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SEMIANNUAL REPORT
April through September 1959

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BIOLOGICAL RESEARCH

RADIATION BIOLOGY AND RADIATION THERAPY

John H. Lawrence in charge

CELL STUDIES BY ELECTRON MICROSCOPY

Thomas L. Hayes, James Koehler, Walter Birnbaum, and Stafford Daniels

The ultrastructure of several single-cell systems is being studied by use of the electron microscope. Information is sought on the morphological changes produced by such physical agents as radiation on the cell organelles (mitochondria, chromatophores, etc.); such information should be useful in determining the basic mode of interaction between radiation and living matter.

Yeast Cells

The structure of the yeast cell has been the object of several recent investigations. Osmic acid, the standard "stain" used in electron microscopy, has not been very satisfactory for this organism. The physical and biochemical properties of uranyl nitrate, however, suggested that it might be applied advantageously. Uranyl ion, containing a heavy metal, may be expected to act as an effective electron stain when incorporated into a cell or tissue to be observed with an electron microscope. Biochemical data suggest that uranyl may be a relatively specific electron stain for high-molecular-weight polyphosphate compounds such as nucleic acid.

Diploid cells of the X901 strain of Saccharomyces cerevisiae grown in yeast extract and dextrose for 3 days were fixed and stained by placing them in aqueous solutions of uranyl nitrate ranging in concentration from 2% to 50%. The duration of fixation after staining was varied from 3 minutes to 18 hours. Best results were obtained with a 40% aqueous solution of uranyl nitrate, in which the cells were suspended for 1 hour at room temperature.

The cells were imbedded in a mixture of butyl and methyl methacrylate (3-to-1 ratio) and sectioned with a Porter-Blum ultramicrotome equipped with a diamond knife, and the specimens were observed with an RCA-EMU 2E electron microscope. The uranyl nitrate was found to be incorporated into the yeast cells and appeared to be an effective electron-scattering agent.

The cytoplasm of the yeast cells exhibited a granular constitution, and no mitochondria or endoplasmic reticulum could be observed. However, a more lightly staining area was found in almost every cell examined. This area appeared to be the nucleus. Within this nuclear area were observed, in most cases, very darkly stained structures (Fig. 1). These structures were in every case within the more lightly stained area (thought to be the nucleus), and were often paired and symmetrical. The appearance and location of these darkly stained inclusions suggested that they might represent the chromosomal material of the yeast cell. These structures, if chromosomes or chromatin aggregations, would indicate that the yeast nucleus has material within it analogous to the chromosomal material of the cells of other organisms. Since the sections of the cells were cut approximately 400A thick, whereas the yeast cells are 4 or 5 micra thick, the sections represent only about 1/100 of the cell diameter. It was therefore not possible to estimate the number of these structures in the nucleus from single micrographs of the cells.

Diatom Structures

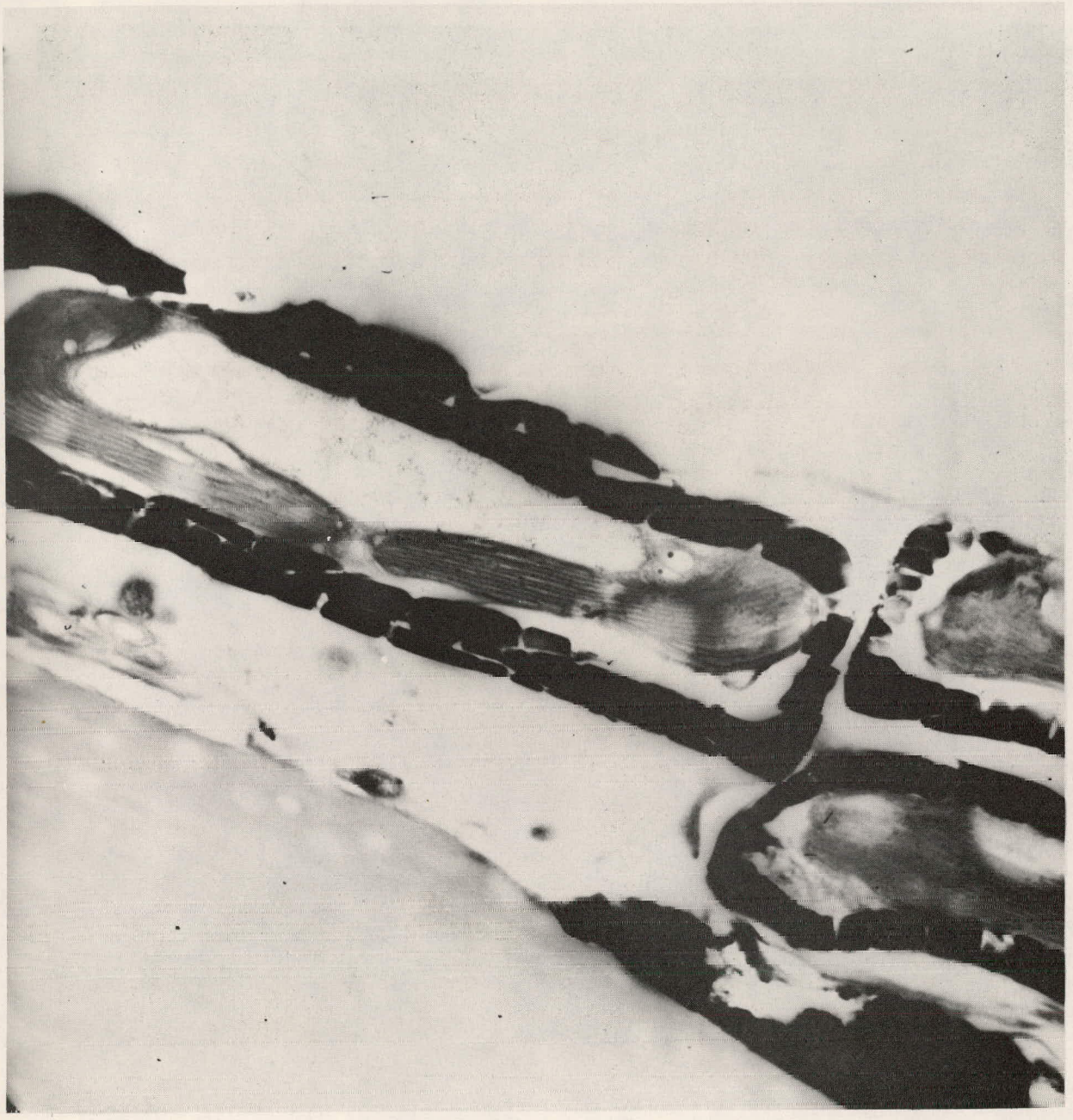
Several fresh-water pennate diatoms belonging to the class Bacillarieae were grown in epiphytic relationship with a green alga, Nitella. Small pieces of the Nitella with the diatoms attached were cut and fixed for electron microscopy (1% osmium tetroxide in veronal buffer, pH 7.4). Figure 2 is a low-power electron micrograph showing several diatoms attached to the cell wall of the Nitella. The siliceous cell wall seems to be completely composed of small blocks of very dense material. Inside the cell wall there is a large chromatophore occupying about half of the cell volume. The chromatophore is a multilamella structure containing dark oval bodies dispersed in an orderly array along the lamellae (Fig. 3). The cytoplasm of the diatoms is in the form of strands around many vacuoles. Several mitochondria were seen, and the nucleus was located in the central portion of the diatom.

Hela Cells

Tissue-culture cells of the stable cell line Hela have been examined in the electron microscope. Work has been done primarily on normal cells, but some experiments are now being completed on the effect of radiation on these tissue-culture cells. After doses of 500 to 1000 roentgens of x-irradiation these cells developed into "giant" forms, which may be ten times normal volume. Electron microscopy has revealed a multitude of bizarre structures (Fig. 4), especially within the nuclear membrane. The effects of intranuclear tritiated thymidine on the cell structure have also been studied. Preliminary work has indicated that very small doses of H^3 thymidine produced traumatic effects on Hela cells as seen in the electron microscope. The specific uptake of this agent by DNA and hence by chromosomes, coupled with the very short range of the H^3 beta particle, provides a means of specific nuclear irradiation.

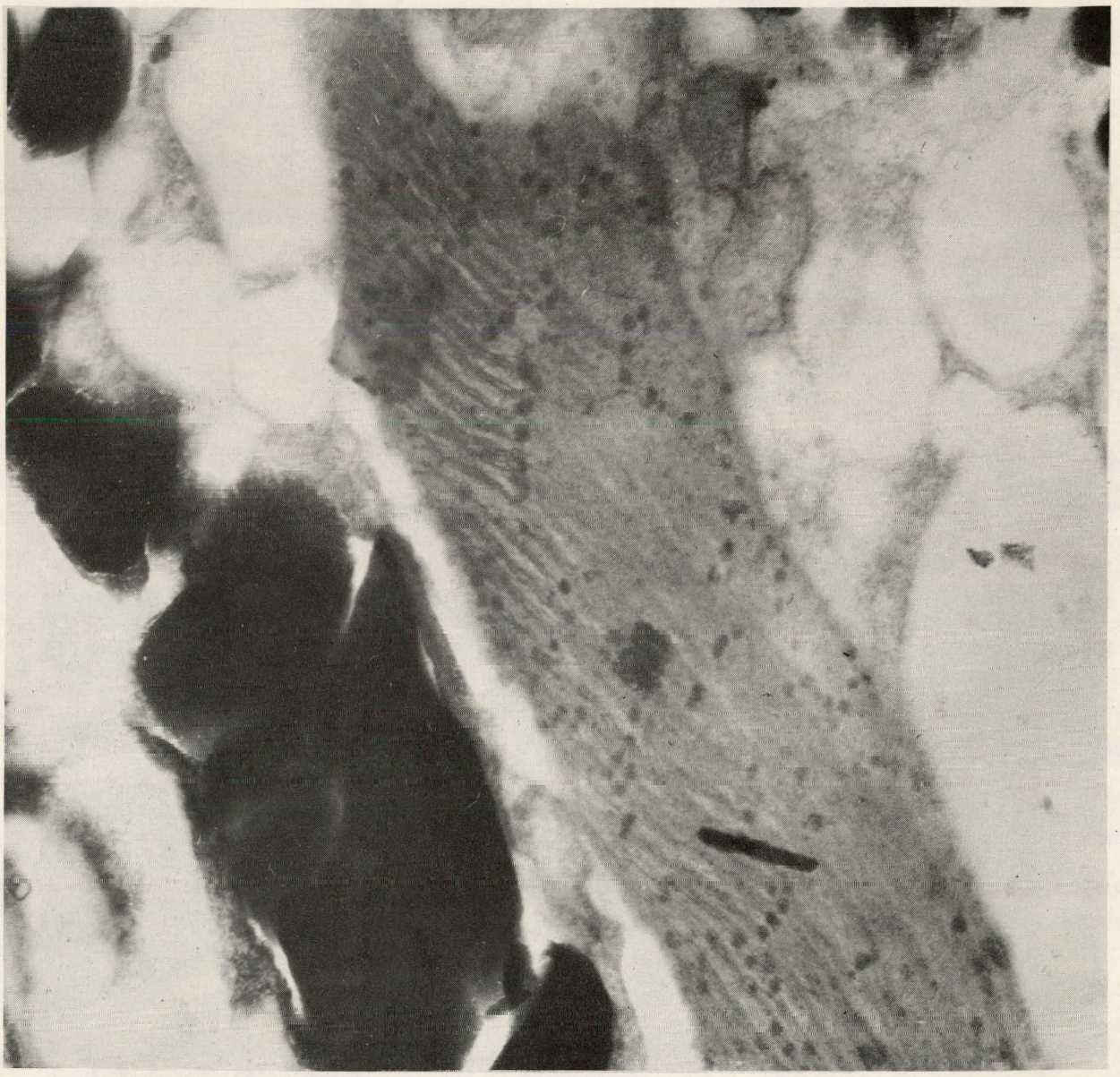
ZN-2319

Fig. 1. Yeast cell fixed in uranyl nitrate.



