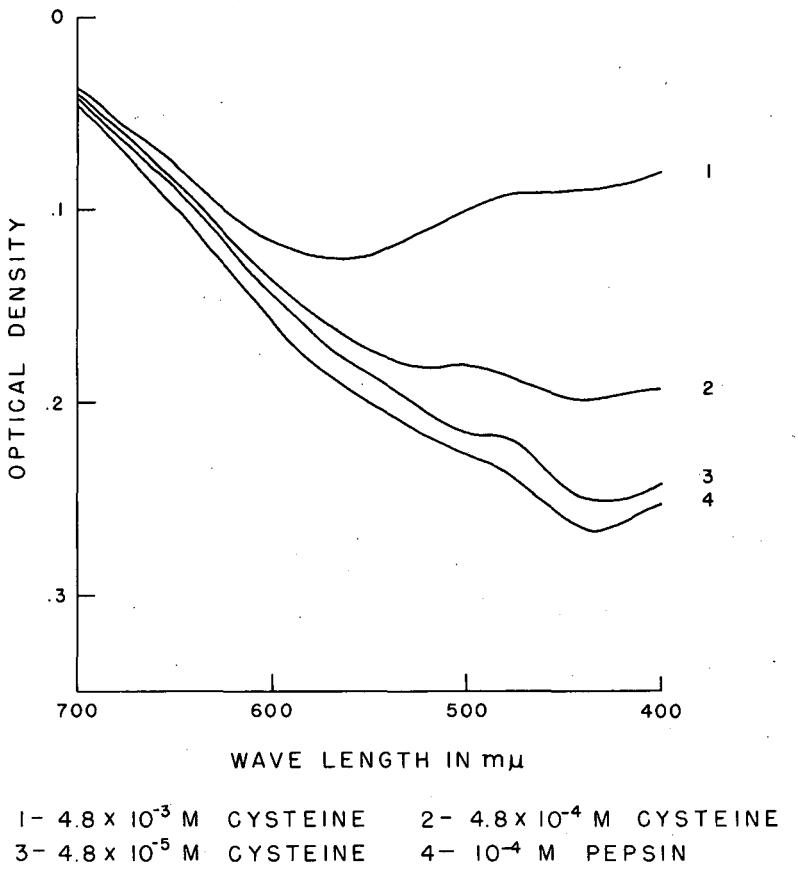

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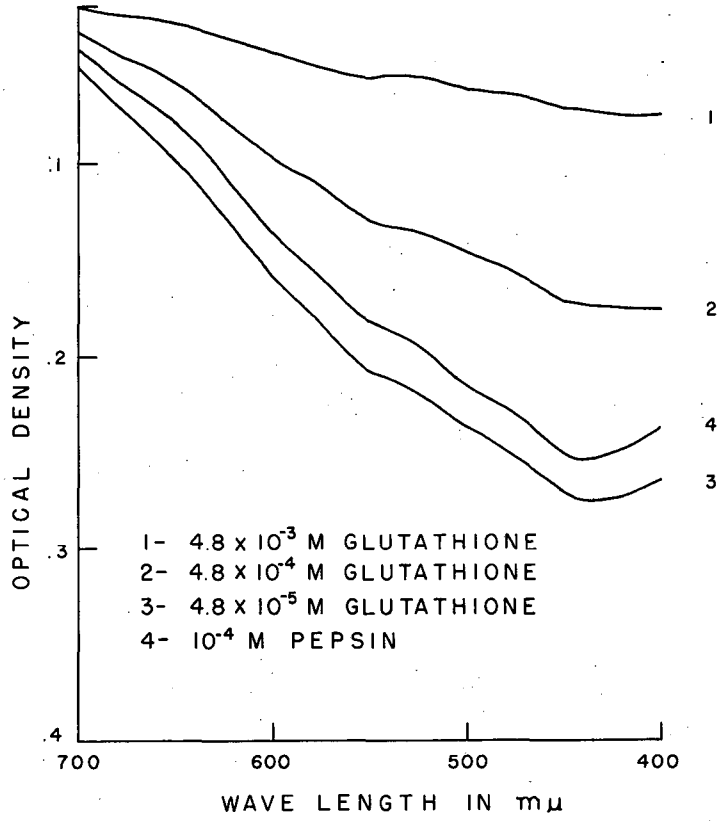
Fig. 3. Effect of cysteine on formation of carbonyl groups in irradiated 10^{-4} M pepsin solutions.



MU-14919

Fig. 4. Effect of glutathione on formation of carbonyl groups in irradiated 10^{-4} M pepsin solutions.

This apparently arises from the decomposition of serine during acid hydrolysis,[8] but was not observed in unirradiated gelatin hydrolyzates, presumably because of the lower serine content of the latter. Most of the keto acid identification studies were therefore made on the irradiated gelatin system. The following keto acid products have been identified: oxaloacetic, α-ketoglutaric, glyoxylic, pyruvic, and phenylpyruvic. Identification was based on the fact that in each case the 2,4-dinitrophenylhydrazone derivatives could not be distinguished chromatographically from the corresponding authentic material.

We have considered the possibility that a transmutation type of reaction

$$[RCH(NH_2)COOH + \dot{R}COCOOH \rightarrow RCOCOOH + \dot{R}CH(NH_2)COOH],$$

may occur during hydrolysis once a particular keto acid has been released. If such a reaction did occur it would, of course, introduce an uncertainty in the identification of the initial carbonyl products. The results of a series of control runs, however, vitiate this possibility. Glyoxylic acid, for example, added prior to hydrolysis of unirradiated pepsin solution, was the only carbonyl recovered--except, of course, for pyruvic acid, which is a normal hydrolysis product as discussed above. Further, that pyruvic acid is observed as the only carbonyl produced in the normal hydrolysis of pepsin also demonstrates the absence of any appreciable transamination. It nevertheless appears that these products represent a major chemical effect of the indirect action of radiation on gelatin and pepsin in oxygenated aqueous solution. This result is interpreted as substantiating the earlier suggestion that the peptide link may be involved in the radiolysis of aqueous protein, and if so, would give rise to terminal carbonyl functions. This proposal was based on the observation that the indirect action of ionizing radiation on diethylamine in oxygenated aqueous solution gives rise to ethylamine and acetaldehyde as principal products. The mechanism of this reaction is written

$$H_2O \rightsquigarrow H,\ OH,\ H_2,\ H_2O_2:$$

$$H + O_2 \longrightarrow HO_2,$$

$$OH + RNHCH_2R \longrightarrow RNH\dot{C}HR + H_2O,$$

$$O_2 + RNH\dot{C}HR \longrightarrow RN{=}CHR + HO_2,$$

$$H_2O + RN{=}CHR \longrightarrow RNH_2 + RCHO,$$

$$2\ HO_2 \longrightarrow H_2O_2 + O_2$$

[8] B. Franck and J. Knobe, α-Keto Acids in Protein Hydrolysates, Chem. Ber. 90, 2450-2459 (1957).

where RN=CHR represents an imino intermediate which is rapidly removed through the hydrolytic reaction. The corresponding reaction at the peptide chain would give rise to the imino derivative -CO-N=C(R)-CO-, which on hydrolysis would yield the keto acid RCOCOOH.

The radiation-induced oxidation of other chemical groups and bonds in pepsin and gelatin also contributes to the over-all carbonyl production. Evidence has been obtained which indicates that this fraction is more complex and less susceptible to analytical investigation, at least with the identification methods now at hand.

OXYGEN-FREE PROTEIN SOLUTIONS

The work of the preceding section represents a specific case in the general use of radical scavengers to obtain information on labile intermediates derived from protein molecules. Oxygen, of course, is of particular interest in view of the radiation biological inference. However, other second solutes could conceivably provide as much or more information on the intermediate processes. Moreover, it is not necessary that the second solute itself react with the protein radicals. For example, consider the radiation chemistry of oxygen-free solution containing protein molecules RH and a simple organic solute R'H. Reaction of H and(or) OH with solute would yield the intermediates R and R'. Regardless of other consideration, there is a certain probability that the combination reaction R + R' = RR' would ensue. We have investigated the radiation chemistry of the system in which RH = pepsin, and R'H = $CH_3COOH(C^{14}H_3COOH)$. The latter has been shown to yield the intermediate CH_2COOH on H and OH attack.[9] Reaction of $C^{14}H_2COOH$ with pepsin radicals R' could yield C^{14}-labeled protein derivatives $R'-C^{14}H_2COOH$. Conventional hydrolysis of this modified protein would yield simple C^{14}-labeled organic compounds. Identification of these would, in turn, provide information on the nature of the free-radical intermediates derived from protein.

Oxygen-free solutions containing acetic acid and pepsin, approximately 10^{-2} M and 10^{-5} M, respectively, were irradiated with 40-Mev helium ions from the Crocker Laboratory 60-inch cyclotron by previously described procedures.[10] The irradiated solutions were distilled to dryness in vacuo to recover C^{14}-labeled acetic acid. The residue was redissolved in 1 N hydrochloric acid and dialyzed for 3 hours in cellophane against running water in the cold. (By our dialysis procedure, C^{14} activity in the irradiated protein solution decreases rapidly to a limiting value that does not change appreciably after about 2 hours.) Retention was essentially proportional to dose over the range 2×10^{18} to 2×10^{19} ev/ml. There was negligible retention of C^{14} activity in a duplicate manipulation of an

[9] Garrison, Haymond, and Weeks, Some Effects of Heavy-Particle Irradiation of Aqueous Acetic Acid, Radiation Research 1, 97-108 (1954).

[10] Garrison, Weeks, Ward, and Bennett, Water Decomposition Yields in the Radiolysis of Aqueous Fromic Acid-Oxygen Solutions by Cyclotron-Produced Radiations, J. Chem. Phys. 27, 1214-1215 (1957).

unirradiated pepsin-$C^{14}H_3COOH$ target solution. The dialyzed material was made 4 N in hydrochloric acid, evacuated, and hydrolyzed on the steam bath for 24 to 36 hours. The hydrolyzate was distilled to dryness *in vacuo*, and chromatographed on a Dowex-50 ion-exchange column by a modification[11] of the method developed by Stein and Moore[12] for separation of amino acids in protein hydrolyzates. The elution curve showed a series of distinct C^{14} activity peaks. A principal one of these (Peak I, Fig. 5), rechromatographed with added amino acid carriers, showed an exact correspondence with authentic aspartic acid. The co-chromatogram is shown in Fig. 6.

The simplest explanation for the formation of C^{14}-labeled aspartic acid under the conditions of this study is that $C^{14}H_2COOH$ radicals combine with protein radicals containing the -NH-CH-CO configuration. That is, aspartic acid is derived through substitution of CH_2COOH for H in the glycine units of the peptide chain. Of course, it might be argued that glycine molecules were introduced into the target solution through protein hydrolysis, and that free aspartic acid formed by combination of $NH_2CHCOOH$ and CH_2COOH radicals was not adequately removed in the dialysis step. However, results of a series of control dialysis experiments with protein-CH_3COOH solutions containing added authentic C^{14}-labeled aspartic acid were found to negate this possibility.

These studies on oxygen-free pepsin-$C^{14}H_3COOH$ solutions lead to a conclusion similar to that arrived at on the basis of the carbonyl measurements in oxygenated solution. Namely, the peptide link is involved as a locus of indirect action, and the various processes can be subjected to detailed inquiry by use of the techniques described.

ORGANIC LIQUIDS

The use of I_2 as a scavenger in the radiolysis of organic compounds is well known. "Yields" for the disappearance of I_2 have been measured in a variety of hydrocarbons,[13] and although this disappearance has been attributed to reaction of iodine with free radicals produced during the radiolysis, there is still some uncertainty as to the precise nature of the reactions occurring. In a few cases, more detailed information regarding the radiolytic cleavage of the hydrocarbons has been obtained by use of radioactive iodine and characterization of the iodides produced.[14]

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[11] Boyd M. Weeks and Warren M. Garrison, Radiolysis of Aqueous Solutions of Glycine, Radiation Research (in press).

[12] S. Moore and W. H. Stein, Chromatography of Amino Acids on Sulfonated Polystyrene Resins, J. Biol. Chem. 192, 663-681 (1951).

[13] Weber, Forsyth, and Schuler, Radiation Research 3, 68 (1955).

[14] R. R. Williams, Jr., and W. H. Hamill, J. Am. Chem. Soc. 72, 1857 (1957).

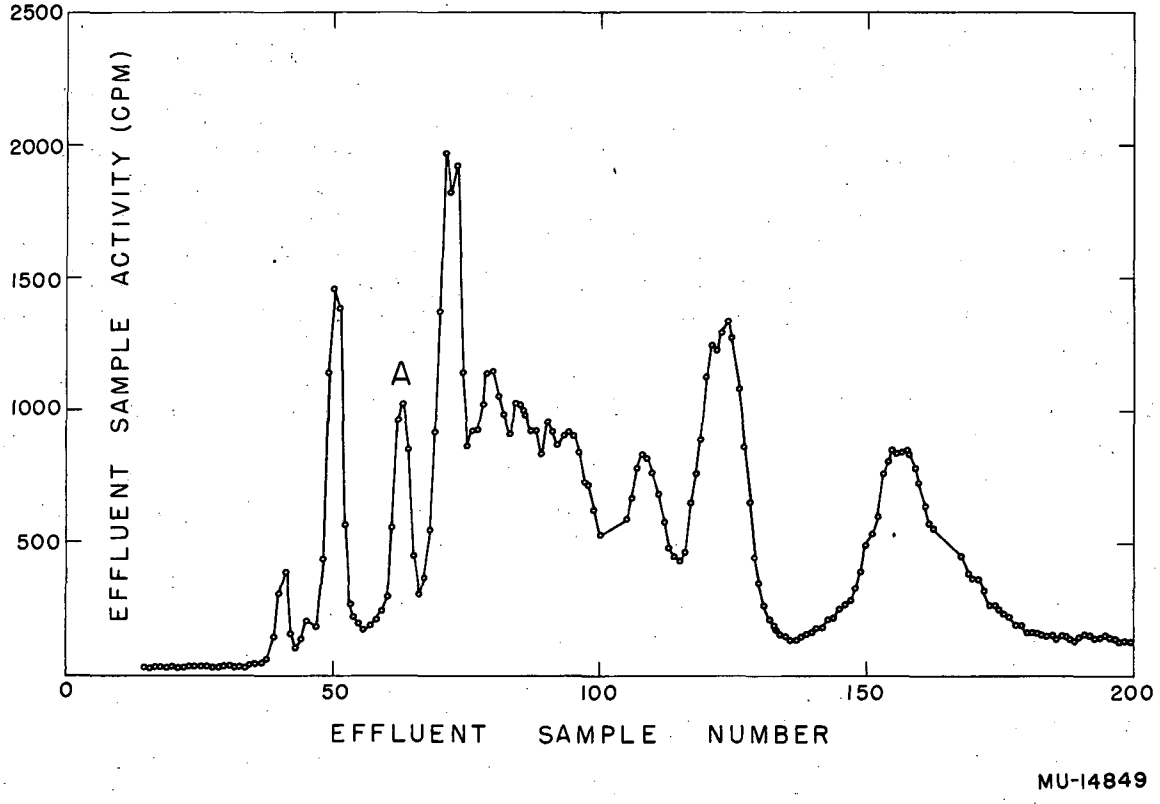


Fig. 5. Typical elution curve for hydrolyzed pepsin-$C^{14}H_2COOH$ derivatives.