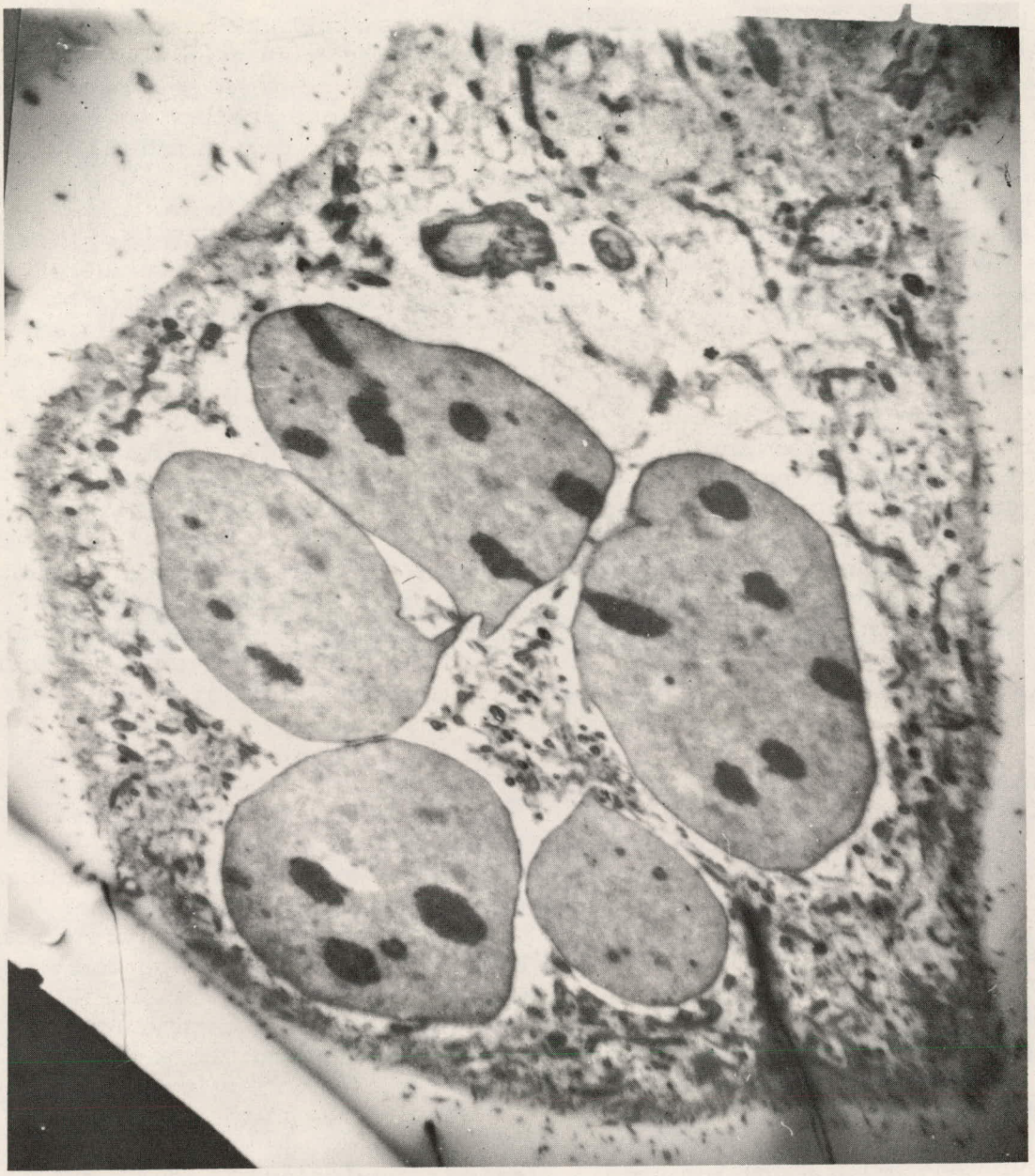
ZN-2320

Fig. 2. Diatoms attached to cell wall of Nitella.



ZN-2321

Fig. 3. Chromatophore of a diatom.



ZN-2322

Fig. 4. Multinucleated Hela cell. 1,000 r x-irradiation.

IRRADIATION OF THE PITUITARY IN HUMANS

James L. Born, John H. Lawrence, and Cornelius A. Tobias

The effects of pituitary irradiation on human metastatic mammary carcinoma and certain endocrine-controlled diseases have been studied. The high-energy particle beam from the 184-in. synchrocyclotron was used, in this instance a beam of 900-Mev alpha particles. These studies were commenced in 1954 and have continued since, including the period from 1955 to 1957 when curtailment of irradiation was necessitated by a renovation of the synchrocyclotron. From the inception of this program in 1954 a total of 115 patients have received irradiation to the pituitary gland. Most of these patients have been women with advanced metastatic mammary carcinoma. A smaller series of patients with pituitary tumors and another series of patients with advanced diabetes mellitus with retinopathy were irradiated.

During the past twelve months 39 patients have received pituitary irradiation and three are currently being irradiated. Of these 26 had metastatic mammary carcinoma, eight had severe diabetes mellitus with retinopathy, two had acromegaly, one had carcinoma of the adrenals, one had malignant exophthalmos, and one had Cushing's disease.

The technique for radiation has remained essentially the same, though several modifications have been made in the procedure. These consist of continuous head rotation and the use of slightly larger apertures in order to irradiate a somewhat larger field. The use of the larger aperture was deemed desirable as studies of the histological sections of the pituitary at intervals up to 14 months following irradiation had shown identifiable cells at the periphery of the gland. All the irradiation of the patients with mammary carcinoma has been at the higher dose level during the past year; irradiation was delivered in multiplanal fractionated doses over a period of 12 days. As a result of the use of the larger aperture combined with the larger dose there has been a rapid onset in the appearance of clinical signs of hypophysectomy. In some of the patients this was noted as early as 2 weeks following the completion of irradiation, at which time the iodine uptake in the thyroid gland had declined to 50% of its preirradiation level. This was accompanied by evidence of adrenal insufficiency necessitating early replacement therapy. This early clinical and laboratory evidence of irradiation hypophysectomy was confirmed by histological findings of extensive destruction of the pituitary, seen as early as 10 and 19 days postirradiation in two far advanced mammary carcinoma patients who died of their metastatic disease at these times.

The 26 patients with mammary carcinoma selected for pituitary irradiation during the past year were in an advanced stage of their metastatic disease; as a result five of those who were far advanced died of progression of their disease within 30 days following irradiation. Of the remaining 21 breast cases 10 have shown an excellent objective response, as shown by regression of soft-tissue lesions and improvement of osseous metastasis. Included among these is one male with breast carcinoma who showed a striking response in the healing of bony lesions and in work rehabilitation. It is known that the remissions obtained in breast carcinoma from pituitary ablation are not permanent, but some patients in the total series have done well for periods of more than a year and are still in a state of remission. The average duration of remission cannot be calculated until recurrence of disease is established on all the patients irradiated. At present 26 of the breast carcinoma cases are living at times ranging from 3 to 52 months after irradiation.

Attempts have been made to evaluate the responses of 70 breast cancer patients to changes in endocrine status brought about by oophorectomy, by combined oophorectomy and adrenalectomy, and by the use of androgenic and estrogenic substances. No definitive conclusions could be reached from these data, as attempts at interpretation are hampered by the small number of patients involved in each category and the lack of any clear-cut response pattern. Thirty-seven of the patients were oophorectomized, but of this group only 18 were premenopausal and were oophorectomized therapeutically for their metastatic disease. Six of these showed a good response to oophorectomy. Six of this group also showed further objective remissions after pituitary irradiation, but only three of this group were among the six who had benefited from oophorectomy. This suggests that a prior response to oophorectomy is not necessary for a later response to irradiation hypophysectomy. Sixteen patients had been adrenalectomized as well as oophorectomized and 14 of them had shown benefit from the combined procedure. Six of this group later showed a further response to pituitary irradiation. Twenty-nine patients were menopausal at the time of pituitary iradiation. Of these 10 showed a good response to irradiation hypophysectomy. Responses to the use of androgenic and estrogenic substances could not be correlated in any definite pattern of response to subsequent ablative endocrine procedures. A more basic approach is being undertaken to the problem of beneficial responses in mammary carcinoma to changes in endocrine status.

Studies on the excretion of urinary estrogens in patients with advanced metastatic mammary carcinoma have shown decreases in total estrogens after oophorectomy and after irradiation hypophysectomy. By use of chemical methods the estrogens were fractionated into estrone, estradiol, and estriol. Changes were also shown in the ratio of these fractions after ablative procedures. Results suggest that either estradiol or estrone, or some equilibrium mixture of the two, is the principal hormone and that estriol is the reduction product. Enzyme methods are being set up to assay the biological activity of these three urinary estrogens on the transhydrogenase in human placenta.

Other studies are being undertaken with C^{14}-labeled testosterone in mammary carcinoma patients to determine metabolic patterns of excretion. Estrogen determinations will quantitate the conversion of testosterone to estrogenic substances.

Mammary carcinomas are being chemically induced in rats, which will afford a means of extending investigative metabolic and therapeutic studies beyond our present limitations.

Investigation of the effects of pituitary irradiation in neoplastic and endocrine-controlled disease will be continued during the coming year. Some modifications will be made in dose schedule, as it is believed that the high-dose range has been well explored. Reductions in total dose levels will avoid the possibility of injury to surrounding cranial nerves. Detailed endocrinological studies including the metabolism of labeled endocrine compounds are expected to yield data of importance on the mediation of responses through the pituitary.

SENSITIVITY OF THE NERVOUS SYSTEM TO IRRADIATION

C. T. Gaffey

Project No. 1. Peripheral Nervous System: Electrophysiological Studies

The primary function of nerve fiber is to serve as the line of communication between receptors and effectors in biological systems. It is one of our projects to study the mode of action of ionizing radiation on frog sciatic nerve. Electrophysiological techniques are being used to investigate bioelectric characteristics (such as threshold, amplitude, latency, conduction velocity, and rates of rise and fall of the action potential) of nerve fiber prior to, during, and after exposure to radiation. It is suggested that knowledge of electrical properties of neural-fiber membrane will provide an explanation for the mechanism of damage in the peripheral nervous system.

The rates of rise and fall of the neural impulse have been shown to be indices of membrane Na-ion and K-ion permeability. By using the Lawrence Radiation Laboratory's 184-inch cyclotron it has been demonstrated that high-energy alpha particles and deuterons preferentially alter the falling limb of the action-potential complex, leaving the ascending limb of the action potential unchanged. Similar results have been found by using soft and hard x-rays. This leads one to suggest that damage in nerve fiber due to ionizing radiation is caused by a selective increase in membrane permeability, first to K ions and then to Na ions.

Single exposures of an isolated nerve fiber to irradiation by high-energy alpha particles and deuterons produce a striking decrease in the amplitude of the neural impulse. For instance, a 40- krad dose of alpha particles causes a degenerate decline in the magnitude of the neural electrical output, which continues until there is a total loss of signal 20 hours after irradiation. A semilog plot reveals a linear relation between the time for onset of irradiation damage (i. e., the period after irradiation for a complete loss of the action potential's amplitude) and the radiation dose. With such a calibration curve, irradiation damage to the peripheral nervous system can be predicted.

Project No. 2. Peripheral Nervous System: Chemical Studies

Alterations in permeability of neural-fiber membrane, as a consequence of interaction with radiation, is an extension of our electrophysiological studies. Neural-membrane permeability is best revealed by sensitive radioisotopic tracer techniques. Modern radioactive counting procedures permit the ready measure of the rate of transport of a particular ionic species through neural-fiber membranes. Membrane-permeability changes probably play a key role in interpreting nonlethal effects of radiation.

Project No. 3. Brain

There is the possiblity that the brain contains a small population of highly radiosensitive cells or nuclei of cells. It is our purpose to use electroencephalographic techniques to analyze brain structures for differential sensitivity of electrical response to high-energy radiation.

The central nervous system is thought to be very resistant to ionizing radiation. This view is based on the observation that in order for total-body irradiation to produce destructuve changes in brain structure, grossly supralethal doses must be administered. However, reports have been published telling of physiological changes after substantially lower doses of irradiation than those required to evoke clearly detectable anatomical damage in brain tissue. E. Gerden (J. Comp. Psychol. 20, 263-290 (1935)), using an auditory stimulus, found consistent changes in the conditioned-reflex response in the dog with doses of 20 to 150 r. A. V. Lebedinsky (Proc. Intern. Conf. Peaceful Uses of Atomic Energy, Geneva, 1955 (United Nations, New York, 1956), Vol. 11, 7-24), has reviewed Russian literature which indicates that functional alterations of the central nervous system may result from x-ray exposure to doses as low as 0.1 r. If these findings are substantiated, it would seem that the central nervous system may contain the most radiosensitive tissue of the body.

The present program is concerned with coupling techniques of permanent brain-implant electrodes with EEG techniques. Computer analysis of the results may be possible.

EFFECT OF RADIATION ON THE RETICULOENDOTHELIAL SYSTEM

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

Previous experiments in this laboratory have demonstrated that a number of substances produce an increase in phagocytic activity in the reticuloendothelial system by causing cell proliferation in the liver reticuloendothelial (RE) cells. Although the RE system has been shown to be relatively radioresistant with respect to phagocytic function, it seemed of interest to determine whether increases in function could be prevented by radiation. Colloidal chromic phosphate has been shown to localize almost exclusively in the liver and spleen, and thus it provides a convenient method for the specific irradiation of these two organs.

Eighteen mice were injected intravenously with chromic phosphate (1μC per gram body wt). Six days later six of these and six controls were injected subcutaneously with 1 mg of estradiol in sesame oil. The rate of disappearance of a test dose of intravenously injected colloidal carbon was determined 4 days after the estradiol injection in order to test the phagocytic capacity.

Table I summarizes the rate constants for carbon disappearance in the different groups of mice (six per group). It may be seen that the high radiation dose to the liver (estimated as 5000 rep) affected the phagocytic efficiency only slightly, in accord with previous findings.[1] However, irradiation prevented the threefold increase in the phagocytic rate constant which is normally produced by estradiol.

The high radiation dose to the liver had very little effect on the general condition of the mice. They continued to gain weight, though at a somewhat reduced rate, and there was only a mild depression in the white blood cell counts. The liver weights remained normal. Spleen and lymph-node weights declined as expected in these radiosensitive tissues.

The prevention of phagocytic stimulation by specific liver irradiation is in agreement with results of whole-body radiation published recently by Benacerraf et al.[2] and lends further support to the concept that increased phagocytic function is primarily due to cell proliferation in the liver.

---

[1] Di Luzio, Simon, and Upton, Effect of X-Rays and Trypan Blue on Reticuloendothelial Cells, A. M. A. Arch. Pathol. 64, 649 (1957).

[2] Benacerraf, King-Rosenberg, Sebestyen, and Zweifach, Effect of High Doses of X-Irradiation on the Phagocytic, Proliferative, and Metabolic Properties of the Reticuloendothelial System, J. Exptl. Med. 110, 49 (1959).

Table I

Rate constants for carbon disappearance in irradiated mice

	Controls	Sesame oil controls	Estradiol	Chromic phosphate	Chromic phosphate	Chromic phosphate plus estradiol
Days after $CrPO_4$	-	-	-	10	11	10
Days after Estradiol	-	-	4	-	-	4
Carbon disappearance rate constant (% per min)	3.4 ± 0.5	3.1 ± 0.3	11.6 ± 1.5	2.2 ± 0.2	2.5 ± 0.3	3.0 ± 0.2
Increase in body wt in 10 or 11 days (g)	6.2 ± 0.5	4.3 ± 0.6	5.6 ± 0.5	2.3 ± 0.8	4.4 ± 0.9	3.5 ± 0.3
Liver wt (as % body wt)	5.9 ± 0.3	5.9 ± 0.3	7.3 ± 0.2	5.8 ± 0.2	6.4 ± 0.2	6.5 ± 0.2
Spleen wt (as % body wt)	0.54 ± 0.03	0.44 ± 0.03	0.69 ± 0.07	0.21 ± 0.03	0.25 ± 0.04	0.35 ± 0.06
Lymph node wt (cerv. and mesen.) (as % body wt)	0.34 ± 0.01	0.31 ± 0.02	0.33 ± 0.02	0.20 ± 0.02	0.22 ± 0.01	0.20 ± 0.01
White cell count (Cells/mm^3 × 10^{-3})	18 ± 1	27 ± 3	19 ± 2	13 ± 2	16 ± 2	13 ± 2

Errors quoted are standard error of the mean

SOME OBSERVATIONS ON THE PHYSIOLOGY OF HOMOLOGOUS DISEASE

James McRae and Lola S. Kelly

The death of mice following the transplantation of homologous hematopoietic tissue has aroused a great deal of interest, but the pathogenesis of the disease is still poorly understood. Studies on the physiology of such mice are in progress.

It has been noted by a number of investigators that homologous disease is associated with an abrupt weight loss in the face of apparently normal or increased food consumption, suggesting an absorption defect. However, we have observed recently that mice developing the disease show a very abnormal behavior towards their food. When presented with food pellets, they chew up two to three times as much as normal mice but swallow only a small fraction of the food. By weighing the food powder on the cage bottom, it could be shown that the decrease in body weight could be partly explained by a decrease in the amount of food swallowed. Attempts to determine the cause of this abnormal behavior are in progress.

In the accounts of the pathological changes found in homologous disease, several authors have mentioned focal areas of liver necrosis. Although no satisfactory explanation has been found for these changes, infections of all types have been held responsible. However, there has been no assessment of liver function in these animals. Since considerable derangement of liver function can occur without any obvious pathology, the ability of wasting mice to detoxify nembutal was investigated. In mice with a body weight loss greater than 15%, sleep times were prolonged in proportion to the weight loss. Sleep times were normal in control animals deliberately starved to produce a weight loss comparable to the wasted mice. Other liver-function tests are currently under investigation in an attempt to shed further light on the homologous disease.

POSTIRRADIATION PROTECTION OF X-IRRADIATED MICE WITH OLIVE OIL

James K. Ashikawa

Olive oil administered intraperitoneally is both protective and therapeutic on whole-body-x-irradiated male Swiss white mice (LRL report). In the three lethal doses tested (LD_{50} and LD_{100}, each at 30 days), involving 464 animals, the benificial effect of this oil was manifested during the bone-marrow phase by an increase in survival, a change in the mortality distribution, and a decrease in weight loss. Figure 5 shows that the effect was most pronounced in the MLD group, in which almost 90% of the postirradiation-oil-treated animals survived.

TOTAL-BODY IRRADIATION VIA INTERNALLY ADMINISTERED ISOTOPES

Saul Winchell and Myron Pollycove

Recent interest in tissue transplantation resulting in suppression of immunological responses, following total-body irradiation, has prompted us to study techniques for total-body irradiation using internally administered isotopes. Such techniques should be more readily available to investigators to permit more uniform irradiation of deep tissues than is allowed by present procedures for administering supralethal doses of radiation externally. Such procedures might prove useful in the management of leukemia and chronic renal disease.

Presently we are studying a chelate of Y^{90} which may be uniformly distributed in the body. Preliminary measurements indicate that the distribution compartment is somewhat larger than the extracellular fluid space. The chelate is relatively inert and is excreted almost exclusively by the kidney in unaltered form. The half time of this excretion, after equilibration has occurred, is approximately 2 hours. Since the concentration of the Y^{90} chelate in urine is significantly higher than in plasma, the possibility of kidney damage by excessive radiation is being investigated.

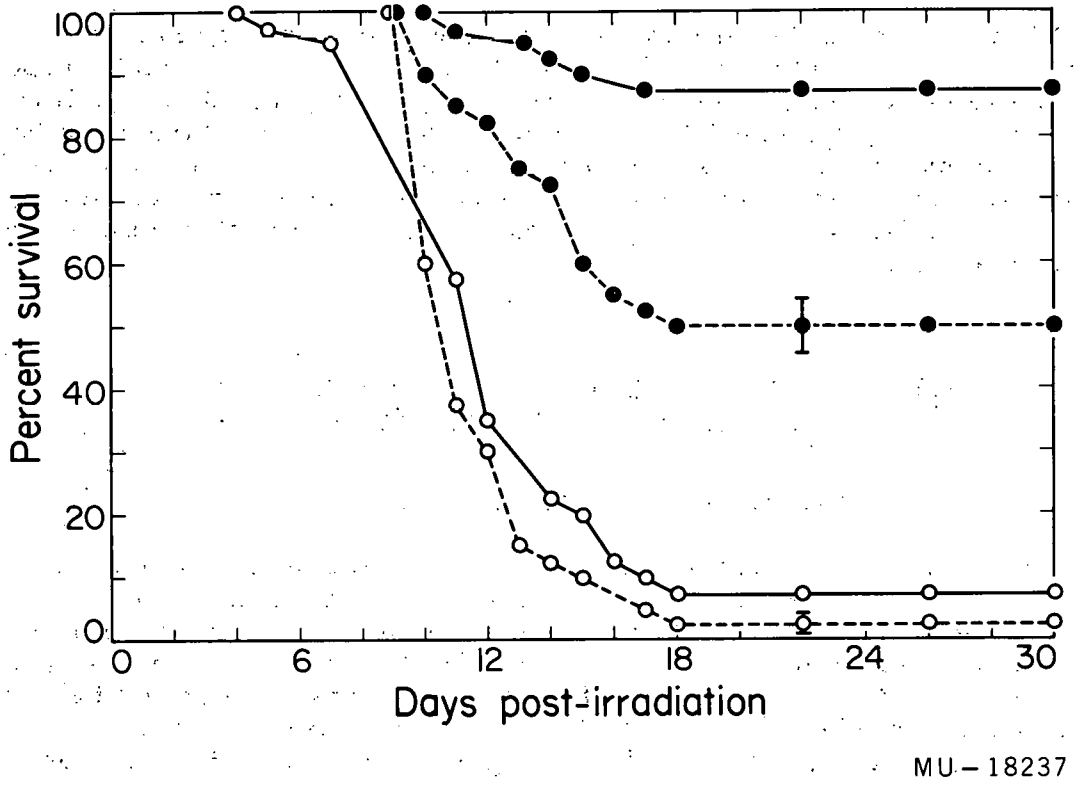


Fig. 5. Effect on male Swiss white mice of 1 ml olive oil injected intraperitoneally within a half hour after whole-body x-irradiation. Forty animals per group.