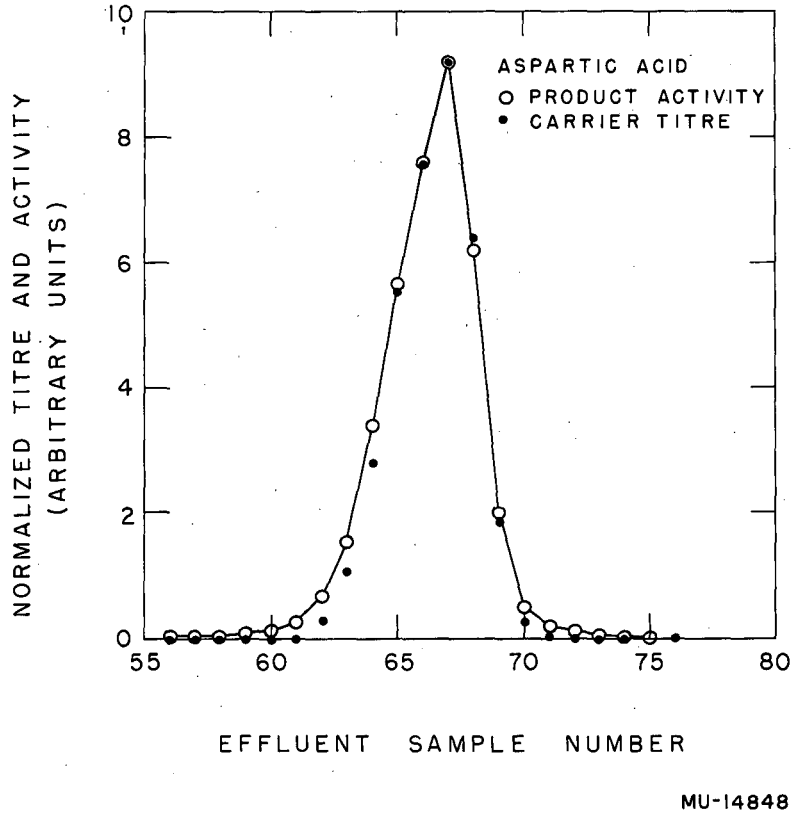


Fig. 6. Co-elution curve of authentic aspartic acid with product I (Fig. 5).

It was thought desirable to examine the possibilities of using ICN as an alternative scavenging agent. Since, in general, R-CN bonds are stronger than the corresponding R-I bonds, it was expected that reaction of a radical R with ICN would proceed via

$$R + ICN \longrightarrow RCN \text{ (or } RNC) + I$$

rather than

$$R + ICN \longrightarrow RI + CN.$$

Iodine should then be produced during the radiolysis and would be a convenient measure of the total "radical" yield. It was also possible that the initial cleavage of the hydrocarbons could be more readily studied in that the products could be hydrolyzed to give either RCOOH or RNH_2, for both of which convenient analytical techniques have been developed for their separation and characterization.

Irradiation of 10^{-2} M ICN solutions in a series of organic solvents by Co^{60} γ rays showed that whereas in solutions in methanol, benzene, and toluene I_2 was liberated, there was no visible production of I_2 in solutions in n-hexane, cyclohexane, cyclohexene, and acetic acid. More detailed studies of some of these systems have been made. Yields were calculated on the assumption of $G(-I_2) = 3.4$ for $\sim 5 \times 10^{-4}$ M I_2 in cyclohexane.

Methanol

The rate of production of I_2 is constant and independent of concentration (see Fig. 7). However, at low concentrations of ICN, as the ICN is consumed, secondary reactions of product iodine lead to eventual disappearance of iodine.

Here $G(I_2)$ is 3.7. This is approximately the same as the yield for the disappearance of iodine on irradiation of dilute solutions of iodine in methanol.

Benzene

The initial rate of production of I_2 is constant but increases with increasing concentration of ICN. Thus, at 10^{-3} M ICN, $G(I_2)$ is 0.75 but increases to 2.4 at 3.6×10^{-2} M ICN. At lower ICN concentrations, secondary reactions of product iodine lead to a fall-off in yield at higher doses and ultimate disappearance of iodine (see Fig. 8).

n-Hexane and Cyclohexane

Since the behavior of these two solvents appears to be essentially the same, they can be discussed together.

There is no visible production of I_2 upon irradiation, although the irradiated solution shows the appearance of a new absorption peak at

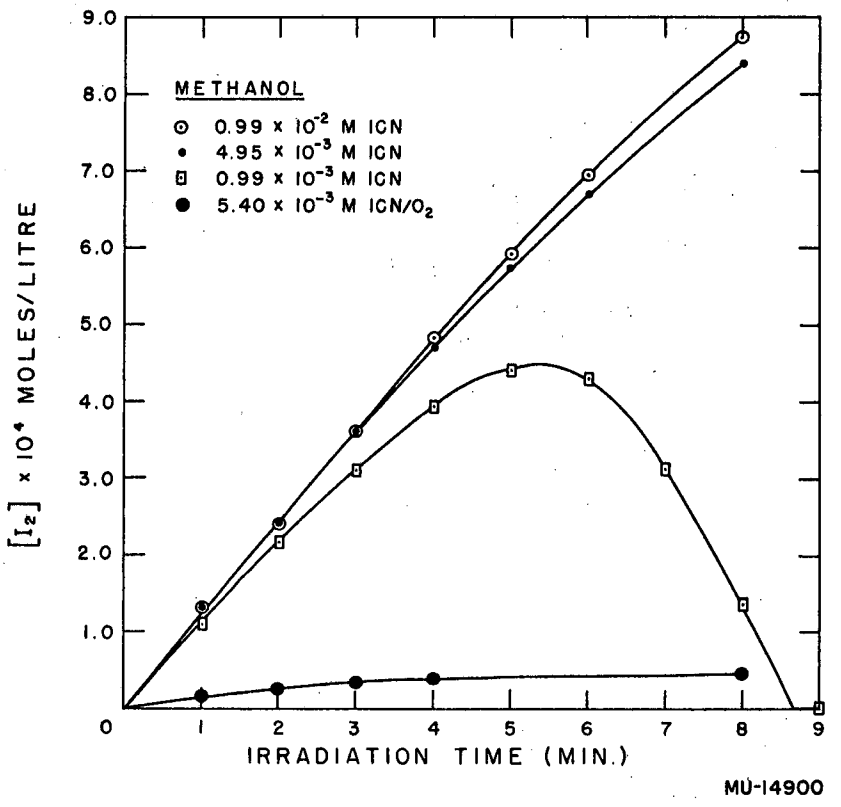


Fig. 7. Production of I_2 in ICN-methanol solutions.

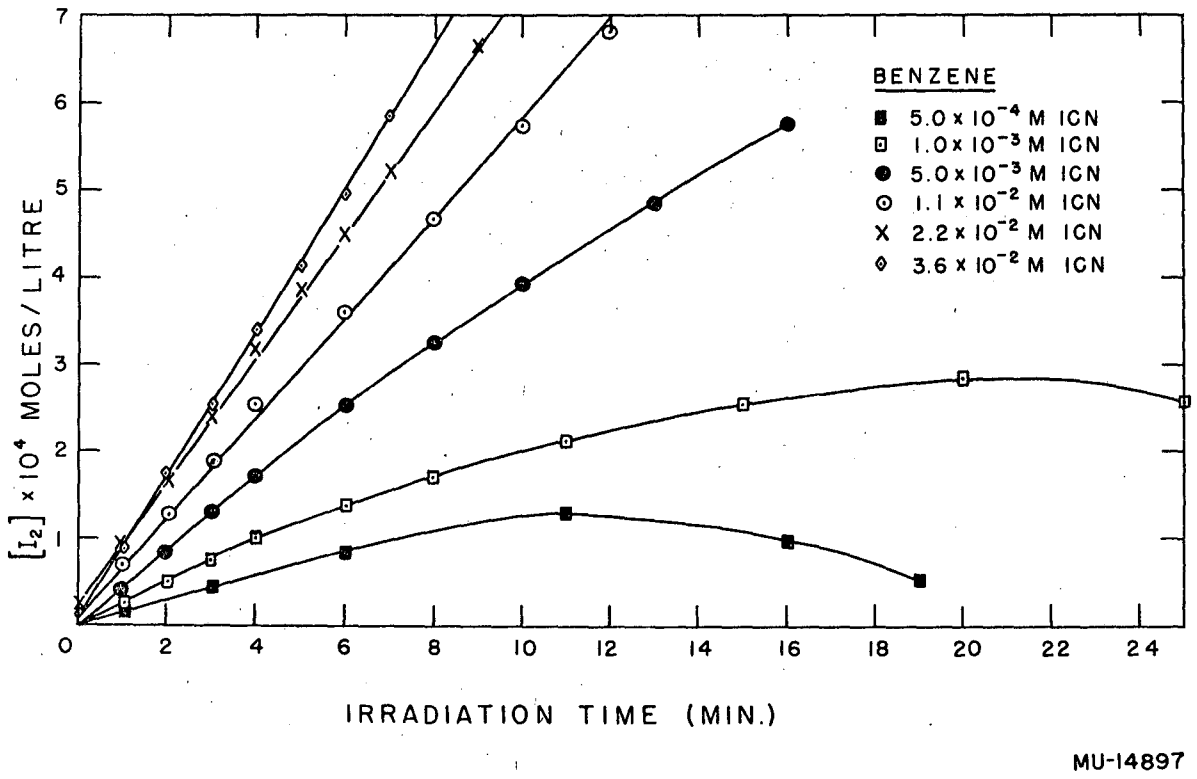


Fig. 8. Production of I_2 in I_2-benzene solutions.

λ = 260 mμ, at which wave length organic iodides usually exhibit absorption maxima. Irradiation of solutions of ICN and I_2 showed a decreased consumption of I_2 from that obtained on irradiation of solutions of I_2 only. The decrease was more marked at higher ICN concentrations, indicating that although ICN did not lead to I_2 production it was, nevertheless, competing for some of the species produced on radiolysis.

Some irradiations were carried out, using ICN labeled with I^{131}, and it was shown that there was uptake of I^{131} by the solvent. The initial uptake of iodine was proportional to dose but increased with increasing ICN concentration (Fig. 9).

Mechanism

It is difficult to reconcile all the above observations in terms of reactions of free radicals only. However, a tentative mechanism can be suggested if it is assumed that ICN does not react with radicals as was first thought, but rather that it is an efficient electron-capturing agent.

Capture of an electron by ICN is followed by dissociation, and consequent charge neutralization of the cyanide ion leads ultimately to the production of an iodine atom and a cyanide radical:

$$RH \rightsquigarrow RH^+ + e,$$

$$RH^+ + e \longrightarrow RH^* \longrightarrow \text{radicals},$$

$$e + ICN \longrightarrow [ICN^-] \longrightarrow I + CN^-,$$

$$CN^- + RH^+ \longrightarrow RH + CN.$$

Whether or not I_2 is produced then depends on the ensuring reactions of the cyanide radical.

In the alkanes, abstraction of H by the cyanide radical produces an alkyl radical which readily reacts with I to give a stable alkyl iodide:

$$CN + RH \longrightarrow R + HCN,$$

$$R + I \longrightarrow RI.$$

Thus, there is no net production of I_2. However, it can be seen that the yield of alkyl iodide increases as the number of electrons captured by the ICN increases, i.e., as the concentration of ICN is increased. The dependence of yield on ICN concentration is consistent with the known behavior of other solutes in cyclohexane.[15]

It might be expected, however, that the CN radical would add to benzene, giving rise to a radical which with I reacts to eliminate HI.

[15] R. H. Schuler, J. Phys. Chem. 61, 1472 (1957).

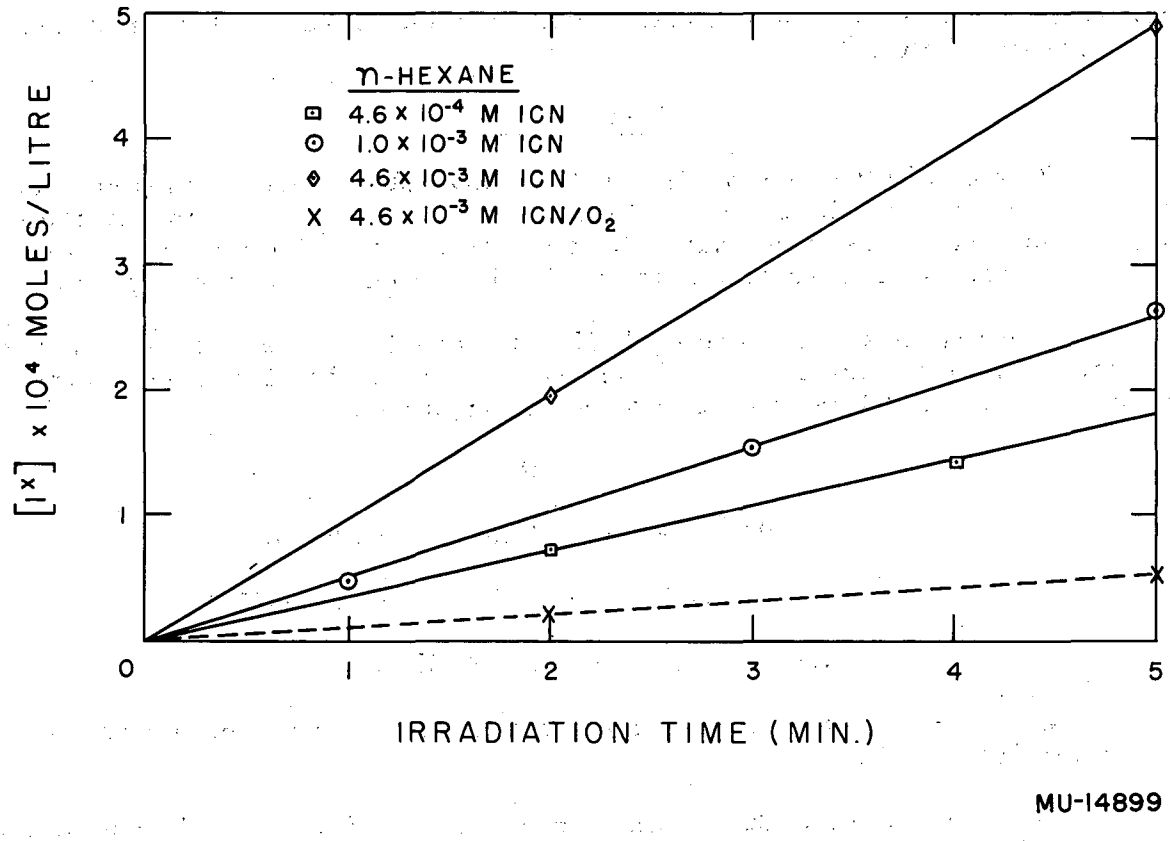
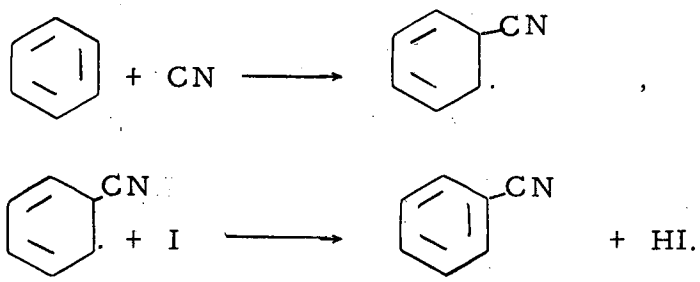


Fig. 9. Uptake of I^{131} in ICN–n-hexane solutions.



Since HI is known to reduce ICN, I_2 is produced:

$$HI + ICN \longrightarrow I_2 + HCN.$$

Here, too, the yield of I_2 increases with increasing electron capture by ICN.

The yields of I_2 observed in benzene are considerably greater than have heretofore been reported for benzene decomposition.[13] At low I_2 concentrations $G(-I_2)$ is 0.33 and does not increase very much at higher concentrations. Such effects, however, would not be observed when I_2 is used as a scavenger, because decomposition of I_2 gives only I atoms, which are unreactive and eventually dimerize so that no net reaction is observed. However, dissociation of ICN yields a cyanide radical which is capable of reacting further and consequently gives a more real measure of the initial radiolytic decomposition.