BIOLOGICAL EFFECTS OF INTERNALLY DEPOSITED RADIOISOTOPES

Patricia W. Durbin and C. Willet Asling in charge

Justine Burg, Ann Hessel, Nylan Jeung, Muriel Johnston, Marshall Parrott, and Marilyn Williams

Mammary Tumors in Normal Female Sprague-Dawley Rats

Mammary tumors are commonly observed in aging female Sprague-Dawley rats. In Davis's colony at Mound Laboratory, Ohio, the spontaneous mammary tumor incidence over the life span of 140 normal rats was 57%; the mean life span was 775 days.[1] In Hartwig's colony at Randolph Air Force Base, Texas, the normal tumor incidence for 36 rats that lived longer than one year was 19.5%; the mean life span in their colony was 511 days.[2] Since March 1953 several groups of normal female Sprague-Dawley rats totaling 137 animals have been observed throughout their life span in our colony. Of 87 animals that lived longer than one year, 22% developed tumors of mammary origin. The mean life span of the various groups in our colony ranged from 442 to 603 days; the mean life span for all groups combined was 500 days.

According to Davis et al. spontaneous incidence of mammary tumor in this strain is highest in the last quarter of the life span. Fifty percent of the tumors they saw appeared after the animals were 540 days old. In Hartwig's colony approximately 50% of the spontaneous tumors were seen after the 540th day. In our colony only 31% of the tumors were first observed after the 540th day. The animals in all three colonies were originally obtained from the same supplier, Sprague-Dawley, Inc., Madison, Wisconsin. Differences in local weather conditions, diet, caging, and handling may account for the discrepancies in life span among the various laboratories. The differences in life span may in turn account for the observed differences in tumor incidence in the same strain.

The life-span tumor incidence may be corrected by actuarial methods for mortality from causes other than mammary tumors. We have chosen to use the method of Berkson and Gage,[3] which allows the tumor incidences to be compared directly regardless of differences in life span. This method is widely used to evaluate treatment of human cancer and takes into account those individuals lost to follow-up for reasons other than death from cancer. The age-specific tumor incidence was calculated as the quotient of the number of tumors first noted during a 100-day interval of age and the population of

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[1] R. K. Davis, G. T. Stevenson, and K. A. Busch, Tumor Incidence in Normal Sprague-Dawley Female Rats, Cancer Research 16, 194-197 (1956).

[2] Q. L. Hartwig, S. P. Kent, and J. A. Sproul, Jr., Effect of Chronic Exposure to Fast Neutrons on the Development of Mammary Tumors in the Rat, Cancer Research 18, 736-39 (1958).

[3] J. Berkson and R. P. Gage, Calculation of Survival Rates for Cancer, Proc. Staff Meetings Mayo Clinic 25, 270-286 (1950).

animals that could be observed for the interval.[4] These ratios were then converted to tumor incidence rates by dividing the percent incidence by the number of days in the interval. For example, 63 normal rats (Table II) were alive at 500 days of age. During the ensuing 100 days two rats developed mammary tumors. Five rats were autopsied on the 502nd day for tissue specimens and were excluded from the data as of the 500th day. Fifteen rats died of various causes, chiefly bronchiectasis, and twelve rats are still alive but are not yet 600 days old. The age-specific tumor incidence rate from 500 to 600 days of age was calculated as $2/[58 - 0.5(15 + 12)] \times 100/100$ days = 2/44.5 = 0.045%/rat-day. The logarithm of the age-specific tumor incidence rate as shown in Fig. 6 increased linearly with age at ages greater than 40 days (the age at which we receive animals from the dealer).

The cumulative tumor incidence (See Table III) was also calculated as described by Berkson and Gage[3] over the observed life span of female rats in our colony. By the 900th day of life 63.7% of the normal animals would be expected to develop a mammary tumor. Actuarial analysis brings our total spontaneous tumor incidence much closer to the 57% reported by Davis et al.[1]

Incidence of Mammary Tumors in Astatine-211-Injected Rats

We have reported an augmented incidence of mammary tumors in female Sprague-Dawley rats after injection with At^{211}.[5,6] The design of more recent experiments was outlined in a previous report.[7] While the initial experiments with At^{211}-injected rats showed a high incidence of mammary tumors at a relatively early age, important unanswered questions remained concerning life-span tumor incidence, dose dependence, and endocrine relationships.

In the present studies we attempted to increase group size using the same total amount of At^{211} by using younger, lighter animals, 43 to 45 days old, rather than the 55- to 60-day-old rats used in the earlier studies. The tumor incidences in five groups of rats given 0.5 μC/g of At^{211} are shown in Table IV. The slopes of the curves of tumor incidence vs age (Fig. 7) of the four groups of older rats, 55 to 60 days old, in Experiments XI through XVI averaged 8.4×10^{-3}/day. The slope of the curve of the younger group, 45 days old, in Experiment XVII was 5.77×10^{3}/day. The experimental

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[4] The observed population was corrected by subtracting from the total population one-half of the sum of nontumor deaths during the interval and the number of rats that are still alive, but who have not yet completed the interval.

[5] J. G. Hamilton, P. Wallace-Durbin, and M. W. Parrott, Comparison of Acute and Chronic Changes Produced in Rats by Iodine-131 and Astatine-211 at Lethal Levels, 2nd Radioisotopes Conf. AERE, Harwell, Vol. I, Medical and Physiological Applications 219-31 (1954).

[6] Durbin, Asling, Johnston, Parrott, and Hamilton, The Induction of Tumors in the Rat by Astatine-211, Radiation Research 9: 378-97 (1958).

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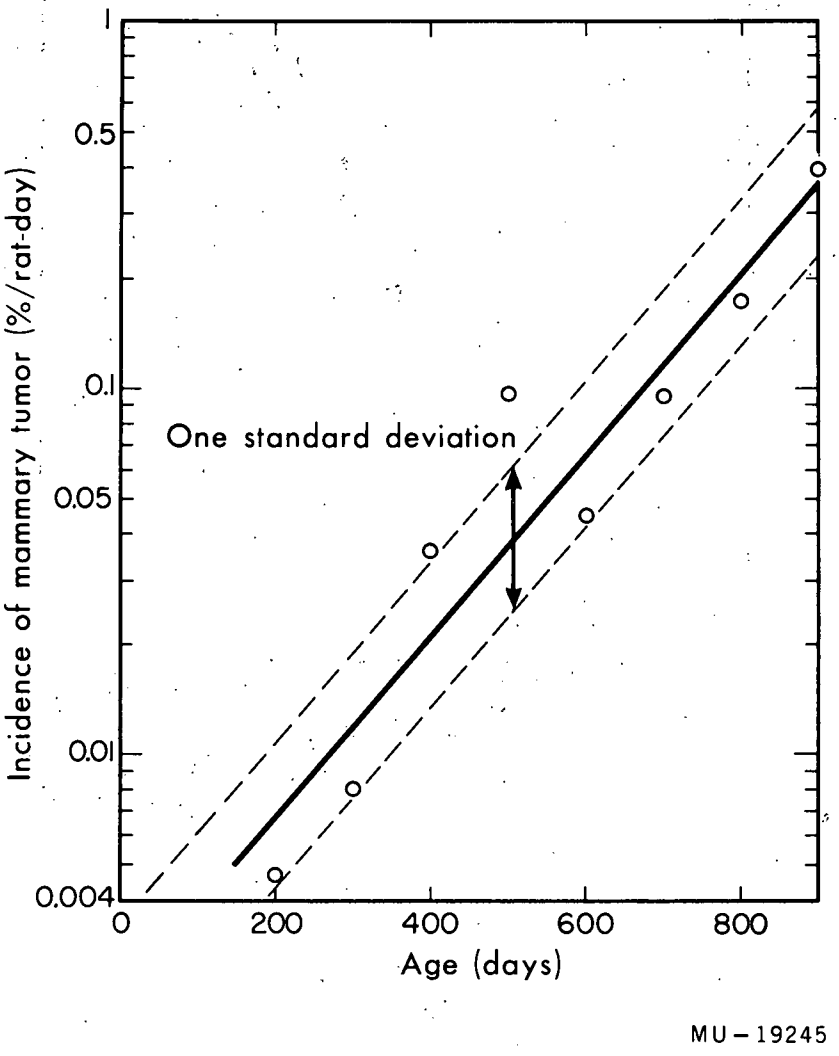

Fig. 6. Age-specific mammary tumor incidence rate in the normal female Sprague-Dawley rat.

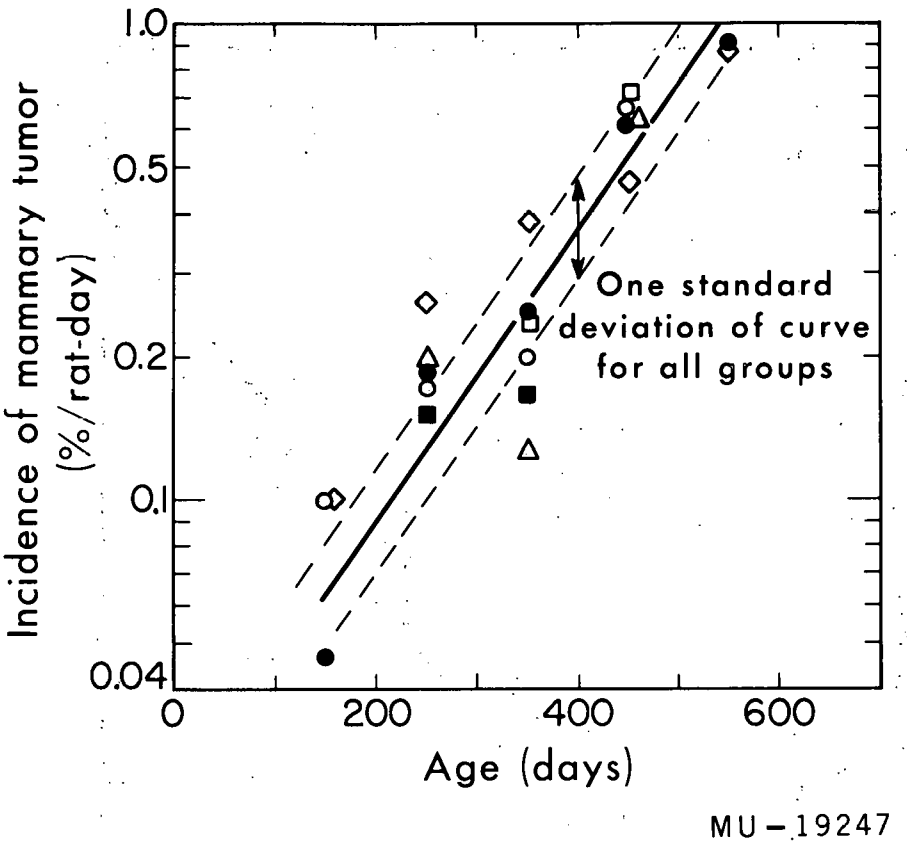

Fig. 7. Age-specific mammary tumor incidence rate of five groups of female Sprague-Dawley rats given 0.5 μC/g of At^{211} at 45 to 55 days of age.

Table II

Calculation of spontaneous mammary tumor incidence rate in normal female Sprague-Dawley rats by the actuarial method of Berkson and Gage.

Interval (days of age)	No. tumors first seen	No. of deaths	Alive on 11/1/59	No. deliberately removed at start	No. alive at start	Rat-exposure days	Tumor incidence (%/rat/day)
40-200	1	0	-	0	132	21,100	0.0047
201-300	1	5	-	5	126	12,350[a]	0.0081
301-400	4	11	-	5	115	10,950	0.0366
401-500	8	24	-	5	95	8,300	0.0965
501-600	2	15	12[a]	5	58	4,450	0.0450
601-700	2	6	-	5	24	2,100	0.0950
701-800	2	3	2	3	13	1,150	0.1740
801-900	1	3	-	2	4	250	0.4000

[a] Animals dying from causes other than mammary tumors, and animals who have not yet completed the full interval are considered, on the average, to have been observed for one-half the interval of interest.

Table III

Calculation of cumulative mammary tumor incidence in normal female Sprague-Dawley rats

Interval	Tumor incidence rate(%/rat-day)	Residual non-tumor-bearing population[a]	Cumulative tumor incidence
40-200	0.0047	100.00	0.47
201-300	0.0081	99.53	1.28
301-400	0.0366	98.72	4.90
401-500	0.0965	95.10	14.05
501-600	0.0450	85.95	18.41
601-700	0.0950	81.69	26.21
701-800	0.1740	73.79	39.06
801-900	0.4000	60.94	63.66

[a] Population at start of interval

Table IV

Mammary tumor incidence of five groups of female Sprague-Dawley rats given 0.5 μC/g body weight of astatine-211 at 45 to 55 days of age

Group	Date	No. at start	Interval (days) 51-100	101-150	151-200	201-250	251-300	301-350	351-400	401-450	451-500	501-550
XI	10/54	20										
Tumors			0	0	2	3	2	2	3	2		
Deaths			0	0	0	0	0	0	0	6 sac.[a]		
XIV	5/55	20										
Tumors			0	2	1	2	1	2	4	2		
Deaths			0	0	0	0	0	0	0	6 sac.[a]		
XV	8/55	17										
Tumors			0	0	0	0	2	2	8	0		
Deaths			0	0	0	0	0	0	1	4 sac.[a]		
XVI[b]	11/56	20										
Tumors			0	0	2	2	1	1	3	3		
Deaths			0	0	0	0	1	0	1	6 sac.[a]		
XVII	10/57	30										
Tumors			2	1	5	2	3	4	1	3	3	1
Deaths			0	0	0	1	2	0	0	1	0	1
Total												
Tumors			2	3	10	9	9	11	19	10	3	1
Deaths			0	0	0	1	3	0	2	23	0	1

[a] Sacrificed for tissue specimens during interval.

[b] Sham ovariectomy before At^{211} injection.

points were too scattered to permit statistical differentiation between the groups injected at different ages. Therefore, we have subsequently treated all five groups as a single large group. The age-specific tumor incidence rates have been calculated for 107 rats given 0.5 μC/g of At^{211} and are shown in Table V. They also appear as the closed circles in Fig. 7.

Dosage levels of At^{211} ranging from 0.095 to 0.76 μC/g were tested to examine the possible dependence of mammary tumor incidence on At^{211} dosage. The work of Bond et al.[8] suggests that for young rats of this strain a linear relationship exists between cumulative mammary tumor induction and x-ray dosage.

Mammary tumor incidence rates for five different At^{211} dosages are shown in Table VI and are plotted in Fig. 8. Except for the group given 0.27 μC/g, the slopes of the curves of tumor incidence rate agree well with one another and with the slope of the curve for spontaneous tumor incidence (Fig. 6). Cumulative tumor incidence (Fig. 9) showed saturation at close to 100% for dosages of 0.27 μC/g and greater. The age at which a specific tumor incidence was reached (Fig. 10) showed a minimum at 0.27 μC/g, and the curve relating tumor incidence rate to a specific age (Fig. 11) showed a maximum at 0.27 μC/g. It therefore appears that a dosage of At^{211} between 0.27 μC/g and 0.5 μC/g is optimal for mammary tumor induction in young Sprague-Dawley rats. The histological material is now being examined and should aid in determining the relationships between At^{211} dosage and pituitary and ovarian structure. Further studies are needed to determine the shape of the first portion of the curve shown in Fig. 11, particularly, whether there is a straight-line relationship between At^{211} dose (up to 0.5 μC/g) and whether such a line passes through the spontaneous-incidence rate at zero At^{211} dose.

The first observations of the biological behavior of astatine were the concentration of this radioelement in the thyroid gland,[9] and the destruction of thyroid tissue with large At^{211} dosages.[10] Within a few weeks of the At^{211} injection, rats given 0.5 μC/g or more are severely thyroid-deficient. Almost three-fourths of the thyroid tissue has been destroyed.[6] The standard

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Table V

Calculation of mammary tumor incidence rate of female Sprague-Dawley rats given astatine-211. Actuarial method of Berkson and Gage. Astatine was given in a single injection, 0.5 μC/g body weight, at 45 to 55 days of age.

Interval (days of age)	No. tumors first seen	No. of deaths	No. deliberately sacrificed	No. alive at start	Rat-exposure days	Tumor incidence (%/rat/day)
51-150	5		-	107	10700	0.047
151-250	19	1	-	102	10150	0.187
251-350	20	3	-	82	8050	0.248
351-450	29	3	22	59	4650	0.623
451-550	4	1	-	5	450	0.890

Table VI

Incidence of mammary tumors in female Sprague-Dawley rats after varying dosages of astatine-211. Astatine-211 was injected intravenously at 45 to 55 days of age.

	No. at start	Interval (days)					
		100-200	201-300	301-400	401-500	501-600	601-700
0.095 μC/g	22						
Tumors		1	3	2	4	2	2
Deaths		0	0	1	1	6	0
Alive at start of interval		22	21	18	15	10	2
Tumor incidence		0.046	0.143	0.115	0.276	0.286	1.00
0.27 μC/g	21						
Tumors		4	6	5	1	2	
Deaths		0	1	0	1	1	
Alive at start of interval		21	17	10	5	3	
Tumor incidence		0.191	0.364	0.50	0.222	0.80	
0.50 μC/g[a]	87	0.047	0.187	0.248	0.623	0.890	
0.60 μC/g	20						
Tumors		2	2	5	4	5	
Deaths		0	0	0	0	2	
Alive at start of interval		20	18	16	11	7	
Tumor incidence		0.10	0.111	0.312	0.364	0.833	
0.76 μC/g	21						
Tumors		1	3	4	7	2	1
Deaths		0	0	3	0	0	0
Alive at start of interval		21	20	17	10	3	1
Tumor incidence		0.048	0.150	0.258	0.70	0.667	1.00

[a] See Tables III and IV.

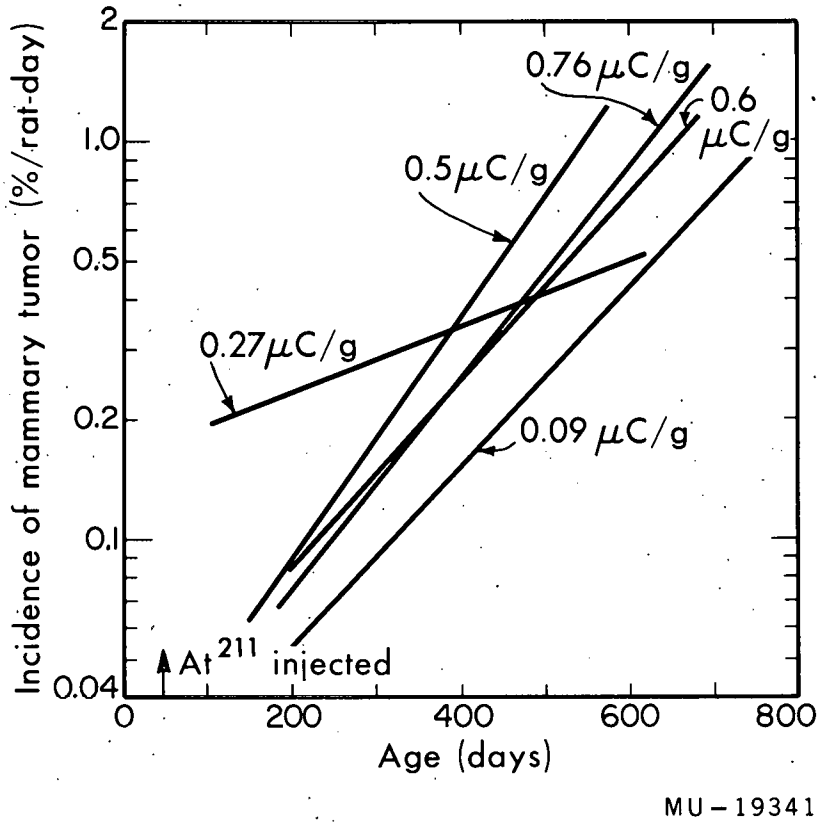

Fig. 8. Effect of At^{211} dosage on the age-specific mammary tumor incidence rate of the female Sprague-Dawley rat.

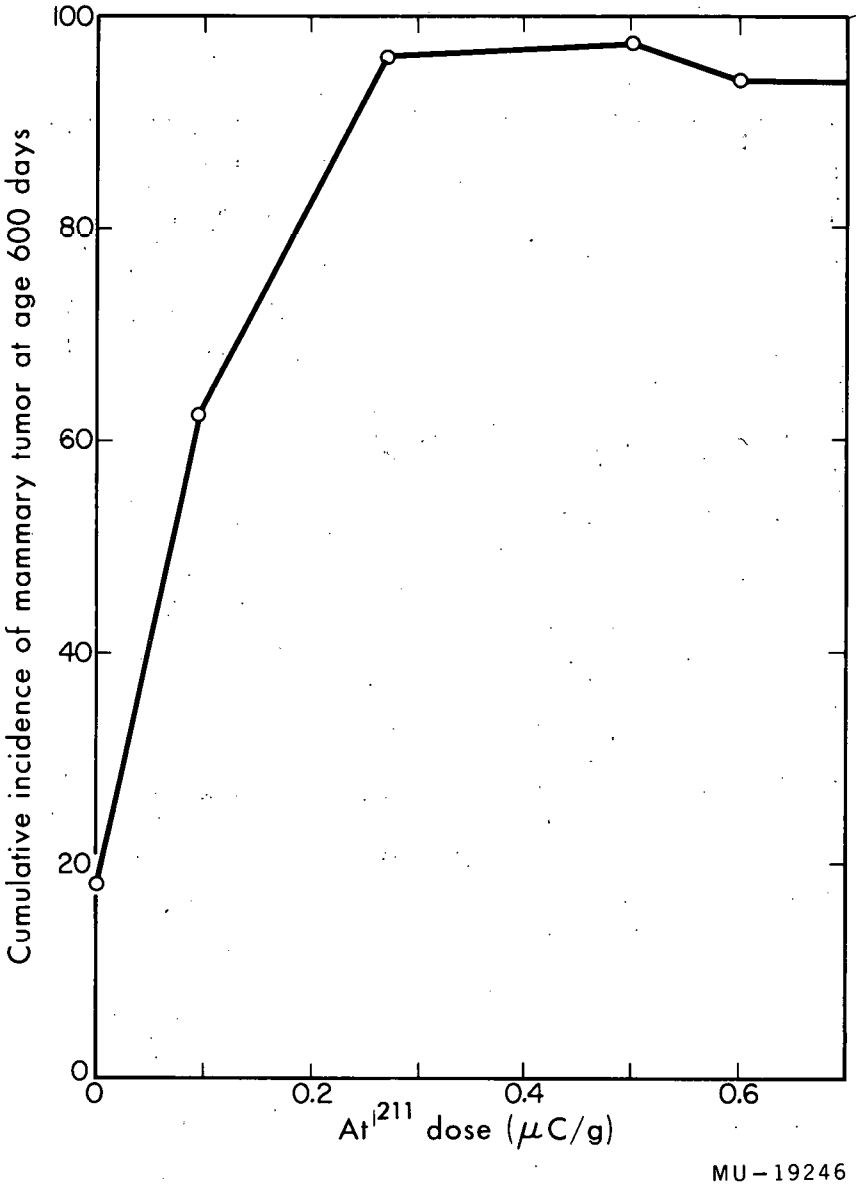

Fig. 9. Variation with At^{211} dose of the cumulative mammary tumors at 600 days of age.