The reaction of a CN radical with methanol leads to the CH_2OH radical, which with I also yields HI, and consequently I_2 is produced:

$$CN + CH_3OH \longrightarrow CH_2OH + HCN,$$

$$CH_2OH + I \longrightarrow CH_2O + HI,$$

$$HI + ICN \longrightarrow I_2 + HCN.$$

If, however, the electron is captured by the parent methanol ion, then an excited methanol molecule is produced which presumably dissociates to radicals, viz.,

$$CH_3OH^* \longrightarrow H + CH_2OH.$$

ICN is known to be readily reduced, and reduction by H atom may then occur,

$$H + ICN \longrightarrow HCN + I,$$

so that the yield of I_2 is the same whether ICN captures a free electron directly or by transfer from a hydrogen atom produced by decomposition of an excited methanol molecule.

Although the above mechanism is by no means certain, nevertheless it provides a convenient working hypothesis on which further experiments are being based.

BIOLOGICAL STUDIES OF RADIATION EFFECTS

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RADIATION THERAPY AND BIOLOGY

WORK IN PROGRESS: SUMMARY OF SELECTED STUDIES

Irradiation of the Pituitary in Advance Cancer With High-Energy Particles from the 184-Inch Cyclotron

With a general irradiation technique described in previous reports 340-Mev protons and 190-Mev deuterons have been employed in localized irradiation of brain and pituitary gland. Recently an improved apparatus was placed in operation at the Berkeley 184-inch cyclotron, utilizing 900-Mev alpha particles; x-ray-television is used for alignment of patients. With these technical improvements it is now possible to deliver intense radiation doses to well-localized, deep-lying volumes of 1 cm^3 in the human body (about 1 mm^3 in the rat), leaving intervening tissues relatively undamaged.

To date 60 patients, 51 with far advanced mammary carcinoma, have received pituitary irradiation with either the proton or alpha-particle beam from the 184-inch cyclotron. This represents the first use of high-energy beams in human therapeutic investigation. The purpose was to learn if high-energy particles could effectively "hypophysectomy," and thus avoid the high morbidity and mortality from surgical procedures. The basic objective was to determine the control exerted by the pituitary on other endocrine organs whose hormones are believed to regulate the growth and spread of breast cancer. The promising results on the first series of 26 cases so treated made the continuation of this work essential. It is believed that 10 of the 26 patients first treated had objective remissions for from 3 to 30 months, manifested by regression of intraabdominal carcinomatosis, of bone lesions, of primary breast tumors, and of lung and skin metastases. Clinical laboratory evidence indicated decreased function of the pituitary and the target end organs following pituitary irradiation. This was corroborated by histological evidence of extensive pituitary destruction.

The shutdown of the cyclotron for 20 months for complete rebuilding, interrupted the studies of patients. The work was resumed in October 1957, using the alpha-particle beam, and an additional 30 patients have recieved pituitary irradiation. Of this group all but two had advanced metastatic breast cancer. It is planned to irradiate a series of 100 patients, in order to make an adequate statistical evaluation of this procedure as a palliative form of treatment for advanced metastatic breast carcinoma. Information from collateral studies is expected to afford a greater knowledge of the

factors involved in carcinoma of the breast, which may in turn afford other therapeutic approaches. At the same time animal studies are being conducted in which various areas of the hypothalamus, which has a neuro-regulatory influence over metabolic activities, are being selectively irradiated. Because of the accuracy with which this can be done without injuring other tissues, it is expected that this procedure will provide further understanding of the hypothalamic control and regulation of body mechanisms. This information promises to have specific application in malignant and endocrine diseases, and in understanding the control of metabolic activities of the body such as weight gain, body temperature, body activity, and water balance. This work will be further reported as studies progress. (Cornelius A. Tobias and James L. Born)

Hypophyseal and Hypothalamic Irradiation in Animals

In conjunction with the human studies, investigations of chronic radiation effects on the hypophysis and hypothalamus of rats are being continued.

It is now ascertained that deuteron irradiation of the pituitary of rats with doses of 950 rad and higher has chronic definitive effects. At the lowest dose, there is relatively little effect on bone growth and target-organ functions. In about a year, however, the animals become chronically obese, even though the hypothalamic "obesity center" received less than 50 rad. In this same period 95% of the rats develop multiple pituitary tumors of all anterior-cell types. At higher doses, at which the pituitary cells die as a result of irradiation, the injury is manifested in gradual regression of bone and body growth and of the functions of the target organ instead of in pituitary tumors and obesity. At 6,000 rad or higher, the over-all tumor incidence in the rats in a 2-year span decreases to about one-half.

Hypothalamic irradiation of young rats results in development of reproducible lesions at the site of irradiation. In the course of development of these lesions one or more of the homeostatically controlled body functions exhibits chronic abnormality. Irradiation in the region of the median eminence by 8,000 rad results in diabetes insipidus, glycosuria, partial gonadal regression, and thyroid malfunction. The same dose given in a small region of posterior hypothalamus, away from the appetite center, has an effect of slowing bone growth in the animals, apparently mediated through the anterior hypophysis. Irradiation of the entire hypothalamus with large doses results in a delayed lethal syndrome frequently accompanied by severe clinical symptoms. (Cornelius A. Tobias and Graeme Welch)

The Influence of Ion Density on Radiobiological Effects

The heavy-ion linear accelerator (Hilac) has provided a unique tool for radiobiological studies at this laboratory. The beams of accelerated nuclei of carbon, oxygen, and neon from the Hilac provide radiations that, together with the beams of protons and alpha particles from the Crocker 60-inch cyclotron, cover a wider range of linear energy transfer (LET) than have previously been available for radiation studies. These beams are now

being used in a systematic study of their relative effectiveness on various biological materials. Thus far the materials that have been studied include the bacteriophage T_1, spores of Bacillus subtilis, and the enzyme lysozyme, all irradiated in the dry state in vacuum. Modifications of the apparatus are now in progress for bombardment of biological samples in air and other gaseous atmospheres.

The information supplied by these studies will bear on the consequences of fission fragments or other recoil nuclei produced within an organism, and on the hazards of heavy primary cosmic-ray particles that will be encountered in space travel. The studies are also of fundamental importance in testing the validity of various theories of the biological action of ionizing radiation.

The studies carried out thus far have been highly productive. For B. subtilis, in which earlier work had shown an increasing relative biological effectiveness (RBE) up to the highest LET available with alpha particles, it has now been shown that the RBE is decreasing at still higher LET. This result parallels that previously obtained in this laboratory for vegetative yeast cells. On the other hand, when the damage to the enzyme lysozyme is described in terms of a cross section per particle for inactivation, the measured cross section continues to rise to a higher value than expected from previous work with other enzymes at lower LET. This result has emphasized the importance of delta rays (low-energy recoil electrons) in any theory of biological action of ionizing particles, and calculations of the contributions from delta rays are now in progress. The ability to irradiate the same biological material with a wide variety of radiations will facilitate the calculation of delta-ray effect in radiation damage to macromolecules. When the same spectrum of radiations is applied to cells in controlled atmospheres, an excellant test will be provided for current theories of radiation injury in living materials. (Robert K. Mortimer, Kwan Hsu, and Tor Brustad)

Acute and Delayed Effects of Radiations on Yeast Cells

Studies of survival of haploid and diploid yeast cells after single exposures to various penetrating radiations led to the recognition of "recessive" and "dominant" lethal damage as operative in radiation-caused inhibition of cell division and to the "migration" model of radiation effects. A single penetrating charged particle (electron, proton, alpha particle, or carbon ion) is capable of producing both types of injury, leading to death in haploid cells and to death or impaired cell division in diploids. However, the biological site of damage is frequently not the actual site of primary ionization or excitation, but appears to be at some distance from the track. Migration of the energy effect appears to be due to diffusion of chemical intermediates--perhaps radicals--or to intramolecular migration of excitation energy. Heavily ionizing particles appear to be capable of producing greater injury farther from the ionizing tracks. In haploid cells lethal damage appears to occur so quickly that so far no postirradiation modifying factors have been demonstrated. In diploid cells, postirradiation factors (e.g., temperature) do influence survival. Sublethal doses of radiation modify these cells for several generations and cause several demonstrable forms of effects. Among

these are: mutations, reduction of growth and cell division rate, respiratory deficiencies, production of nonviable progeny, increased sensitivity to re-irradiation, and recovery. Whereas progeny of a single irradiated cell are usually impaired in their growth rate and metabolic efficiency, the mutation-like recovery process results in rapidly dividing cells again. This entire phenomenon resembles the time development of radiation-induced carcinogenesis in animals, and the underlying factors in the irradiated somatic cells of diploid animal tissue may be very similar.

A number of experiments were carried out studying the effect of continuous x-irradiation on a population of yeast cells in steady-state proliferation in a chemostat. As much as 3000 r delivered in a generation time was well tolerated by the population, which, a few generation times later, reached another steady state characterized by an increased period required for cell division. (Robert K. Mortimer)

Leukemia Induction by Radiation

Some scientists have stated that even very low levels of radiation, such as those involved in irradiation from bomb fallout, will produce leukemia and bone cancer in people, although at a lesser incidence than will very high levels of radiation. This point they have never proved directly, but on the other hand it has never been disproved conclusively, either. Moreover, it has never been proved that levels of irradiation fifty times as high, such as have been experienced by the population for thousands of years, have anything to do with leukemia induction in man. On the other hand, neither has this been disproved.

As a result we have initiated this year a program of measurement of the natural radiations (many times that from fallout) that people are usually exposed to and the corresponding incidence of leukemia. This has required setting up special instrumentation for measuring low radiation levels with the accuracy required. The other aspects of this program are concerned with directly studying any possible relationship of leukemia to such radiation levels. (Hardin B. Jones)

Effects of Radiation on Fat Metabolism

Some time ago it was proved in our laboratory that total-body irradiation of animals results in a derangement of fat metabolism which leads to marked elevation in concentration of certain of the fat-bearing lipoproteins of the blood. The important feature of this effect of radiation is the high degree of association between the extent of the disturbance in fat metabolism evident soon after the radiation and the chance that the animal will not survive the irradiation. Since this effect on fat metabolism seems so crucially related to survival of radiation injury, it is being actively investigated. (John Hewitt)

Fallout and Related Subjects

A study of the metabolism of Sr^{90} and its relation to calcium metabolism is in progress, in order to improve evaluation of the exposure

due to Sr^{90} in fallout and to seek methods of reducing absorption and of increasing elimination of Sr^{90}. The occurrence of I^{131} in cattle thyroids due to fallout has been noted and has led to a continuing program of measurement of this activity, both in cattle and in autopsied human thyroids, in the San Francisco area.

During the flurry of increased fallout that resulted from the coincidence of heavy rains shortly after a series of Soviet nuclear tests, Donner Laboratory collaborated with the California Department of Public Health in obtaining and assembling data on contamination of various kinds, especially with respect to vegetable crops, and in evaluating the resultant hazard.

The long-term effects of low-level radiation are extremely difficult to determine. Studies aimed at evaluation of these effects in human populations of sufficient size to give results of reasonable statistical validity have been initiated. Particular attention is being given to those effects involving the blood-forming systems.

Study of the excretion pattern of Sr^{90} in primates (rhesus monkeys) now includes several new individuals. A preliminary analysis of Sr^{90} excretion has been derived from data on two animals that have been followed for 4 years, three followed for 1 year, and three followed for 1 month. The observed pattern of excretion is believed to represent removal of Sr^{90} from the blood stream and the soft tissues, exchange of radioactive atoms deposited on bone surfaces, turnover of mineral in spongy bone, and turnover of mineral in compact bone. The last of these mechanisms has a half time of 980 days, which agrees well with Sr^{90} turnover calculated for human beings from recent fallout measurements in other laboratories.

Because most of the experimental work with radioisotopes of the alkaline earths has been performed with laboratory rodents, related studies are under way using the double-labeling technique (Ca^{45} and Sr^{90} in the same animals) in adult rats. Injected rats of both sexes, whose skeletal growth patterns differ, are being followed during their life spans for (a) excretion, (b) retention in the whole skeleton and selected bones of varying growth activity, and (c) skeletal histopathological changes. Retention curves are complete to 8 months for young females (65 days old at the time of injection) and to 4 months for adult females (110 days old). Initial retention was greater in the younger animals; however, it appears that the processes representing long-term turnover of mineral in the compact bone are similar for the two age groups. On the whole the retention curves for the rat appear to be in agreement with those obtained for the monkey when a correction is applied for the difference in life expectancy and skeletal growth during adult life. (Hardin B. Jones, Patricia W. Durbin, and Margaret R. White)

HEMATOLOGICAL EFFECTS OF LOW-LEVEL RADIATION DOSES IN MAN

R. Lowry Dobson, Mary M. Chupp, Nylan Jeung, and Howard G. Parker

An increased incidence of binucleated lymphocytes in the peripheral blood of humans exposed chronically to levels of radiation averaging 200 mrem per week has been confirmed at this laboratory. This phenomenon requires clarification, particularly with respect to (a) relationship of response to dose and (b) the time pattern of response after single and multiple exposures.

A group of seven healthy individuals working at the Nevada Test Site during an operational period was chosen for study. In the course of his work each individual received a single total-body exposure of gamma radiation in a period of 15 to 30 minutes.

Hematological studies were conducted during a control period prior to exposure and for 11 days after exposure. On each subject five blood counts were taken daily at 2-hour intervals for a period of 3 days before exposure. The same procedure was followed on the day of exposure and 1, 2, 4, 6, 9, 10, and 11 days postexposure. Blood films made at the same time were treated with Wright's stain for binucleated lymphocyte counting. The slides were examined at 200 × magnification. To avoid possible bias, the slides had been independently coded with random numbers before they were examined. The total numbers of white blood cells and binucleated lymphocytes were recorded. On each slide 6,000 to 10,000 white blood cells were counted. Positional coordinates for each binucleated lymphocyte were recorded.

Preliminary results of this study are presented in Table I. These represent binucleated lymphocyte counts for the control period of two pre-exposure days and for two selected postexposure periods. Data from the third pre-exposure day, the day of exposure, and the fourth and sixth post-exposure days are not available at this time. The first and second post-exposure days were considered together as Period I, and the ninth, tenth, and eleventh postexposure days together as Period II. Significant increases in binucleated lymphocytes were seen in the two subjects who received the highest doses: the subject who received 2.4 r showed an increase during the second postexposure period, and the subject who received 2.6 r showed an increase during the first postexposure period. This increase in binucleated lymphocytes was demonstrated at the 5% significance level by computation of the ratio of the means. Although a dose effect is suggested, since the persons receiving exposures of less than 2 r failed to show a significant response, the fact that each of these two individuals showed a response at only one of the postexposure times indicates that caution should be used in relating the binucleated-lymphocyte response to the radiation alone.

It is also of importance to note that the incidence of binucleated lymphocytes seen at control times in this group is rather higher than we have observed in previous experiments. It is possible that the erratic pattern of daily living of all seven subjects during the period of observation was responsible for the high incidence of binucleated lymphocytes seen during

the control period; furthermore, otherwise detectable responses to the smaller radiation doses may have thus been obscured.

Although the results of this experiment are still incomplete, the apparent pattern is of great interest. Experiments are now being designed to further confirm these results and to explore the nature of this phenomenon. This information should be of value in furthering our understanding of the radiosensitivity of the lymphoid system, and may help in developing a biological method for observing small doses of radiation in the human.

Development of an automatic electronic counter to permit more rapid compilation of larger volumes of data on binucleated lymphocytes is being considered. It will probably be an essential tool in this work in the future.

Information of this kind should be of value in advancing our understanding of the radiosensitivity of the lymphoid system and may help in developing a biological indicator of small doses of radiation in man.

Table I

The incidence of binucleated lymphocytes (BnL) among leukocytes in the peripheral blood of seven normal persons before and after exposure to single doses of gamma radiation

Subject	Dose (r)	Number of leukocytes counted			Number of BnL seen			Calculated number of BnL per 50,000 leukocytes		
		Control Period	Period I[a]	Period II[b]	Control Period	Period I[a]	Period II[b]	Control Period	Period I[a]	Period II[b]
A-1	0.7	115,729	80,000	90,000	5	8	1	2.2	5.0	5.6
A-2	0.7	136,331	90,000	110,000	12	9	9	4.4	5.0	4.1
A-3	1.4	140,000	85,898	89,406	15	7	12	5.4	4.1	6.8
A-4	1.5	140,000	90,000	110,000	12	13	9	4.3	7.2	4.1
A-5	1.7	119,911	50,000	100,000	26	9	17	10.8	9.0	8.5
A-6	2.4	152,382	90,000	100,000	10	14	21	3.3	7.8	10.5
A-7	2.6	170,000	90,449	110,000	55	50	33	16.2	27.8	15.0

[a] First and second postexposure days were considered together as Period I.