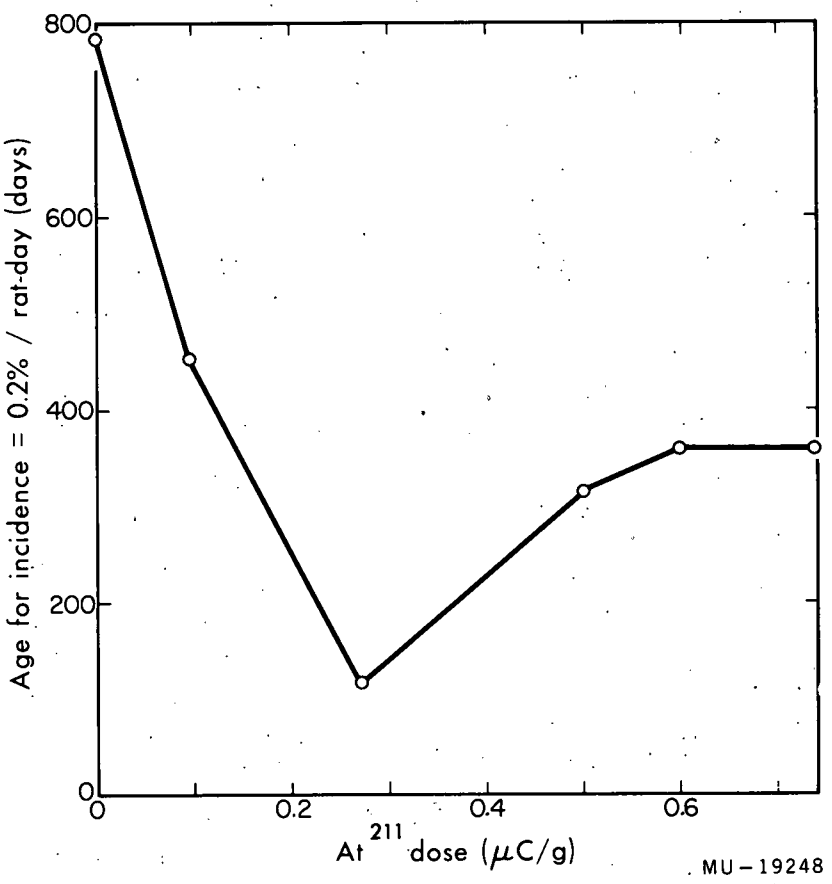

Fig. 10. Variation with At^{211} dose of the age at which the mammary incidence is 0.2%/rat-day.

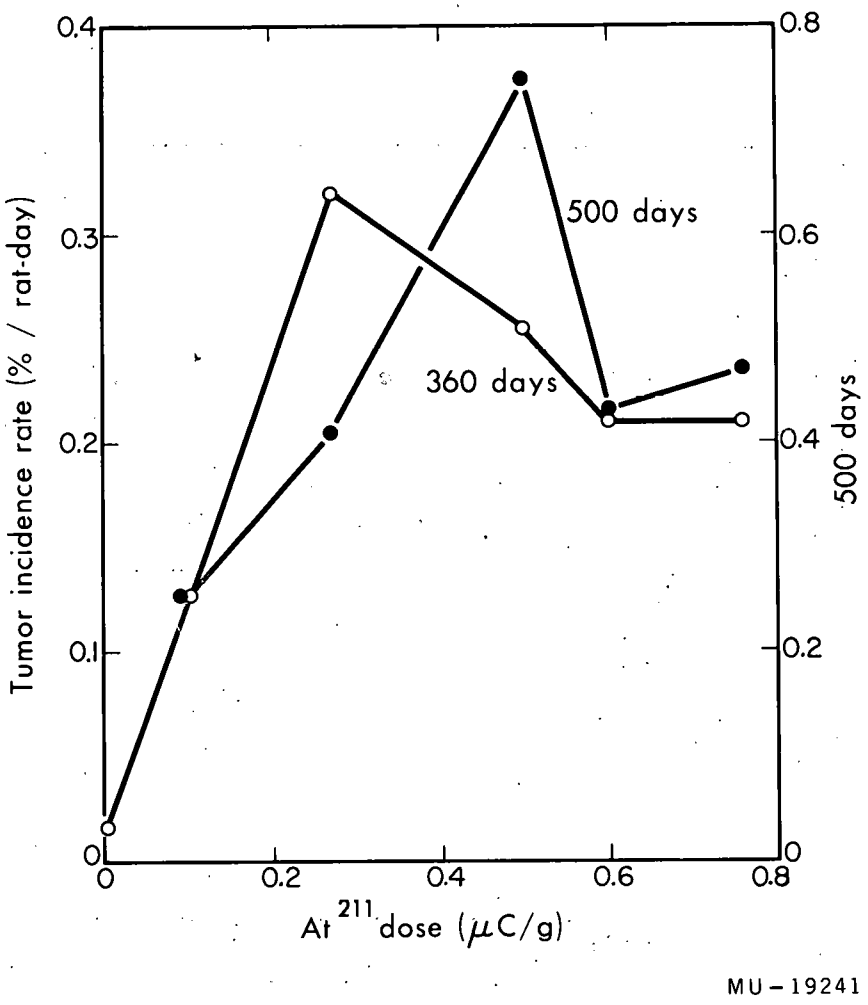

Fig. 11. Mammary tumor incidence rate as a function of At^{211} dosage at age 360 days(left-hand scale) and 500 days (right-hand scale).

metabolic rate (SMR) is very low.[6,11] The anterior pituitary shows structural changes associated with thyroid deficiency.[6] Pituitary tumors in irradiated mice have been attributed to thyroid insufficiency.[12] Therefore, it was important to examine the role of the thyroid gland in the production of mammary tumors. In previous studies l-thyroxine was given to two groups of rats for several days prior to the At^{211} injection to reduce the amount of At^{211} taken up by the thyroid tissue. This type of pre-irradiation treatment provided only partial protection of the thyroid tissue. The SMR was closer to normal than for animals that received only At^{211}. More thyroid tissue was present several months later at autopsy, and an I^{131} uptake test indicated that the remaining thyroid tissue was probably functioning normally. A third group of rats was given l-thyroxine in the drinking water from the day after the At^{211} injection, but the amount was so small that there was no improvement in the SMR, and pituitary histology was only slightly improved. The 1-year cumulative mammary tumor incidences in these three groups of rats (Experiments XI, XIV, and XV) were no different from their At^{211}-injected counterparts that were markedly thyroid-deficient.[6]

Three injections per week of 14 μg of l-thyroxine (equivalent to 3 to 5 μg/day) were found to reproduce a normal SMR in rats whose thyroid glands had been completely destroyed by a combination of surgery and radiation with I^{131}.[13] A group of 15 rats was maintained on l-thyroxine at this dosage for the remainder of their lives after At^{211} injection. The mammary tumor incidences are shown in Table VII for all four groups of rats whose thyroid status was presumably better than that of rats receiving only At^{211}. The age-specific tumor incidence rates are plotted in Fig. 12. The slopes of the curves for tumor incidence rate vs age were similar, and all four groups have been combined. The curves for tumor incidence and composite tumor incidence rate are shown for the 72 rats that either were protected by thyroxine pretreatment or received thyroid therapy. The slope of the composite rate curve was 6.41×10^{-3}/day, very close to that for severely thyroid-deficient At^{211}-irradiated rats. Both curves are reproduced in Fig. 13 - they are almost identical. It appears that the functional state of the thyroid tissue (the level of circulating thyroid hormone) has little influence on the production of mammary tumors in the At^{211}-irradiated rat. The same conclusion can be drawn from the studies by Bond et al.[8] and Shellabarger et al.,[14] who observed the occurrence of mammary tumors in female rats of this strain exposed to 200 to 600 r of x-irradiation. Although there is at least partial ovarian derangement in this range of x-ray dose, there is no question about the adequacy of thyroid function.[8,15,16]

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Table VII

Effect of improved thyroid status on mammary tumor incidence in astatine-211 injected Sprague-Dawley female rats. Astatine-211 was administered at a level of 0.5 μC/g body weight at 45 to 55 days of age.

Experiment & Treatment	No. at start	Interval (days) 101-200	201-300	301-400	401-500	500-600
XI. Thyroxine pre-treated	20					
Tumors		0	6	5	3	
Deaths		0	0	0	6[a]	
XV. Thyroxine pre-treated	18					
Tumors		1	7	3	4	
Deaths		0	0	1	2[b]	
XIV. l-thyroxine in water	19					
Tumors		1	2	3	0	6
Deaths		0	2	2		3
XVIII. l-thyroxine, 3 injections/wk	15					
Tumors		3	0	4	1	5
Deaths		0	0	1	1	0
Total - all groups	72					
Tumors		5	15	15	14	5
Deaths		0	2	4	12	0
No. alive at start		72	67	50	29	5
Rat-exposure days		7200	6600	4800		500
Tumor incidence (%/rat/day)		0.069	0.227	0.312	0.610	1.00

[a] Rats deliberately autopsied during interval.

[b] Rats deliberately autopsied at beginning of interval.

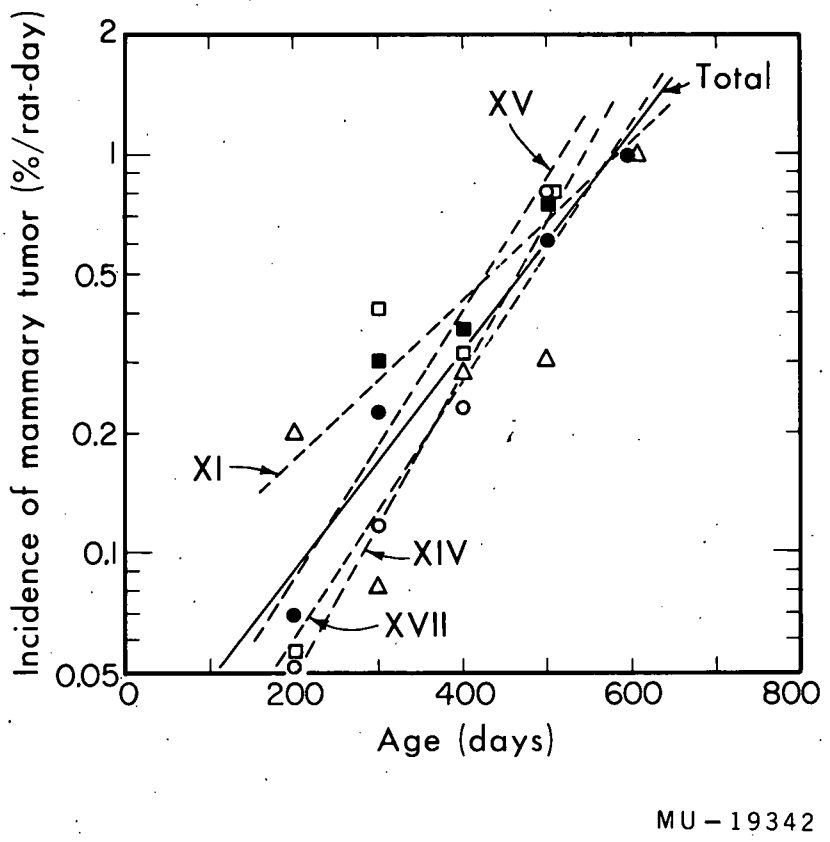

Fig. 12. Effect of thyroid status on age-specific mammary tumor incidence rate. Experiments XI and XIV, l-thyroxine pretreatment; Experiment XV, l-thyroxine in drinking water; Experiment XVII, l-thyroxine injected three times per week.