[b] Ninth, tenth, and eleventh postexposure days were considered together as Period II.

PHYSICAL BIOCHEMISTRY AND ITS APPLICATIONS

WORK IN PROGRESS: SUMMARY OF SELECTED STUDIES

Introduction

Studies have continued on methodology for the large molecules of the blood--including those bearing the various lipids (fatlike materials)--and the serum proteins. Special methods evolved for study of the serum proteins are free of many of the types of errors that were present in the older methods available. The new methods have been used for measurements of the amounts of these important biological substances in persons in health, those with several forms of cancer and leukemia, and those with liver disease. These findings may help sharpen understanding of certain metabolic abnormalities of cancer and other states as they affect proteins.

Studies of the lipids and lipoproteins of blood (of importance in radiation injury and in heart disease) have continued with respect to analytical methods for the determination of some of the subconstituents, known as fatty acids, that help make up the lipoproteins of the blood.

The electron microscope, the ultracentrifuge, spectroscopy, and radioisotopes have been very advantageously utilized in the study of the blood lipoproteins.

Electron Microscopy

Following the direct visualization of the serum lipoprotein macromolecules by means of this laboratory's electron microscope (RCA, EMU 2-E), a study of the submicroscopic morphology of coronary artery tissue was undertaken. In view of the strong correlation between elevation of certain serum lipoproteins and atherosclerosis, it was of interest to investigate the affected tissue by techniques that would permit visual examination of these macromolecules.

It was first necessary to establish techniques for the fixation, staining, and sectioning of such tissue. A method has been set up in this laboratory using osmuim fixation, methacrylate embedding, and sectioning on a Porter-Blum ultramicrotome, which yields satisfactory ultrathin sections for viewing in the electron microscope.

Preliminary results suggest that this is a reasonable way to investigate early submicroscopic changes in the arterial tissue as well as the mechanism by which submicroscopic particles might be transported from the blood to the arterial wall. Such information would be of value in testing any theory of the genesis of atherosclerosis. (Thomas T. Hayes)

Influence of Diet on Serum Lipoproteins

The significant relationships between serum lipoproteins and heart disease have directed a major part of our research effort toward an evaluation

of the underlying metabolic factors responsible for abnormal lipoprotein concentrations in the blood. As one of the steps in this evaluation, the role of dietary composition in lipoprotein metabolism was ascertained. When present in excess in the diet, animal fats (saturated fats) and carbohydrates were found to influence two chemically distinct lipoprotein groups. Animal fats increased the concentrations of the major cholesterol-bearing lipoproteins, while the carbohydrates increased the concentrations of the major fat-bearing lipoproteins. Elevated concentrations of these lipoproteins were reduced by lowering the animal fat and(or) the carbohydrate content of the diet, depending upon which one of the major lipoprotein groups was elevated. Vegetable oils like cottonseed oil were found to be effective substitutes for animal fats or carbohydrates. They did not increase lipoprotein concentrations and served as sources of palatable calories.

Utilizing such variables as animal fats and carbohydrates, we can now set up controlled experiments to uncover some of the metabolic bases for lipoprotein elevations in the blood. Radioactive tracer experiments with labeled fat are being developed by which some of the metabolic pathways during particular conditions of diet can be followed. Likewise, chemical methods--in particular gas chromatographic techniques--are being set up for studying the types of fats in the various lipoprotein fractions during such experimental periods of diet. We expected to combine the above-mentioned techniques to study interconversions between the various fats in serum lipoproteins. (Frank T. Lindgren and Alex V. Nichols)

Ultracentrifuge Studies of Serum Lipoproteins

As part of the continuing program in the study of the physical and chemical properties of serum lipoproteins, extensive ultracentrifugal experiments are in progress to establish techniques for isolation of homogeneous lipoprotein fractions. Such fractions then serve as precisely characterized materials for chemical analyses and electron microscopy. It is the aim of these studies to investigate the fine structure of particular lipoprotein groups and relate them to biochemical as well as to clinical abnormalities. These ultracentrifugal techniques utilize detailed knowledge of the salt gradients occurring in the lipoprotein solutions during ultracentrifugation. (Frank T. Lindgren and Alex V. Nichols)

Infrared Spectroscopy

A new and simplified type of infrared spectrophotometric analysis for serum lipids has been developed and is being evaluated on both milligram and submilligram levels of lipid-sample size. The chromatographic separation employed in our earlier procedures is not necessary with this method, but less information is obtained. An infrared filter photometer is under development for the purpose of measuring total lipids from very small amounts of serum (less than 0.1 milliliter). The principal applications of such simplified procedures can be foreseen as screening tests for large numbers of samples.

Analysis of the phospholipid components of blood serum has been made possible by a combined chromatographic and infrared-absorption method.

For the more complex mixtures of phospholipids from various tissues, experience thus far indicates that this procedure is a good general approach for this class of compounds, but that more basic spectral information is needed, as well as refinements in the chromatographic procedure.

Some progress has been made in the use of the infrared microspectrometer for examining the lipid deposits in arterial or other tissue. A few reasonably good spectra of tissue sections have been obtained, and they appear to be at least partially interpretable in terms of lipid content. Efforts will be continued in this area.

The effects of various fats on experimental atherosclerosis in rabbits is being studied in feeding experiments, with special emphasis on the "unnatural" isomers of fatty acids that are produced in commercial hydrogenation processes. The depostion of such fatty acids in the aortae of rabbits has been demonstrated, but insufficient chemical data have been accumulated for statistical comparison with the behavior of other fatty acids. (N. Keith Freeman)

BLOOD AND BLOOD CIRCULATION

RED BLOOD CELL STUDIES

Myron Pollycove

Radioiron (Fe^{59}) was used to study red blood cell production and destruction and iron metabolism in 38 patients with a wide variety of blood disorders. Serial measurements of radioiron in plasma, red cells, bone marrow, liver, and spleen were performed to quantitate hemoglobin synthesis, mean maturation time of red blood cells, red cell life span, and the amount of iron exchanging with storage iron. Sites of red cell destruction were determined; in many cases these measurements led to selection of successful therapy.

The survival of white cells, red cells, and platelets in circulating blood was determined in five normal subjects and in four patients with blood disorders. P^{32}-diisopropylfluorophosphonate, a compound which rapidly and irreversibly binds various intracellular enzymes, was used to label the blood cells and platelets. In two patients survival of granulocytes in the circulation was markedly shortened to approximately 1 day (normal 5 to 15 days). The results of these studies were correlated with results of iron kinetic studies.

In another approach to this problem, studies are continuing on the kinetics of red blood cell destruction in the rat when it is returned to sea-level pressure after acclimatization to the pressure at 15,000 ft. These have been further correlated with red cell life span measurements. More measurements of this type will be done. It is also planned to reinvestigate some aspects of the response of red cell volume to increased pO_2 in a box built for the purpose, as well as to decreased pO_2 in the altitude chamber.

STUDIES ON ERYTHROPOIETIC SUBSTANCES IN URINE

Donald Van Dyke and Joseph F. Garcia

Highly potent erythropoietin (the material that stimulates red blood cell formation) has been concentrated from the urine of two patients with severe anemia. This material has been used for both chemical and biological investigation. Erythropoietin is neither precipitated nor inactivated on boiling, is not completely destroyed by prolonged digestion with trypsin, is precipitated at pH 3, and is present in the residue remaining after ultrafiltration which contains a large amount of carbohydrate. These findings, suggesting that the urinary material may be a mucoprotein, have formed the basis for the isolation procedure used.

This material concentrated by ultrafiltration and injected into normal rats daily for 14 days, has caused a 60% increase in the total circulating red cell volume. It also has produced a polycythemia in normal mice and rabbits and one monkey. A single injection of this material has been found to produce profound effects on iron metabolism in five normal human beings. Further purification is in progress so that the therapeutic possibilities may be investigated.

By use of continuous paper electrochromatography, progress has been made toward separation of the erythropoietic activity in ultrafiltrates of urine of certain patients. Other physical methods such as ultracentrifugation are also being used to characterize this material. Present indications are that activity may be associated with more than one homogeneous subfraction of the starting material. In order to single out the specific subfractions that are capable of stimulating red cell formation, development of new methods and separation procedures for preparation and purification of biological samples is being continued. Starting from known possibilities of gradient electrophoresis, a project is under way which would make possible such separations as a rapid continuous process, especially for larger-scale preparations, in free-boundary liquid systems.

EFFECT OF HIGH ALTITUDE ON RED CELL FORMATION

Will Siri and Charles Webster

The investigation of red cell formation was further augmented by measurements of the rate of radioiron incorporation that were performed in Bolivia on native miners who were fully acclimatized to an altitude of 17,000 ft. At this altitude the rate at which radioiron is exchanged in plasma and incorporated in red cells is about twice as great as at sea level. Since the rate of formation of red cells is greatly increased at high altitude, ultrafiltrates of urine from these subjects were tested for erythropoietic activity.

HEART FUNCTION AND CIRCULATION

Myron Pollycove

Heart function in four normal subjects and in five patients with anemia or polycythemia was studied with I^{131} albumin. Simultaneous measurements were performed of body surface, heart blood radioactivity and arterial blood radioactivity. Blood outputs of the heart per minute were calculated independently from both heart and arterial data and found to be in good agreement. Heart-output determinations were performed accurately in this way without arterial puncture. In addition, this procedure permitted measurement of the circulation time from vein to right heart and from right heart to left heart. Continuous recording of blood pressure was used for calculation of mean body arterial resistance to blood flow. Correlation of heart output with blood content of hemoglobin and red blood cells was begun.

AUTORADIOGRAPHIC STUDIES OF MOUSE LEUKOCYTES

Bernard J. Bryant and Lola S. Kelly

Studies have been continued during the last year on leukocyte maturation and life spans in mice by means of the autoradiographic technique. Autoradiography makes possible a measurement of the activities of individual cells that have incorporated a radioactive precursor. This paper reports data based on analyses of the labeling seen in the different classes of white cells in the blood and blood-forming tissues. Such analyses are not possible by the biochemical methods usually employed.

The adenine C^{14} used here is a convenient label for cells synthesizing new DNA during an interval at the start of the experiment. It is now generally agreed that once DNA has been synthesized from the nucleotides, it remains and is stable for the life of the cell or until it is segregated at the next division. Active DNA in a cell is thus a permanent label for the cell.

Young adult male A strain mice were injected intraperitoneally with 8.5 μC each of adenine C^{14}. The animals were sacrificed at regular intervals following the injection and smears were taken from various tissues--tail blood, thymus, lymph nodes, and femoral marrow. Adenine is quickly taken up in the cells in the form of nucleotides and at some later time is synthesized into both RNA and DNA. The smears were fixed in absolute methanol for 1 hour, then washed in distilled water for 1 hour to remove unpolymerized nucleotides. Duplicate smears were also digested with ribonuclease to remove RNA. After this treatment the only remaining activity is due to adenine incorportaed into DNA.

The smears were next prepared with autoradiographic stripping film (Pelc, Intern. J. Appl. Radiation and Isotopes 1, 172 (1956)) and exposed for 30 days. At the end of this time the slides were photographically processed and subsequently stained through the film with Giemsa stain.

Figure 1 shows the strikingly different courses of adenine incorporation into DNA in the three major classes of white cells in peripheral blood. Labeled neutrophils rise sharply from the second day after injection to day four, by which time more than half the cells are labeled. This is followed by a rapid decline. Labeled small lymphocytes, on the other hand, show a plateau at 2 to 3% that lasts for at least 8 days, and have disappeared by the 14th day. This indicates that most of the small lymphocytes were formed before the start of the experiment, in contrast to the neutrophils. Correspondingly, the life of the small lymphocyte is long compared with that of the neutrophil. Labeled medium and large lymphocytes reach a maximum at 1 to 2 days and have disappeared by the fifth day.

The question arises, however, whether neutrophils disappear from the body at the end of a life span or randomly with respect to their ages. To find the answer one must know the data on the immediate precursors in the marrow. Figure 2 shows data on the last two of the maturation stages of neutrophils--the band and mature neutrophils in the marrow--togehter with the peripheral neutrophils. It should be noted that these values are from bone marrow smears not extracted with ribonuclease, since this treatment would make marrow-cell identification impossible. Peripheral neutrophils contain little RNA label, however, since RNase treatment does not measurably lower the activity of this class. The same may be true of mature marrow neutrophils, since they have lost the staining property for RNA in the cytoplasm.

The data of Fig. 2 suggest that the band neutrophil has a definite maturation time, but that once the neutrophil has matured, it is released to the circulation randomly in time. More complete data will be necessary to estimate band cell maturation time and the time spent by mature neutrophils in the marrow.

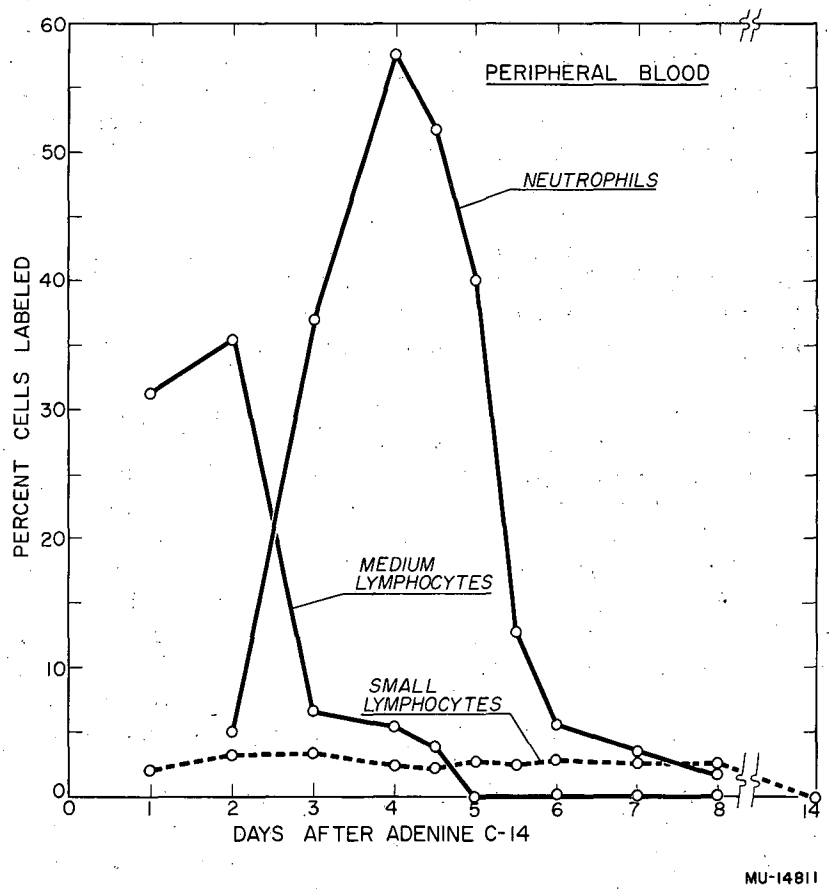

Fig. 1. Percent of circulating white cells with labeled DNA as a function of time after a single injection of adenine C-14.