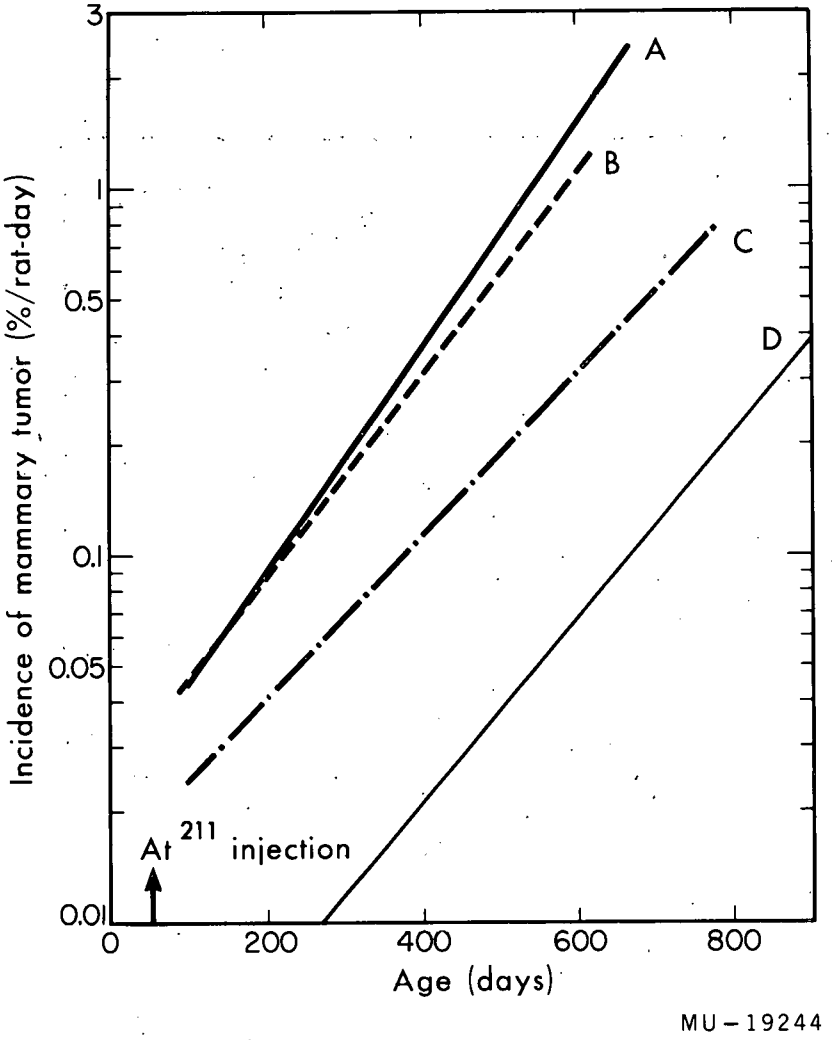

Fig. 13. Comparison incidence rate of mammary tumor in normal female Sprague-Dawley rats, and the effect of improved thyroid status or ovariectomy in rats given 0.5 μC/g of At^{211}.

A: 0.5 μC/g At^{211} only; T_{ID} = 98 ± 17 days.

B: At^{211}, improved thyroid status; T_{ID} = 108 ± 15 days.

C: At^{211}, ovariectomized; T_{ID} = 133 ± 22 days.

D: normal controls; T_{ID} = 122 ± 28 days.

The well-established relationship between mammary tumor production and the ovarian hormones[17] indicated that the ovary was involved in the development of mammary tumors in our young At^{211}-injected rats. Indeed, Cronkite et al. suggest that mammary tumors arise in x-irradiated female rats of this strain only when "functional" ovaries are present.[18] The functional status of the ovaries of At^{211}-irradiated animals is not clear. Female rats given At^{211} at dosages greater than 0.5 μC/g are sterile as a direct result of radiation damage in the ovaries.[19] Microscopically, the ovaries--although small and empty of follicles--still retain their glandular character and are predominantly cellular rather than fibrotic.[6]

The effect of ovariectomy on mammary tumor induction in the At^{211}-irradiated rat is shown in Table VIII and in Fig. 13. One group of rats (Experiment XVI) was ovariectomized 2 to 3 days before the At^{211} injection. The other group (Experiment XVIII) was ovariectomized within a week after At^{211} was administered. The data from both groups have been combined. The slope of the curve of tumor incidence for rate ovariectomized At^{211}-injected rats was similar to those for At^{211}-injected intact rats and for normal controls. Tumors appeared at a more advanced age in the ovariectomized irradiated group, but earlier in life than in normal controls. A group of 13 ovariectomized control rats (no At^{211}) has been observed for 600 days. Only four rats are still alive, and to date no mammary tumors have been seen. Removal of the ovaries will undoubtedly reduce mammary tumor incidence below normal control levels, and a much larger group will have to be ovariectomized to assure observation of the small number of tumors expected. The results of these studies are summarized in Table IX and Fig. 8, and from these summaries the following conclusions may be drawn:

1. Mammary tumors arise spontaneously in normal female Sprague-Dawley rats at a rate that increases with age. The rate of change of the logarithm of the rate of mammary tumor incidence is a linear function of age, $N_{t2} = N_{t1} e^{\lambda(t2 - t1)}$, with a slope λ of approximately 5.7×10^{-3}/day.

2. The age-specific rate of mammary tumor incidence in female rats of this strain irradiated with At^{211} at 45 to 55 days of age was 15 to 23 times that for normal rats of the same chronologic age. The slope of the curve for incidence rate vs age was the same for normal and irradiated rats. Irradiation with At^{211} advanced the mammary tumor induction process by about 450 days--almost one-half the life expectancy of this species.

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3. The total spontaneous mammary tumor incidence is calculated as 63.7% over the life span. When rats were given 0.27 μC/g or more of At^{211}, the cumulative mammary tumor incidence was close to 100% by the 500th day of life.

4. The functional status of the thyroid gland does not seem to play an important role in the induction of mammary tumors by At^{211}. Induction rates and total tumor incidence were similar for severely thyroid-deficient rats and rats whose thyroid status had been made more nearly normal, either by pre-irradiation protection with thyroxine blocking or by postirradiatior thyroid hormone therapy.

5. Ovariectomy within a few days of At^{211} injection postponed the appearance of mammary tumors by about 270 days. The age-specific tumor incidence rate was one-half to one-third that observed in intact At^{211}-injected rats, but was still six times that for normal rats of the same age.

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Table VIII

Effect of ovariectomy on mammary tumor incidence in female Sprague-Dawley rats given astatine-211. Ovariectomy was performed two to five days before injection of 0.5 μC/g body weight of astatine-211 at 45 to 55 days of age.

Experiment & Treatment	No. at start	Interval (days) 40-200	201-300	301-400	401-500	501-600	
XVI. Ovariectomy	19						
Tumors		0	2	0	0	-	
Deaths		0	4	1	12[a]	-	
XVIII. Ovariectomy	24						
Tumors		0	1	5	2	3	(7 alive at 610 days)
Deaths		1	0	2	2	1	
Total - Ovariectomy	43						
Tumors		0	3	5	2	3	
Deaths		1	4	3	2	1	
Alive at start		43	42	35	15	11	
Rat-exposure days			4000	3350	1400	1050	
Tumor incidence (%/rat/day)			0.075	0.149	0.143	0.286	

[a] Twelve remaining rats autopsied at 408 days and excluded from data as of the 400th day.

Table IX

Comparison of mammary tumor incidence rates in the normal and At^{211}-treated female Sprague-Dawley rat.

Group	Mean life span	Longest-lived survivor (days)	Cumulative life-span tumor incidence %[a]	Age on reaching 0.2%/day tumor incidence	Slope of tumor-incidence-rate curve ($days^{-1}$)
Control	500	950	63.7	785 days	5.69×10^{-3}
At^{211}-irradiated					
0.095 μC/g	553	731	100	453 "	4.98×10^{-3}
0.27 μC/g	326	570	96	116 "[b]	1.98×10^{-3}
0.50 μC/g	380	550	97.6	315 "	7×10^{-3}
0.60 μC/g	489	660	94	360 "	5.72×10^{-3}
0.76 μC/g	408	726	100	360 "	6.35×10^{-2}
0.5 μC/g l-thyroxine pre-treatment or therapy	350	550	100	330 "	6.41×10^{-3}
0.5 μC/g ovariectomized	450	>600	>51.6	510 "	5.2×10^{-3}

[a] Not corrected for control tumor incidence.

[b] Slope of tumor-incidence-rate curve in doubt.

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HEMATOLOGY, CIRCULATION, AND BLOOD BIOCHEMISTRY

FATTY ACIDS IN SERUM LIPIDS

Alex V. Nichols and Frank T. Lindgren

The metabolism of serum lipids in health and disease has been extensively studied in this laboratory. Our interest in fatty acids stems from our investigations into fatty acid absorption and transport, using tritium-labeled stearic acid. In these studies specific lipids and lipoprotein groups were shown to be responsible for the transport of the radioactive fatty acids in the blood. Such data have prompted us to examine in greater detail the specific fatty acid composition of serum lipids and lipoproteins.

Many detailed analyses have now firmly established the broad lipid composition of serum and serum lipoproteins.[1, 2, 3] Cholesteryl esters, triglycerides, phospholipids, unesterified fatty acids, and unesterified cholesterol have been determined in these biological materials, and considerable data are available on their concentrations in various metabolic states.[4]

With the single exception of unesterified cholesterol, all these lipid compounds contain one or more fatty acid molecules. These fatty acid molecules are an integral part of lipid compounds and play a major role in determining their physical and chemical properties. The great diversity of fatty acids present in lipid compounds was recognized early, but methodological problems prevented their quantitative determination. For example, it was well understood that, in the broad chemical class of cholesteryl esters, compounds such as cholesteryl laurate, cholesteryl oleate, cholesteryl linoleate, etc., were present. However, until recently the available methods allowed at best only an approximate estimate of the content of any cholesteryl ester fraction. This may likewise be said for the triglyceride and phospholipid compounds, which as lipid classes contain many different fatty acids and as individual molecules also may contain several different fatty acid molecules.

The present interest in research on fatty acid composition and metabolism stems from new developments in different areas of investigation. Workers in lipid metabolism have recently demonstrated marked differences in serum lipid concentrations resulting from alterations in dietary fatty acid composition.[5, 6, 7, 8] Individuals on diets high in saturated fatty acids were found to exhibit elevated serum lipoprotein and lipid concentrations. Isocaloric substitution of the saturated fatty acids by unsaturated fatty acids resulted in lower values of serum lipoprotein and lipid. These results showed a most

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remarkable influence of the dietary fatty acid composition on both lipid transport and metabolism. Other workers in lipid metabolism have called attention to the important role of albumin-bound fatty acids in the over-all energy metabolism of the body.[9] They have determined a very rapid turnover for serum fatty acids in humans and have indicated a definite relationship of serum fatty acid levels to carbohydrate metabolism. From the biological standpoint just these recent developments are more than sufficient reason for more careful study of the detailed composition of the serum fatty acids.

However, even though the above observations on lipid metabolism indicated the value of studying serum fatty acid composition and metabolism, more sensitive biophysical instrumentation was essential. With most biological work, especially in humans, only small samples of blood or tissue are usually available. Conventional methods of analyzing for fatty acids required amounts of biological samples many times those attainable in practice. In recent years outstanding progress has been made in the chromatographic analysis of fatty acid molecules.[10,11] For rapid resolution of fatty acid mixtures, chromatographic procedures are now carried out on special column materials at high temperatures (150° to 200°C) and with gas flow instead of with conventional liquid solvents. For tremendous gains in sensitivity, electronic detectors have been designed allowing stable responses to microgram amounts of fatty acid mixtures. Such improvements have been incorporated into a method for fatty acid analysis which is termed gas-liquid chromatography of fatty acids. These developments in biophysical instrumentation stimulated the present intensive research on fatty acid composition.