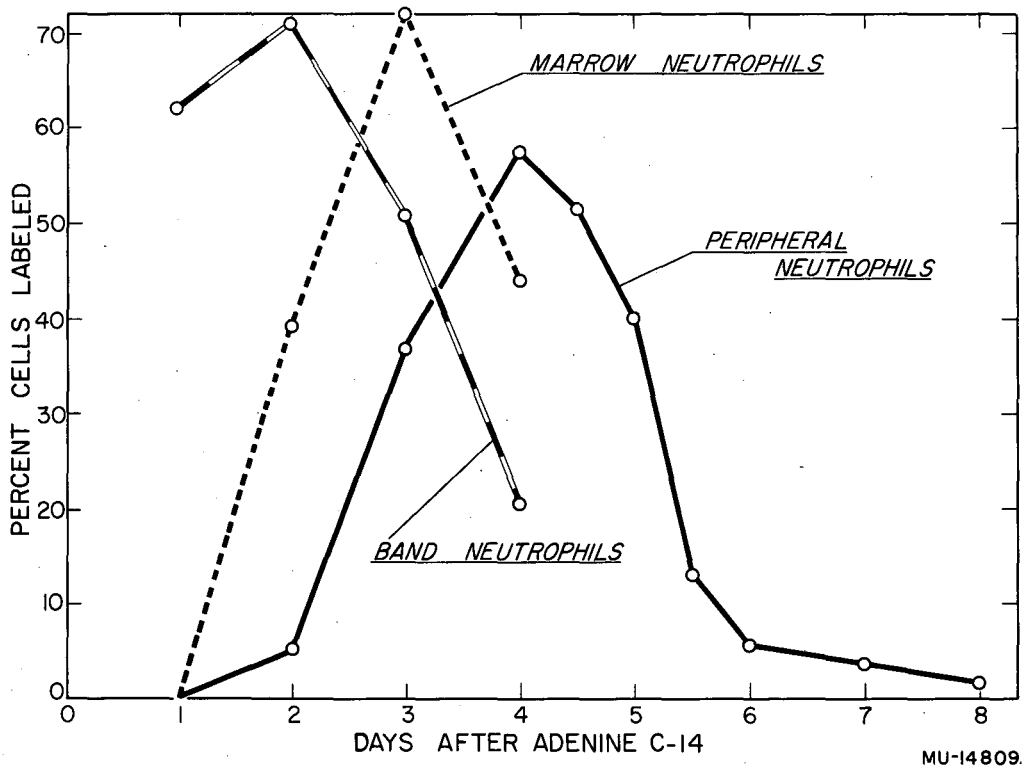

Fig. 2. Percent labeled neutrophils seen in marrow and peripheral blood as a function of time after a single injection of adenine C-14.

A conclusion on whether neutrophils, once released from the marrow, disappear from the body randomly or at the end of a life span can be approached by comparison of experimental blood values with theoretically derived values. The derived values are based on the assumption that mature marrow neutrophils are released randomly to the circulation.

Values for levels of labeled neutrophils in the blood calculated on the basis of the random-disappearance model fit the experimental data poorly regardless of the assumed rate of disappearance. If a two-day life span is assumed, however, the calculation for expected levels of labeled cells in the circulation agrees closely with the experimental values. This calculation fits the experimental data whether one chooses the entire population of labeled neutrophils or only those having a high grain count. The shape of the curve is confirmed, in that calculations for $1\frac{1}{2}$- and $2\frac{1}{2}$-day life spans fall symmetrically on either side of the experimental data. Since data are not yet available for the marrow past the fourth day, none of these calculations has been carried past day $4\frac{1}{2}$. The tentative conclusion then is that neutrophils have a 2-day life span after release from the marrow. A consideration of relative pool sizes leads one to conclude that a large part of this time is spent extravascularly. This agrees with data published recently by several authors (for review see Patt, Blood 12, 777 (1957)).

Data on events in the lymphoid tissues are presented in Fig. 3. The percentage of labeled cells in the nodes is an average from values obtained in the axillary, cervical, and mesenteric nodes, since no significant difference was found between them. The most striking finding is the high activity of the mouse thymus in comparison with the lymph nodes. By the third day, however, essentially all the active cells have disappeared from the thymus, indicating that the turnover time for thymus cells is in the range of 2 to 3 days. The lymph nodes reach a much shallower peak and retain a comparatively large proportion of active cells for at least 4 days. This suggests that the lymph nodes have a turnover time that is long compared with the thymus. These turnover times are in good agreement with our biochemical estimates from P^{32} incorporation into DNA--2 days for thymus and 15 days for lymph nodes in A-strain mice.

In peripheral blood, labeled small lymphocytes plateau at 2 to 3% for the first 8 days and have disappeared by the 14th day. The life of the small lymphocyte is thus placed somewhere between 8 and 14 days. A large part of this time is probably spent in the organized or diffuse lymphoid tissue rather than in the circulation.

In all tissues, medium and large lymphocytes represent about 10% of the total lymphocyte population. Medium and large lymphocytes in the blood reach a peak at 1 to 2 days and have essentially disappeared by the fourth day. The life of these cells is therefore on the order of 2 to 3 days. This corresponds to the data of Ottesen (Acta. Physiol. Scand. 32, 75 (1954)), who has reported that in human subjects about 10% of the lymphocytes have a mean age of 3 days while the remainder are much older. It is proposed that his results correspond to medium and large lymphocytes and to small lymphocytes respectively.

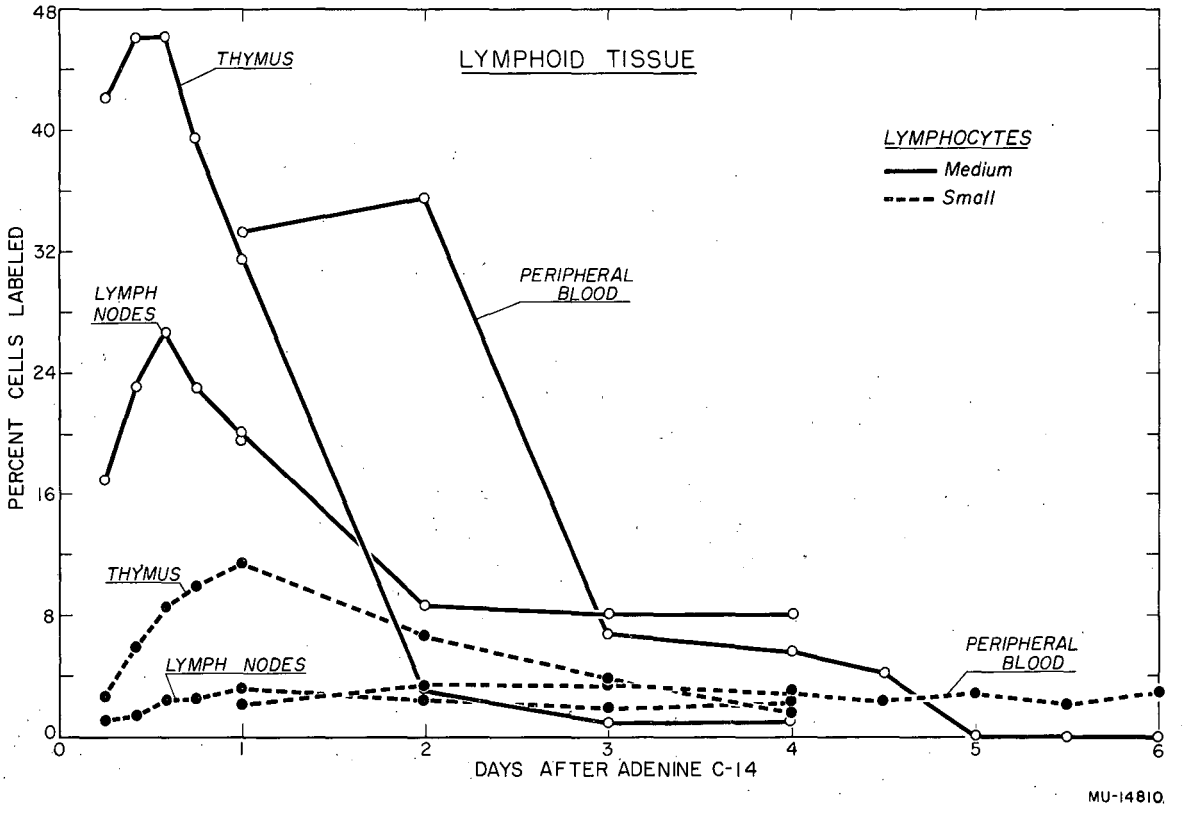

Fig. 3. Percent DNA labeled lymphocytes seen in lymphoid tissues and peripheral blood as a function of time after a single injection of adenine C-14.

These preliminary estimates are summarized as follows: (a) peripheral neutrophils have a 2-day life span; (b) the life of medium and large lymphocytes is in the range of 2 to 3 days; (c) the life of small lymphocytes is between 8 and 14 days.

OTHER PHYSIOLOGICAL AND MEDICAL STUDIES

PROLIFERATION OF THE LIVER RETICULOENDOTHELIAL SYSTEM

Lola S. Kelly, Caroline R. Finney, J. Dorothy Hirsch, and Ernest L. Dobson

One of the functions of the liver is the removal of cellular debris and particulate matter from blood by the phagocytic action of cells of the reticuloendothelial (RE) system.

Following the temporary blockade of the RE system by injection of colloids, the phagocytic function of the liver gradually returns to normal. Also, phagocytic activity can be increased above normal by the injection of yeast cell walls (Zymosan) or endotoxins. It has been suggested that formation of new RE cells occurs under both of these conditions.[1,2] The experiments reported herein were undertaken to test this hypothesis.

Synthesis of desoxyribosenucleic acid (DNA) as measured by the incorporation of radioactive phosphate, has been used as a sensitive indicator of new cell formation. Approximately 25 μC of P^{32} were injected into Swiss male mice at various times after the injection of a blockading dose of colloids (carbon or saccharated iron oxide) or of Zymosan. Two hours after the P^{32} injection the mice were sacrificed and liver DNA was isolated for the determination of specific activity. The phagocytic activity was measured by the rate of removal from the blood of intravenously injected colloidal carbon.[3]

Figure 4 shows the results obtained after a blockading dose of saccharated iron oxide (Proferrin, 1.5 mg/mouse). It can be seen that after an initial reduction the rate of removal of carbon returns to normal by the third day. During this recovery period the liver DNA specific activities rise steeply, demonstrating new cell formation. Similar results were obtained when colloidal carbon was used as the blocking agent.

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[1] Benacerraf, Halpern, Biozzi, and Benos, Brit. J. Exptl. Path. 35, 97 (1954).

[2] B. Benacerraf and M. M. Sebestyen, Federation Proc. 16, 860 (1957).

[3] Kindly supplied by Dr. B. Benacerraf.

Figure 5 shows the same measurements at various times after a yeast cell wall preparation (Zymosan, 1 mg/mouse). As Benacerraf has demonstrated, Zymosan injection results in a marked increase in the phagocytic activity. The striking increase in the incorporation of P^{32} on the second and third days leaves little doubt that very active new cell formation occurred. Concomitantly the liver size increased approximately 20% by the third day. Nuclear counts were made in randomly selected fields of liver sections to determine if there had been a selective increase in one cell type. It was found that per hundred parenchymal cell nuclei the littoral cells increased from 72 in the controls to 119 on the third day after Zymosan.

A preliminary experiment has been performed to define the cell type primarily responsible for the increased DNA synthesis and cell division. Tritium-labeled thymidine, a specific precursor for DNA, was injected into Zymosan-treated mice and radioautographs of liver sections were prepared. Whereas control livers show essentially no "positive" cells, each microscopic field in the treated livers contained several littoral cells that had incorporated thymidine into DNA. No positive parenchymal cells were seen, indicating that cell proliferation is limited to the littoral cells.

The finding that new cell formation is involved in reticuloendothelial system function may help to resolve the current uncertainty as to its relative radiosensitivity.

THE REGULATION OF THE VOLUME OF THE BODY FLUIDS

Ernest L. Dobson, Dorothy M. Adamson, Donna Betz, and Fredrick Mustell

The hypothesis has been advanced by others, and extensively reviewed by Smith,[1] that changes in plasma and extracellular fluid volume activate a "volume receptor," causing changes in salt and water excretion. On the other hand we have proposed as an alternative hypothesis that the changes in salt and water excretion that accompany isotonic expansion or contraction of the body fluids are caused by alterations in liver and kidney blood flow rather than by stimulation of a specific "volume receptor."[2,3] Such changes in body fluid volume probably do not activate the osmoreceptor described by

[1] Homer W. Smith, Salt and Water Volume Receptors, Am. J. Med. 23, 623-652 (1957).

[2] E. L. Dobson, Homeostatic Regulation of Body Fluid Volumes: Role of a Blood-Flow-Dependent Control Mechanism, Federation Proc. 16, 31 (1957).

[3] E. L. Dobson, Relation of Hormone Dynamics to Body Fluid Volume Homeostasis, Federation Proc. 17, 36 (1958).

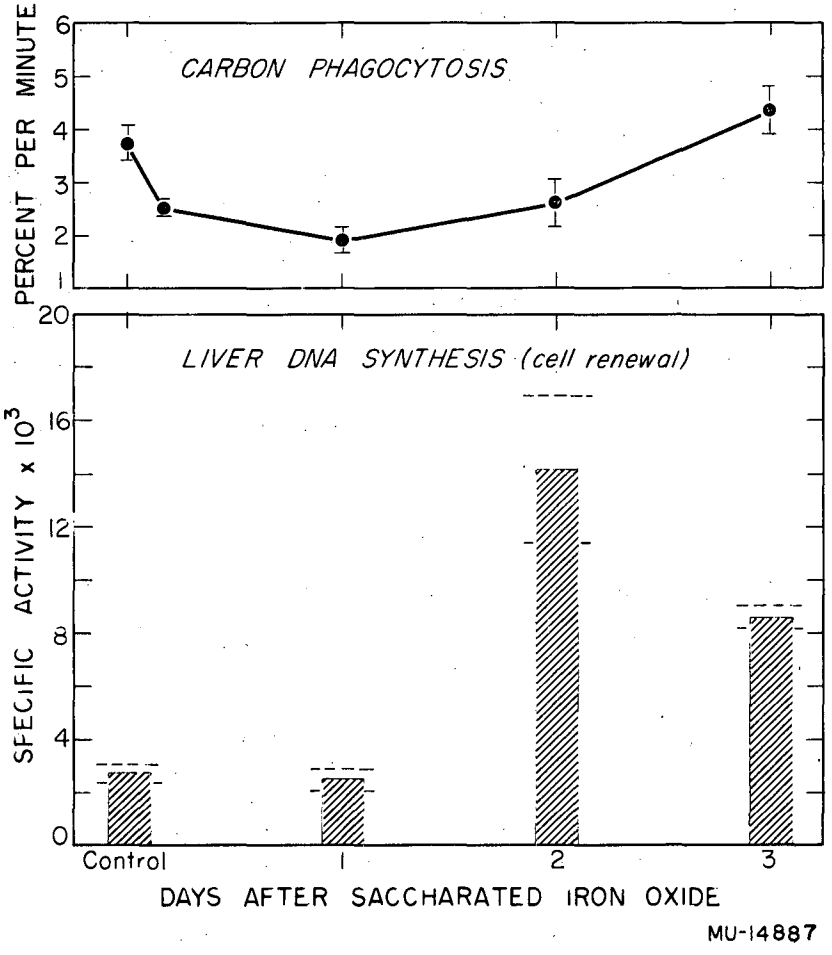

Fig. 4. Phagocytic function and DNA synthesis at various times after intravenous Proferrin injection.
(Specific activity = (cpm/mg P)/cpm injected/g).

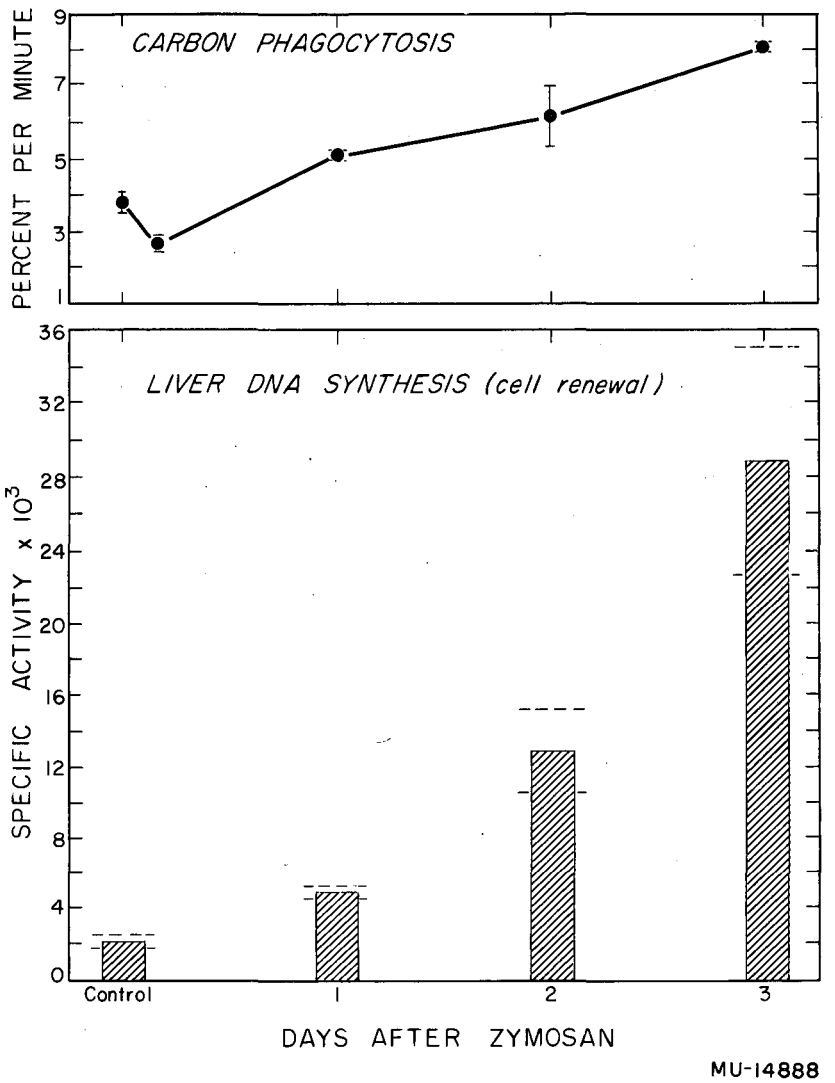

Fig. 5. Phagocytic function and DNA synthesis at various times after intravenous Zymosan injection.
(Specific activity = (cpm/mg P)/cpm injected/g).

Verney[4] because they involve no changes in the osmolarity of the tissue fluids. Nevertheless, volume changes through their effect on blood flow, can alter blood hormone levels by changing rates of destruction. This is because the blood flow to the liver and kidney is a rate-limiting step in hormone inactivation. Thus the infusion of isotonic saline or plasma increases the liver and kidney blood flow, which increases the rate at which the hormones are destroyed, causing a fall in the hormone concentrations in the blood. A decrease in antidiuretic hormone concentration permits a more rapid loss of water, and a decrease in aldosterone concentration permits a more rapid loss of salt. This increased salt and water excretion falls off gradually because the liver and kidney blood flow return toward normal as normal fluid volume is restored. A decrease in the volume of the blood plasma effected by bleeding causes the reverse effect, namely a decreased blood flow and a consequent increased hormone level and an avid retention of salt and water until the deficit in volume is made up by intake.

Data supporting this hypothesis are presented in Table II. This table shows how changes in the blood flow to liver and kidney are related to the rates of salt and water excretion in the dog.

The infusion of isotonic saline or plasma (Items 1 and 2, Table II) caused a marked increase in liver and kidney blood flow, and more than doubled the rate of salt and water excretion. In some cases the infusion of plasma produced a reaction, (Item 3, Table II) characterized by edematous spots on the skin. This reaction is accompanied by circulatory disturbances and by a loss of plasma, apparently into the edematous swellings. In the case cited as Item 3, Table II, the loss of plasma was no greater than the amount infused, so that the final measured plasma volume was equal to the animal's original plasma volume. There was, however, a very marked decrease in the circulation to the liver and kidney and a reduction in salt and water excretion to one-quarter of the prereaction value.

The infusion of red cell mass (78% hematocrit blood) caused no change in liver plasma flow but a 30% increase in renal plasma flow, resulting in a slight increase in total flow to both liver and kidneys. This resulted in a diuresis, but its magnitude was markedly less than that obtained by an infusion of equivalent volumes of either saline or plasma (Items 1 and 2). Previous work has indicated that blood flow may be increased without net volume changes by exchange of plasma or dextran for whole blood, and possibly decreased by volume-for-volume exchange of red cell mass for whole blood.[5] Such isovolemic exchange experiments (Items 5 and 6, Table II) indicate that salt and water excretion are altered by changes in liver and kidney blood flow without concomitant changes in volume. In these experiments volume-for-volume exchange was carried out with red cell mass (99% hematocrit blood) and the plasma expander dextran. These results show that when there is a net increase in liver plus kidney plasma flow, the excretion of salt and water increases, and when liver plus kidney blood flow decreases, the excretion of salt and water decreases.

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[4] E. B. Verney, The Antidiuretic Hormone and the Factors Which Determine Its Release, Proc. Roy. Soc. (London) Ser. B. 135, 25-106 (1947).

[5] E. L. Dobson, G. F. Warner, C. R. Finney, and D. M. Adamson, Early Circulatory Disturbances Following Experimental Thermal Trauma, UCRL-2987, May 1955.

Table II

Effect of changes in blood flow on salt and water excretion

	Percent Change in				
Infusions (10 ml/kg)	Liver plasma flow	Renal plasma flow	RPF and LPF	Urine flow	Salt excre-tion
1. 0.9% Saline	+12	+24	+15	+162	+140
2. Plasma	+18	+ 3	+12	+187	+106
3. Plasma (reaction)	-39	-24	-34	- 73	- 75
4. Red cell mass	0	+30	+ 8	+ 77	+ 72
Isovolemic Exchanges (16 ml/kg)					
5. Red cell mass for whole blood	-18	-24	-22	- 7	- 54
6. Dextran for whole blood	+56	+21	+52	+ 44	+ 26

Figures 6, 7, and 8 show the salt- and water-excretion patterns together with blood-flow patterns produced by saline and plasma infusion. Figure 8 shows an initial increase in excretion followed by a pronounced fall in excretion when the circulation is impaired as a result of an "allergic" reaction to the infused plasma.

Figure 9 compares the excretion response to an infusion of plasma with the excretion response to an infusion of an equal volume of high-hematocrit blood (78% cells). In the former a marked increase in blood flow is accompanied by a greatly increased urine flow. In the latter a much smaller increase in blood flow results in a very much less marked diuresis.

Figure 10 compares the effect on water excretion of a volume-for-volume exchange of dextran for whole blood (a manipulation that increases the liver and kidney plasma flow without changing the blood volume) with the effect on water excretion of a volume-for-volume exchange of red cell mass (a manipulation that decreases the liver and kidney plasma flow without changing the blood volume).

Thus the relationship observed by others between isotonic expansion or contraction of the body fluids and salt and water excretion appears to be mediated through changes in liver and kidney blood flow produced by these volume changes rather than through the stimulation of a specific "volume receptor." Elimination of the need for a "volume receptor" simplifies considerably the explanation of the response of salt and water excretion to isotonic expansion and contraction of the volume of the body fluids.

CARBOHYDRATE METABOLISM

Myron Pollycove

Glucose metabolism was studied with C^{14} glucose. Following intravenous injection of a tracer amount of this substance, body-pool size, turnover rate, and oxidation to carbon dioxide were determined. These aspects of carbohydrate metabolism were measured in three normal subjects and two diabetic patients, without treatment and following the administration of insulin and new oral compounds such as orinase and DBI, which lower blood sugar. Preliminary results indicate that orinase, unlike insulin, does not increase oxidation of glucose, but that under certain circumstances DBI may increase glucose oxidation.

ESTROGEN DETERMINATIONS IN CARCINOMA OF THE BREAST FOLLOWING PITUITARY IRRADIATION

Anne S. Cleveland

It is generally believed that most types of cancer of the breast are hormone-dependent, although there is evidence that indicates that at some phase in their growth, through a process yet unknown, these cancers may become autonomous. If cancer of the breast is hormone-dependent, the study of levels of estrogen, as the hormone most directly implicated, would seem of primary importance.

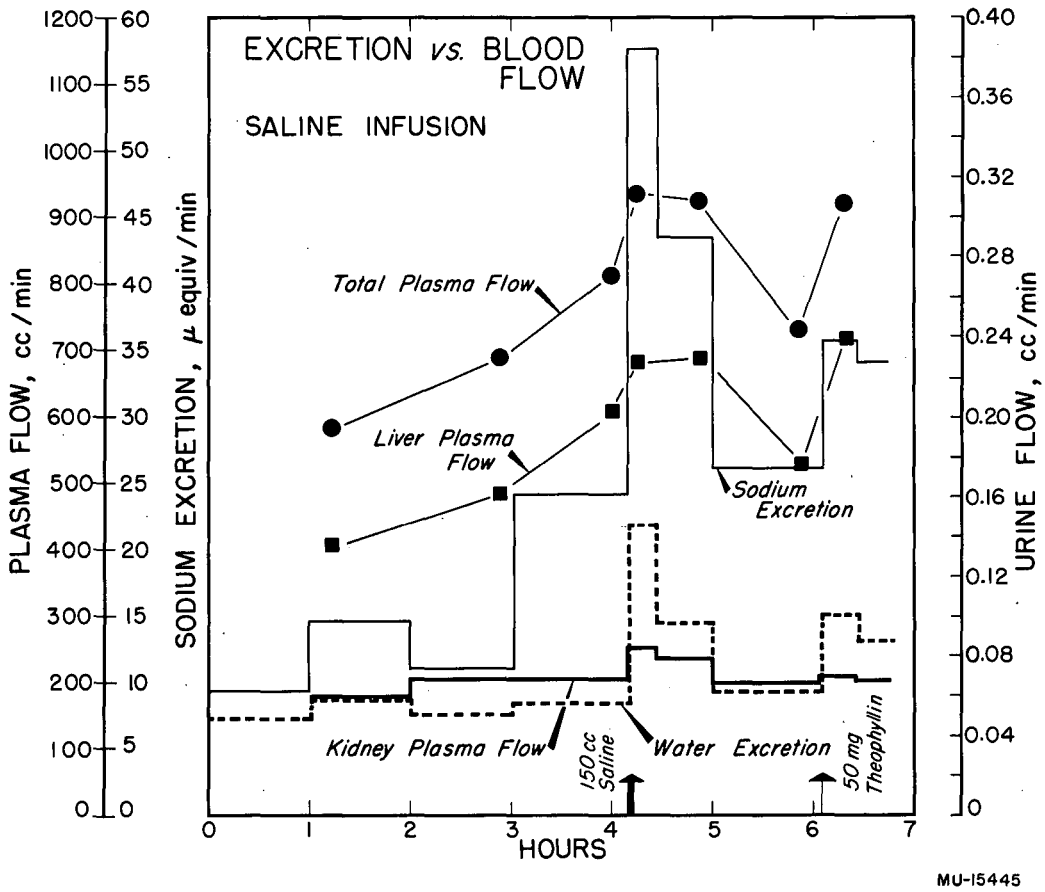


Fig. 6. The effect of saline infusion on liver and kidney plasma flow and the corresponding response of salt and water excretion. Note that changes in blood flow produced by the intravenous injection of 50 mg of Theophyllin caused corresponding changes in salt and water excretion.

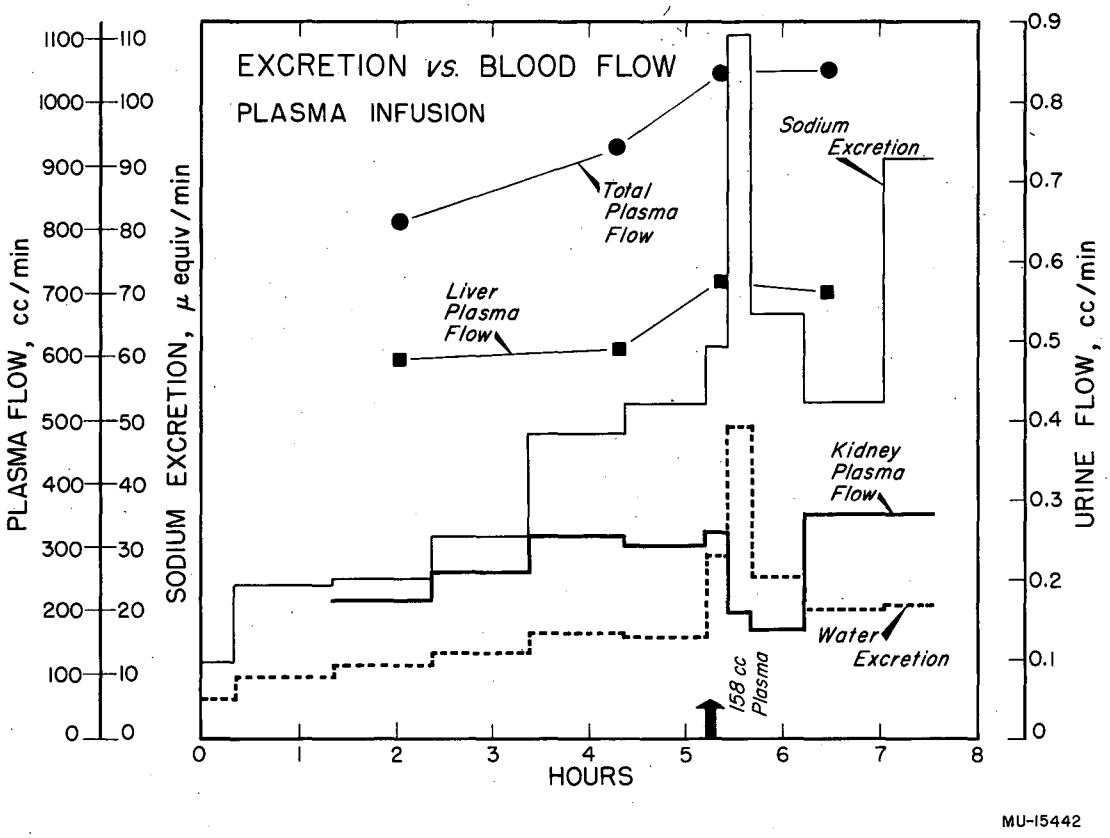


Fig. 7. The effect of plasma-volume expansion by the infusion of plasma on liver and kidney plasma flow, and the corresponding response of salt and water excretion.

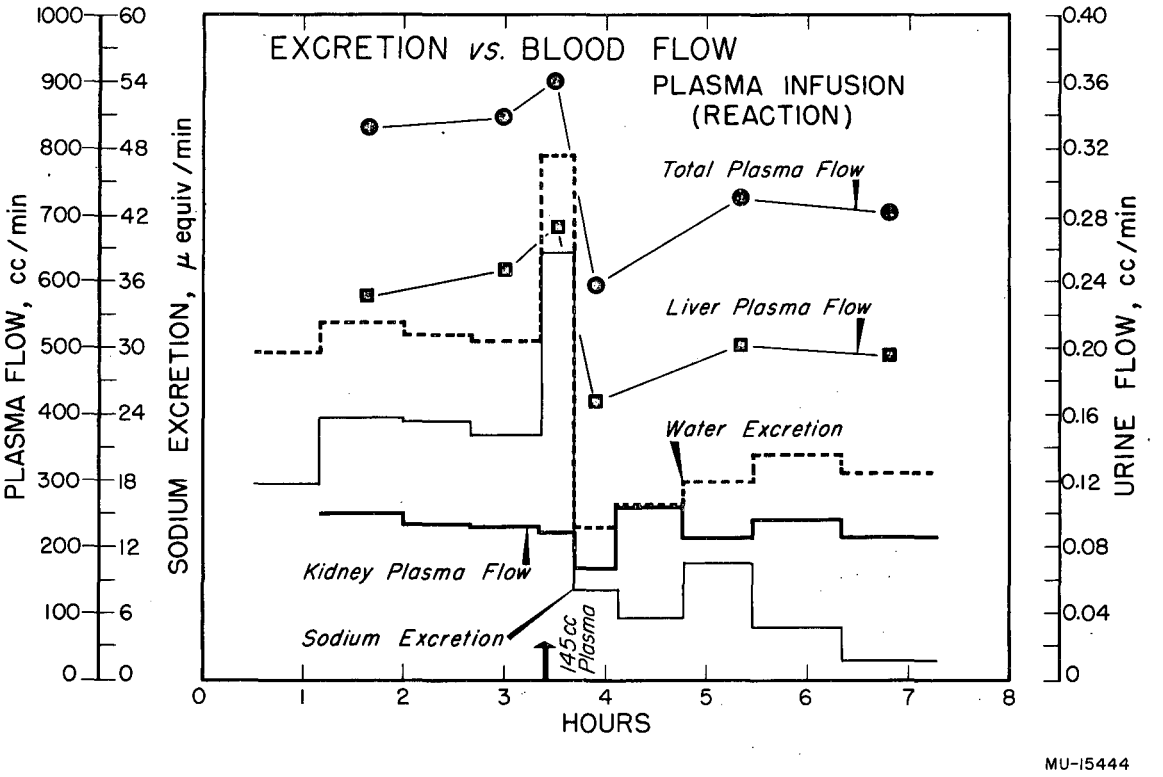


Fig. 8. The effect of an "allergic" reaction to infused plasma. This is the same dog as presented in Figs. 6 and 7. The temporary increase in blood flow produced a temporary increase in both salt and water excretion. Development of the reaction to the infused plasma produced a marked fall in both salt and water output. Plasma-volume measurements showed a temporary increase and then a fall, but the plasma volume did not fall below the preinfusion value. Therefore, the decreased rate of excretion of salt and water is considered to be a result of the decreased blood flow.

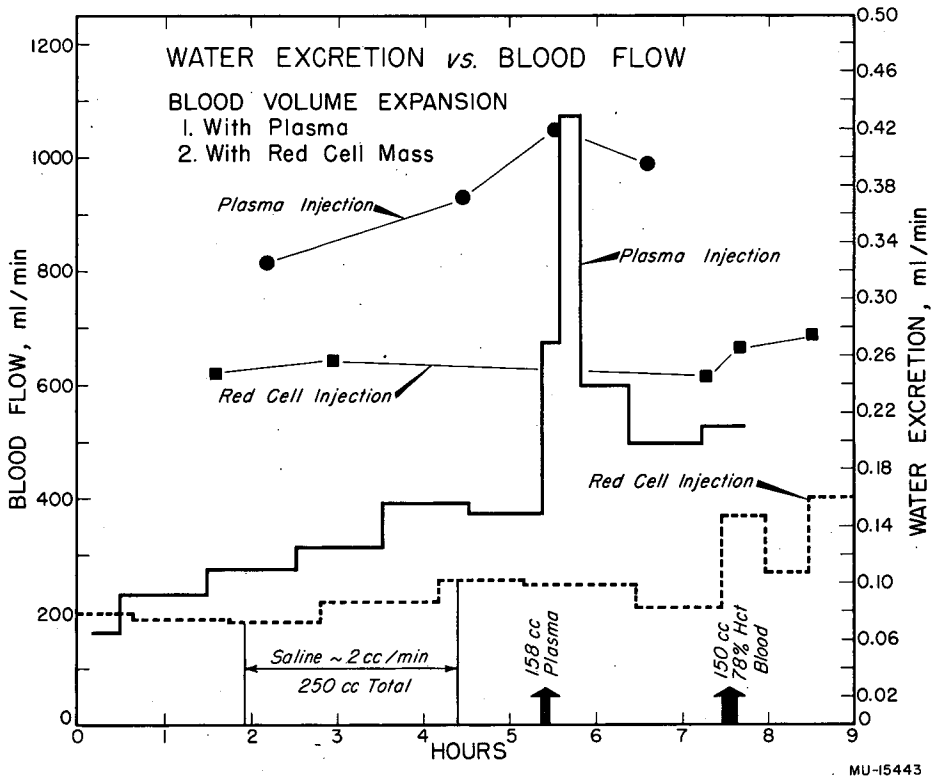


Fig. 9. This shows the very marked difference in diuretic response to volume expansion by plasma infusion and red cell infusion. Infusion of 158 ml of plasma nearly tripled the rate of water excretion, while an equal volume of high-hematocrit blood (78% cells) caused an increase of only about 50% in the water-excretion rate. Total liver plus kidney plasma flows are indicated by the dots, plasma infusion by circles, and red cell infusion by squares. Water excretion is indicated by the line graphs, plasma infusion by the solid line, red cell infusion by the dotted line.

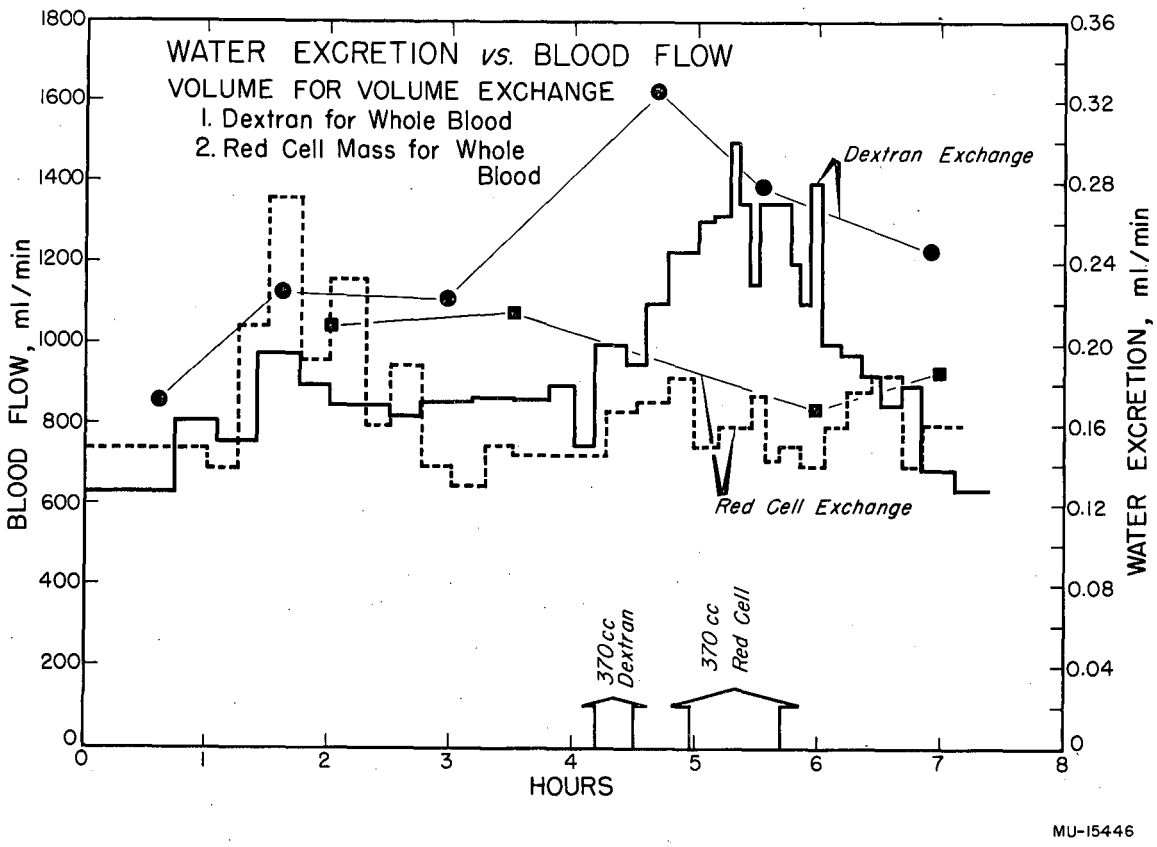


Fig. 10. This shows the diuretic response and blood flow increase resulting from isovolemic exchange of dextran for whole blood. When an isovolemic exchange of red cell mass for whole blood is carried out, the blood flow decreases, and there is no diuresis; in fact, an antidiuresis can be calculated. Total liver plus kidney plasma flows are indicated by the dots, dextran exchange by the circles, red cell exchange by the squares. Water excretion is indicated by the line graphs, dextran exchange by the solid line, and red cell exchange by the dotted line.

The significance of urinary estrogens has not been conclusively established and little is known of circulating estrogens. However, patterns of urinary estrogens seem to reflect their production. Unitl recently, estrogens in the urine have been estimated by various bioassay methods such as the vaginal smear or by the uterine weight technique. These assays are dependent on many factors--the animals used, the number of injections, the purity of the injected compound, etc. Chemical methods have now been developed and are being studied. It has not been a simple task because the amount of estrogens present in normal and postmenopausal urines is small and various medications and pathological states may interfere.

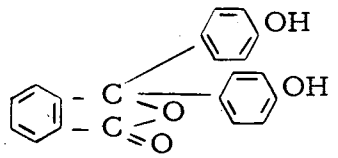
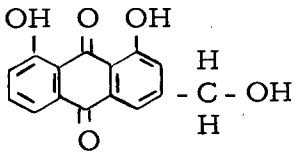
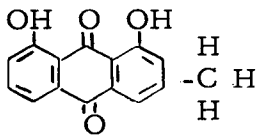
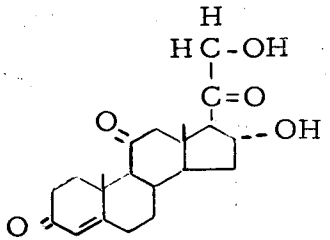
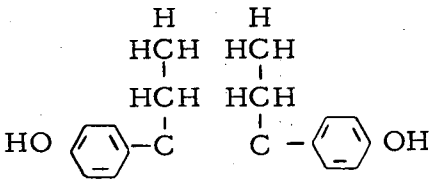
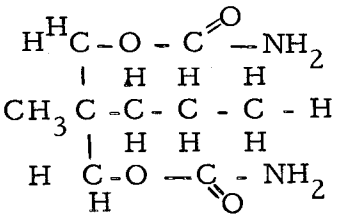
I have elected to use Brown's (Edinburgh) chemical method for the assay of estriol, estradiol-17β, and estrone in the urine of patients with cancer of the breast, to note any change pre- and postirradiation of the pituitary gland at the cyclotron. The method has been checked and used by many groups and all are in agreement that it is specific for the three estrogens.

The procedure is, of necessity, a lengthy one. (The 3-methyl ethers of estriol, estradiol-17β, and estrone are not available, therefore I prepared and purified samples for the standard curves. The solvents used must all be redistilled.) The glucuronide and sulfate conjugates of the urinary estrogens are hydrolyzed, and the estrogens extracted with ether. The ether extract is washed free, in various steps, of bile acids and some colored products from the urine. It is then concentrated and the residue is dissolved in a mixture of benzene and petroleum ether. Extraction with water removes the estriol fraction. Extraction with alkali removes the estrone-estradiol fraction. At this point (according to a recent publication by Brown), an additional purification step has been incorporated. Urinary phenolic impurities are so modified by saponification that they may be removed by solvent partition. This step was introduced to eliminate interference by stilbestrol, cortisone, phenolphthalein, meprobamate, and other substances. The original procedure is resumed and the two fractions--estriol and estrone-estradiol--are further purified by methylation to their respective 3-methyl ethers. These derivatives have solubilities different from estrogens and are thus separated from impurities that have been carried along with the estrogens. The methyl ethers are separated chromatographically on alumina columns, the eluates concentrated in vacuo, the Kober colors developed, the optical densities measured at three wave lengths, and the resulting data corrected for nonspecific absorption by Allen's method. To be significantly different (using Brown's data), two estimations must differ by more than 1.7 γ per 24° for estriol, 1.3 γ per 24° for estrone and 1.2 γ per 24° for estradiol..

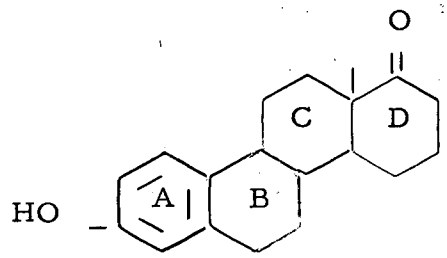
Any chemical method must be checked by bioassay because a chemical determination must necessarily assay one type of compound. It may, for example, be an assay for estrogens with a steroidal structure, but there are various estrogenic compounds that are not steroidal. Within the steroidal group itself, estradiol-17β is biologically very active, but its isomer, estradiol-17α, is not. Colorimetry in a chemical determination does not necessarily show the difference, as would bioassay. The particular color reaction in Brown's method makes no distinction.

Table III

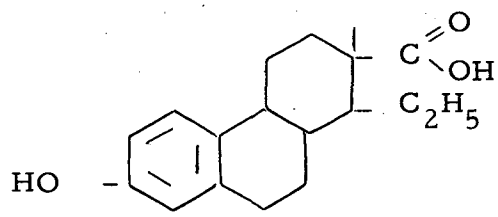
Effects of various drugs on the amounts of estrogen found in urine

Drug		Dose (mg per 24°)	Method	γ per 24° Estrone	Estradiol -17β	Estriol
Phenolphthalein (purgative)		240	A	1.5	19.0	-1.2
			B	1.2	0	0
Senna (purgative)		400	A	1.4	5.0	0
			B	2.0	5.8	0
Cascara (purgative)		240	A	0	-6.4	0
			B	0	0	0
Cortisone (corticoid)		200	A	0	-1.0	-6.6
			B	0	0	+1.3
		75	A	3.2	-8.2	3.3
			B	2.8	1.5	5.6
Stilbestrol (estrogen)		10	A	0	-11.1	1.3
			B	0	-5.0	2.0
Meprobamate (tranquilizer)		400	A	7.7	2.1	49.6
			B	6.8	2.5	8.1

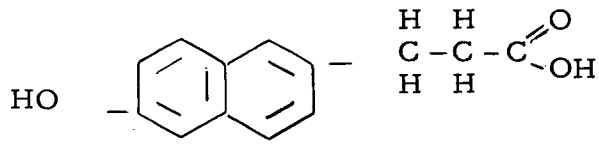
We do not know what makes a compound estrogenic. The structure can be quite different from the steroidal form of estrone. Meischer and his group have altered ring D of the steroid structure to prepare a compound, bisdehydrodoisynolic acid, which is still active estrogenicly. Horeau and Jacques went on to change the C ring and prepared active allenolic acid. The alterations are indicated as follows.



estrone

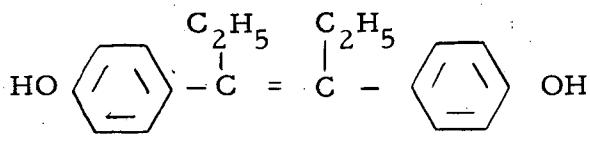


bisdehydrodoisynolic acid



allenolic acid

Sir Charles Dodds and his group have synthesized compounds such as stilbestrol, hexestrol, etc., that have a high estrogen activity but are not steroidal in nature:



stilbestrol

Table III indicates the effects of various drugs on the amounts of estrogen found in the urine as recently reported by Brown. Method A is the original method. Method B incorporates the extra purification step. The presence of some of the drugs has resulted in negative readings in Method A (confirmed by me). Senna still gives high results using Method B. I have included the structural formulae to draw attention to the phenolic groups, the ring systems, and the dissimilar structure of meprobamate. Other drugs and pathologies must also be considered. One might be mindful of the possibility of metabolism of compounds to estrogens.

Lawrence

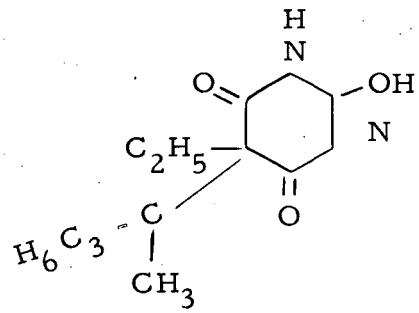
Some preliminary data, resulting from the procedure before modification (Method A), are given in Table IV.

Table IV

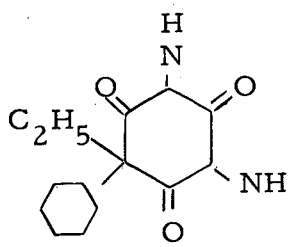
Patient and age	Dates	State	micrograms per 24 hrs Estriol	Estrone	Estradiol-17	Total
W 43	10-23	preovariectomy	4.6	5.5	2.7	12.8
	1-14	postovariectomy	0.6	10.7	0.0	11.3
	1-22					
	to 2-14	26,400 rad				
	2-13	postirradiation	2.0	2.6	0.7	5.3
	3-24	postirradiation	3.0	1.4	0	4.4
M 61	1-13	preirradiation	5.3	2.8	8.7	16.8
	1-17					
	to 2-7	24,000 rad				
	2-5	postirradiation	3.6	2.9	1.0	7.5
D 65	12-20	preirradiation	18.4	11.4	6.3	36.1
	12-20					
	to 1-24	24,000 rad				
	1-20	postirradiation	9.6	13.6	6.4	29.6
	3-19	postirradiation	22.3	11.7	4.9	38.9

Patient D was receiving testosterone. It is known that testosterone may be metabolized to estrogen. Patient M, during the week of 1-9-58 to 1-15-58 received various preparations containing the following drugs, one or more of which might account for the high total yield.

nembutal



phenobarbital



Lawrence

Medication during the week of 1-29 and on discharge was noted as nembutal and A. P. C. with codeine.

Bulbrook and Greenwood (Clinico-pathological Laboratories, Imperial Research Fund, London) have shown, as have others, that estrogen excretion continues postoophorectomy. They suggest that continued excretion post-oophorectomy-adrenalectomy may be due to incomplete removal of adrenal tissue or to accessory adrenal tissue. (In the trimming of adrenal tissue, I have three times noted accessory tissue.) Posthypophysectomy, no urinary estrogen was detected in four of ten cases. The estrogen levels were unaltered in three cases, and increased in three cases. The suggestion is offered that perhaps in only four of the ten cases was the amount of circulating trophic hormone reduced below the amount needed for estrogen production by the target organs. In the other six cases perhaps not all of the pituitary tissue was removed, perhaps there was some rapid regeneration of tissue, perhaps pharyngeal pituitary tissue can secrete trophic hormone posthypophysectomy.

Precise determination of urinary estrogens is important because if they are indicative of the circulating estrogen level and if they are excreted posthypophysectomy, the cancer may still be hormone-dependent. One may doubt that the hormone-independent cancer exists.

THE SCINTILLATION CAMERA

Hal O. Anger

By means of the scintillation camera, pictures are being taken of a series of patients before they undergo thyroid operation. The camera maps the distribution of a tracer dose of radioiodine previously given to the patients, and shows the location of the functioning areas of the thyroid. The immediate object of this study is to compare the information gained from the scintillation camera with the findings of the surgical operation.

The pictures taken by the scintillation camera compare favorably with those taken by conventional scanners at the present time, but work is still proceeding to improve the definition and contrast of the pictures obtained. The object of improving the resolution is to enable the user to see smaller voids in the thyroid and, therefore, to detect thyroid abnormalities at an earlier stage in their development.

LOW-BACKGROUND HUMAN WHOLE-BODY COUNTER

Howard G. Parker

Preliminary plans for a low-background whole-body counter are being worked out. This counting facility will most probably begin with a steel-shielded room containing a large NaI crystal for gamma-ray spectroscopy, roughly duplicating facilities available at Argonne and Los Alamos. Such facilities will soon be essential to the operation of any major center using radioactivity or handling diagnosis of contamination cases for a large area.

The counter will be planned for use also in diagnoisis of radioactive contamination cases. It will be operated most of the time in research in counting methods, fallout problems, and natural body radioactivity, --this last in conjunction with other body-composition studies at this laboratory.

REPORTS ISSUED

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

Hal O. Anger, Mapping the Distribution of Gamma-Ray-Emitting Isotopes with the Scintillation Camera, UCRL-3992 Abstract,* Jan. 1958.

Paul C. Blanquet and Cornelius A. Tobias, Hypothalamus and Thyroid, UCRL-8095 Abstract, Dec. 1957.

Ernest L. Dobson, Tracer Measurements of Blood Flow in Studies on the Homeostasis of Body Fluids, UCRL-3998 Abstract,* Dec. 1957.

R. Lowry Dobson and Mary M. Chupp, Hematologic Effects in Man of Low-Level Radiation Exposure, UCRL-3996 Abstract,* Dec. 1957.

L. H. Frommhagen, N. K. Freeman, and C. A. Knight, The Lipid Constituents of Influenza Virus, Chick Allantoic Membrane, and Sedimentable Allantoic Protein, submitted to Virology UCRL-8052, Nov. 1957.

D. Fluke, Ann C. Birge, Joseph Sayeg, Tor Brustad, and Cornelius A. Tobias, Radiobiological Studies with Stripped Carbon Nuclei and Other Heavy Ions, UCRL-8078 Abstract,* Dec. 1957.

Paul Howard-Flanders, The Effect of Nitric Oxide on Radiosensitivity and the Mechanism of Radiation Injury; UCRL-3994 Abstract,* Jan. 1958.

Hardin B. Jones, Estimation of Effect of Radiation Upon Human Health, UCRL-8082 Abstract,* Dec. 1957.

Hardin B. Jones and Patricia W. Durbin, Estimation of the Turnover Equation of Strontium-90 for Human Bones, UCRL-8083 Abstract,* Dec. 1957.

Lola S. Kelly, DNA Synthesis in Irradiated Animals, UCRL-3995 Abstract,* Dec. 1957.

John H. Lawrence, Joseph F. Garcia, and Van Dyke, Biological and Chemical Characteristics of Urinary Erythropoietin, UCRL-3997 Abstract,* Dec. 1957.

*Abstract for the International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958.

31 Robert K. Mortimer and R. Gunther, Genetic Effects of Radiation on Yeast Cells, UCRL-3993 Abstract,* Jan. 1958.

34 Myron Pollycove, Isotopic Measurements of the Life Span of Human Erythrocytes, Leukocytes, and Platelets, UCRL-3999 Abstract,* Dec. 1957.

39 Tobias, Lawrence, Born, Carlson, Toch, Van Dyke, Garcia, Thorell, Anger, Welch, Riggs, and Anderson, Hypophyseal and Hypothalamic Irradiation Using 340-Mev Protons and 900-Mev Alpha Particles, UCRL-8077 Abstract,* Dec. 1957.

40 Cornelius A. Tobias and Graeme Welch, Acute and Delayed Effects of Radiations on Yeast Cells, UCRL-8109 Abstract,* Jan. 1958.

41 Cornelius A. Tobias, Radiation Hazards in Space Flight, UCRL-8115, Jan. 1958.

42 Cornelius A. Tobias, Howard C. Mel and David G. Simons, A Summary of the Berkeley Conference on Biological Effects of Cosmic Rays and Accelerated Heavy Ions, UCRL-8201, March 1958.

*Abstract for the International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1958.

PAPERS PUBLISHED

During this period papers from Donner Laboratory have been published as follows:

Hal O. Anger, Scintillation Camera, Rev. Sci. Instr. 29, (1), 27-33 (Jan. 1958).

Berlin, Beeckmans, Elmlinger, and Lawrence, A Comparative Study of the Ashby Differential Agglutination, Carbon-14, and Iron-59 Methods for the Determination of Red Cell Lifespan, J. Lab. Clin. Med. 50, No. 4, 558-69 (Oct. 1957). (Reprint 1957-202)

E. L. Dobson, Relation of Hormone Dynamics to Body Fluid Volume Homeostasis (Abstract); Fed. Proc. 17 (1), No. 137, Pt. 1, 36 (March 1958).

J. W. Gofman, Diet in the Prevention and Treatment of Myocardial Infraction, Am. J. Cardiology 1 (2), 271-83 (Feb. 1958).

T. L. Hayes and J. E. Hewitt, Visualization of Individual Lipoprotein Macromolecules in the Electron Microscope, J. Appl. Physiol. 11 (3), 425-28 (Nov. 1957).

P. Howard-Flanders, Effect of Nitric Oxide on the Radiosensitivity of Bacteria, Nature 180, 1191-92 (Nov. 30, 1957).

B. S. Jacobson, Evidence for Recovery from X-ray Damage in Chlamydomonas; Rad. Res. 7, No. 4, 394-406 (Oct. 1957) (UCRL-3893; Reprint 1957-205).

L. S. Kelly, Effect of Radiation on DNA Synthesis in Mammalian Cells, Progr. in Biophys. and Biophys. Chem., 8, 143-63 (1957).

L. S. Kelly; Proliferation of the Liver Reticuloendothelial System (Abstract), Fed. Proc. 17 (1), No. 332, Pt. 1, 84 (March 1958).

Lawrence, Van Dyke, Garcia, and Contopolous, Studies of the Regulation of Red Cell Production aided by Isotopes; UNESCO; Intern. Conf in Radioisotopes, in Sci. Research Proc., No. 159 (1957).

R. K. Mortimer, X-Ray-Induced Homozygosis in Diploid Yeast; Rad. Res. 7, No. 4 (Oct. 1957). (Reprint 1957-208)

Strisower, Gofman, Strisower, and Krog, Tyrosine, Iodine, and Human Serum Lipoproteins, Proc. Soc. Exptl. Biol. Med., 97 (3), 698-700 (March 1958).

Tobias, Lawrence, Born, McCombs, Roberts, Anger, Low-Beer, and Huggins; Pituitary Irradiation with High-Energy Proton Beams: A Preliminary Report, Cancer Research 18, (2), 121-34 (Feb. 1958).

45 Van Dyke, Garcia, and Lawrence, Concentration of Highly Potent Erythropoietic Activity from Urine of Anemic Patients, Proc. Soc. Exptl. Biol. Med. 96 (2), 541-44 (Nov. 1957).

46 G. Welch, Effects of Chronic Exposure to X-Rays on a Steady-State Population of Saccharomyces cerevisiae (Abstract); Rad. Res. 7, No. 4 (Oct. 1957). (Reprint 1957-204)

RADIATION DETECTION AND PROTECTION

Radiation Laboratory
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Berkeley, California

HEALTH CHEMISTRY

Nelson B. Garden in charge

Reported by Rosemary J. Barrett

APPARATUS AND EQUIPMENT

Processing equipment in the Bldg. 70 6-in. lead caves, 2-in. shielded boxes, and auxiliary gloved boxes was set up for the handling of an irradiated transuranic sample received from the MTR in October, and again for five samples received in March; two from the latter group were subjected to separation processes, and the curium therefrom combined, repackaged, and reinserted in the reactor. The first hot-run testing of the refrigerated-charcoal bomb (for fission gases arising in the closed-dissolver treatment of these pile slugs), as developed by the Airborne Activity Control group, was--as predicted by pilot runs--completely successful. The method eliminates such gases from the stack-effluent problem, which problem has been under study for two years. Study by this group disclosed that the peaks of alpha activity in the off gas from 6-in.-cave processes occur during (a) evaporations and (b) foil packaging of powdered product.

Health Chemistry created the equipment for and participated in the conversion of 100 curies of tritium to tritiated water, achieved by oxidation of the tritium over copper oxide in an all-glass enclosed system; the material was packaged for use and storage. Use of a 4-curie aliquot for chromatographic studies (involving transfer, centrifuging, and evaporation) showed (a) no detectable losses to environment and (b) approximately a 35-mC loss via duct to outside atmosphere. This fraction of a percent of the material could have been captured, but in view of the quantities liberated elsewhere (to the general atmosphere for dilution) it did not seem economically justified to spend money at this point, where no health hazard was presented.

An improved chain-drive manipulator and pipetter for general use was designed and fabricated, to obviate manual pipetting of radioactive material and hence to reduce exposure.

An isotope-storage cabinet, under negative pressure, providing 15 individually vented, locked storage areas, has been made for use by researchers, who justified these private storage areas on the basis of the value of their individual material and possibility of accidental disturbance or contamination. Each area has room for 20 quart-size ice cream cartons. The researchers' radioactive samples, already in closed containers and cartons, are placed in storage after being further sealed in plastic bags.

Two new shipping containers have been put in use in transporting irradiated samples from Argonne National Laboratory and the MTR. The containers are made of stainless-steel-canned uranium, of 1-in. wall thickness. With the addition of hevimet liners, combinations can be made to provide up to 2.75 inches of lead equivalent. The boxed containers weigh approximately 90 and 110 pounds.

Alarms were installed on each of the two high-level Co^{60} radiation units. If active material is released into the treatment well, a warning is given to alert the personnel present of the possibility of exposure.

Forty-five enclosures--gloved boxes, lead-shielded boxes, special setups, etc.--were fabricated, assembled, and fitted to order.

Forty-four radioactive sources were made on request from researchers by the Special Problems group, each meeting the desired energy and quantity specifications. They included the following: seventeen Cs^{137}, nine $Sr\text{-}Y^{90}$, two U, one Na^{22}, one Co^{60}, ten Pu, one Ra, two C^{14}, one Ru^{106}. Progress was made in this group in increased efficiency in source radioassay techniques and source-packaging technology.

The Instrumentation group, in addition to routine survey-meter management, developed an enlarged edition ($4\frac{1}{2}$ by 9 in.) of the standard UCRL air proportional alpha probe, for floor and clothing surveys. This same type of probe is being applied to an automatic counter-and-recorder unit for filter papers from air sampler, the mechanical features of which were completed in this period.

CONTROL OF AIRBORNE ACTIVITY

The Airborne Activity Control group was engaged, among other activities, in the following:

(a) Studies on emanation control. Absorptive media used revealed that the presence of variable moisture is an important factor in validity of data.

(b) Investigation of control of gaseous At^{211}. The results indicate that air-sampling efficiency of activated carbon is at least a thousand times that of fibrous media.

(c) Continuous modification to close-capture control systems on the various heavy-element targets bombarded on the Hilac.

(d) Design of an automatic counter-recorder for air-sample filter papers. This apparatus, to count and record 100 filter-paper samplings, both for alpha and for beta-gamma determination, in 4 hours, is under fabrication.

(e) Consultation on the rebuilding and improving of the Crocker Laboratory ventilation facilities, effected mainly to decrease chemical fume hazards.

(f) Design of a new box scrubber assembly, based on 3 years' operating and experimentation.

(g) The first detection (from routine environmental surveys) and reporting on the heavy fallout-rainout, which started March 20, 1958, and which was later confirmed elsewhere.

HEALTH PHYSICS

Burton J. Moyer in charge

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

Beta-gamma meters	22
I D L meters	21
Juno ion chamber	20
Abacus logarithmic ion chamber	30
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	2,000
Total man-days' coverage with electroscopes	10,800
Total man-days' coverage with dosimeters	4,500
Total man-days' coverage with slow-neutron chambers	4,500

Bevatron	Man-days	Number per day (approx.) for 6 mos.
Electroscopes	4500	25
Bldgs. 53 and 80		
Slow-neutron chambers	2700	15
Dosimeters	2700	15
Electroscopes	2700	15
Bldg. 71		
Electroscopes	900	5
Bldg. 70		
Electroscopes	900	5
Bldg. 2		
Slow-neutron chambers	1800	10
Dosimeters	1800	10
Electroscopes	1800	10

Cases of Monthly Exposure above 0.5r*

Including neutron exposures:

Monthly film expos. above	184-inch area	60-inch area	Bldg. 51	Chem	Other	Total
0.5	0	11	30	4	9	54
1.0	0	1	3	3	2	9
1.5	0	0	1	2	1	4
2.0	0	0	0	0	0	0
3.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0

Gamma readings only:

Monthly film expos. above	184-inch area	60-inch area	Bldg. 51	Chem	Other	Total
0.5	0	11	1	4	3	19
1.0	0	1	0	3	0	4
1.5	0	0	0	2	0	2
2.0	0	0	0	0	0	0
3.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0

*Excluding Livermore

Moyer

Information Division
sa