In this laboratory we have designed and set up a gas-liquid chromatographic unit for the study of serum lipids, serum lipoproteins, and other biological materials. The design and performance of this instrument are described in another report.[12]

This report presents the data on the fatty acid composition of the cholesteryl esters, triglycerides, phospholipids, and unesterified fatty acids of seven normal individuals. Other workers have reported on fatty acid composition of phospholipids, unesterified fatty acids, and a pooled fraction containing triglycerides plus cholesteryl esters.[13,14] The present study thus provides a more complete analysis of the fatty acid composition of blood lipids in normal subjects.

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Methods

Samples of venous blood were collected from 6 normal males and 1 female. Prior to drawing of blood, subjects maintained their regular eating patterns. Lipids were extracted from each of the serum samples by a modification of the method of Sperry.[15] Separation of the lipids into cholesteryl esters, triglycerides, phospholipids, and unesterified fatty acids was carried out according to the procedures described by Freeman.[16] After fractionation the lipids were methylated by the method described by Stoffel.[17] Gas-liquid chromatography of the methylated fatty acids was performed by methods reported by Upham.[12]

Fatty acids were identified by comparison of the elution times of serum fatty acid with elution times of known calibrated standard fatty acid mixtures. Some overlap of standard fatty acid elution times with elution times of unidentified fatty acids can occur, and this should be resolved by additional procedures such as bromination and hydrogenation of the fatty acid mixtures followed by rechromatography. In this paper the serum fatty acids are reported according to the terminology proposed by Dole.[14] By this terminology palmitic acid would be reported as 16:0. The number on the left designates the number of carbon atoms while the number to the right of the colon gives the number of double bonds in this fatty acid. Other notations, such as branching or isomeric form, usually precede the numerical listing.

Results

The results are presented in Tables X—XIII for each of the major fatty-acid-containing lipids in serum. Thus, cholesteryl esters, triglycerides, phospholipids, and unesterified fatty acids are listed for each serum sample whenever available. The term "pre 16:0" designates all fatty acid peaks observed prior to the appearance of palmitic acid (16:0). Most of the short-chain fatty acids in the lipid fractions are included in this fraction, and the predominant contributions to this value are made by lauric acid (12:0) and myristic acid (14:0). Unidentified peaks on the chromatograms were calculated in terms of calibration factors for fatty acids theoretically expected to appear at those peak elution times.

Discussion

The results show a definite pattern for the fatty acid composition in the serum lipid fractions of seven normal subjects. Each lipid fraction appears to have a fairly characteristic distribution of fatty acids. Although there is some variation between individuals, a basic pattern of fatty acid distribution is clearly evident.

In serum cholesteryl esters the predominant fatty acids are linoleic (50%), oleic (19%), palmitic (10%), and arachidonic (6%). These values certainly establish cholesteryl esters as an important transport form for the more unsaturated fatty acids. Serum triglycerides present a different fatty acid pattern. The major fatty acids in this lipid are oleic (41%), palmitic (28%),

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Table X

Composition of Serum Cholesteryl Ester Fatty Acids

(composition reported in percentage by weight)

Components	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	AV1-7
(pre 16:0)[a]	7.09	3.71	4.46	2.64	3.12	2.22	7.93	4.37
16:0(palmitic)	13.73	9.91	9.64	9.78	9.49	9.98	10.40	10.36
16:1(palmitoleic)	7.60	2.57	2.76	2.86	2.19	2.84	3.44	3.40
(16:1-18:0)[b]	2.94	0.78	2.15	0.83	2.14	0.78	2.67	1.66
18:0(stearic)	1.66	1.03	1.23	0.97	1.33	0.92	2.18	1.34
18:1(oleic)	25.66	18.47	16.85	16.73	14.62	20.97	18.48	18.76
18:2(linoleic)	35.04	56.12	54.32	47.91	56.42	54.27	48.26	50.41
(18:2-20:4)[c]	4.40	1.62	3.02	8.64	3.11	3.01	2.29	3.02
20:4(arachidonic)	7.33	5.39	5.06	8.71	7.11	4.50	4.34	6.05
(post 20:4)[d]	0.93	0.37	0.49	0.93	0.49	0.49	-	0.61

[a] Unidentified short-chain fatty acids with 12:0 and 14:0 as major contributors

[b] Unidentified acids measured on chromatograms between 16:1 and 18:0.

[c] Unidentified acids measured on chromatograms between 18:2 and 20:4.

[d] Unidentified acids measured on chromatograms after 20:4.

Table XI

Composition of Serum Triglyceride Fatty Acids

(composition reported in percentage by weight)

Components	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	AV 1-7
(pre 16:0)[a]	5.08	4.12	6.09	6.01	2.59	1.93	3.85	4.21
16:0(palmitic)	30.98	25.93	27.94	30.53	25.91	29.07	24.92	27.90
16:1(palmitoleic)	5.19	3.74	3.39	4.15	3.45	3.36	3.45	3.85
(16:1-18:0)[b]	1.50	1.29	1.20	1.50	0.99	0.69	0.89	1.10
18:0(stearic)	4.32	4.71	4.11	4.75	4.58	3.44	4.90	4.41
18:1(oleic)	40.10	40.82	36.96	36.99	41.07	45.19	43.83	40.74
18:2(linoleic)	9.04	16.50	18.29	12.38	18.07	14.30	14.60	14.79
(18:2-20:4)[c]	2.99	1.54	1.11	2.10	1.53	1.20	1.97	1.76
20:4(arachidonic)	0.79	1.12	0.90	1.58	1.78	0.78	1.56	1.24
(post 20:4)[d]	-	-	-	-	-	-	-	-

[a] Unidentified short-chain fatty acids with 12:0 and 14:0 as major contributors

[b] Unidentified acids measured on chromatograms between 16:1 and 18:0

[c] Unidentified acids measured on chromatograms between 18:2 and 20:4

[d] Unidentified acids measured on chromatograms after 20:4

Table XII

Components	Composition of Serum Phospholipid Fatty Acids (composition reported in percentage by weight)							
	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	AV 1-7
(pre 16:0)[a]	2.83	3.24	2.50	3.82	4.09	1.96	4.58	3.31
16:0(palmitic)	28.78	30.39	34.65	31.43	30.53	29.31	28.85	30.47
16:1(palmitoleic)	1.76	1.02	1.11	1.39	1.39	1.09	1.48	1.29
(16:1-18:0)[b]	0.89	0.98	0.89	0.98	1.19	0.78	1.18	0.98
18:0(stearic)	14.93	14.48	12.88	13.76	15.27	12.03	14.51	13.92
18:1(oleic)	18.02	9.86	10.94	9.86	10.32	12.25	13.89	12.08
18:2(linoleic)	19.31	22.02	23.45	17.54	20.79	21.51	21.04	20.72
(18:2-20:4)[c]	3.70	7.05	3.49	4.89	4.58	3.32	3.48	4.33
20:4(arachidonic)	9.76	9.08	8.34	14.84	11.45	7.86	8.76	9.93
(post 20:4)[d]	-	1.86	1.74	1.49	0.37	9.89	2.23	2.96

[a] Unidentified short-chain fatty acids with 12:0 and 14:0 as major contributors

[b] Unidentified acids measured on chromatograms between 16:1 and 18:0

[c] Unidentified acids measured on chromatograms between 18:2 and 20:4

[d] Unidentified acids measured on chromatograms after 20:4.

Table XIII

Composition of Serum Unesterified Fatty Acids

(composition reported in percentage by weight)

Components	Subject 1	Subject 2
(pre 16:0)[a]	10:45	10.71
16:0(palmitic)	20.62	27.59
16:1(palmitoleic)	5.26	3.69
(16:1-18:0)[b]	1.90	2.52
18:0(stearic)	9.03	8.47
18:1(oleic)	29.70	26.40
18:2(linoleic)	12.21	13.99
(18:2-20:4)[c]	4.29	2.67
20:4(arachidonic)	3.03	3.06
(post 20:4)[d]	3.52	0.89

[a] Unidentified short-chain fatty acids with 12:0 and 14:0 as major contributors

[b] Unidentified acids measured on chromatograms between 16:1 and 18:0

[c] Unidentified acids measured on chromatograms between 18:2 and 20:4

[d] Unidentified acids measured on chromatograms after 20:4

linoleic (15%), and stearic (4%). Here, there is an increase in the more saturated fatty acids in serum. In the phospholipids there again appears a different fatty acid pattern. The major fatty acids are palmitic (30%), linoleic (21%), stearic (14%), and oleic (12%). The phospholipids apparently transport a high percentage of the saturated fatty acids like palmitic and stearic acids. However, they are also highest in arachidonic acid (9%) in comparison with the other lipid fractions. The unesterified fatty acid fraction, which is principally transported in serum bound to albumin, shows yet another fatty acid distribution. The major fatty acids in this fraction are oleic (28%), palmitic (24%), and linoleic (13%).

We would interpret these results to represent the average fatty acid composition of normal individuals on their regular home diets. The acute influence on serum fatty acids after an ingestion of moderate amounts of various fats has been studied by Dole.[14] He has observed the serum fatty acid composition to be relatively stable to such fat loads. From this we would gather that meals eaten by our subjects prior to blood drawing had little, if any, influence on the fatty acid patterns observed. It is interesting to note how similar are these distributions among the subjects, particularly in the light of data reported by Ahrens showing significant differences in distributions when different fats were used during specific dietary periods.[18] We would thus infer from our data that such normal individuals must be on reasonably similar dietary patterns to exhibit such comparable fatty acid distributions. Whether abnormalities in lipid metabolism alter such distributions will be further investigated in this laboratory.

Conclusions

1. Fatty acids in the various serum lipids exhibit highly characteristic distributions.

2. Normal individuals on regular "home" diets show very similar fatty acid distributions in serum lipids.

3. The major fatty acids in serum are distributed among the various lipids (cholesteryl esters, triglycerides, phospholipids, and unesterified fatty acids) in a manner broadly suggesting that a specific lipid form is necessary for the transport and metabolism of certain fatty acids.

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HEMODYNAMIC ASPECTS OF ATHEROGENESIS

Wei Young

Atherosclerosis is generally regarded as a disease process which is mainly due to disorder of metabolic factors--lipoproteins, hormones, heparin, etc. Such factors may operate to deposit certain of the circulating serum lipoproteins into the tissue site at which a plaque develops. Direct evidence suggesting this mechanism is that lipoproteins having properties similar to those of the low-density serum lipoproteins can be isolated from plaques. Further, studies using tritium-labeled cholesterol[1] demonstrated that significant quantities of ingested dietary cholesterol actually entered the arterial plaques.

The metabolic factors affecting atherosclerosis have been extensively studied, especially with respect to lipoproteins. However, careful examination upon dissection of the arteries very frequently shows that plaques are scattered all along an arterial branch. An artery of considerable length, uniformly and concentrically coated with lipid material without localized regions of sclerosis, seems to be quite rare. On the contrary, plaques are in the majority of cases deposited eccentrically. Such an eccentric deposition of lipid materials and the topographic scattering of plaques cannot be explained readily by general metabolic factors alone. Thus our attention has been directed to the local factors, such as pressure, mode of flow, etc. By quantitative grading of sclerosis and a systematic analysis of coronary arteries with respect to relative position and severity of plaques, we were able to demonstrate that local factors are significantly related to atherogenesis.

Atherosclerosis at Bends of an Artery

Table XIV shows the effect of the bend of the anterior descending branch of the left coronary artery of a rabbit. The sclerosis for the prebend was measured as 47.5, 59.8, and 54.9 I/E units for the bend and 48.4, 42.7, and 32.9 I/E units for the postbend. The higher value at the bend of the artery than in the prebend or postbend speaks for itself: the bend of an artery is more susceptible to deposition.

[1] Max W. Biggs and David Kritchevsky, Circulation 4, 34 (1951).

Table XIV

The effect of arterial bend on atherogenesis

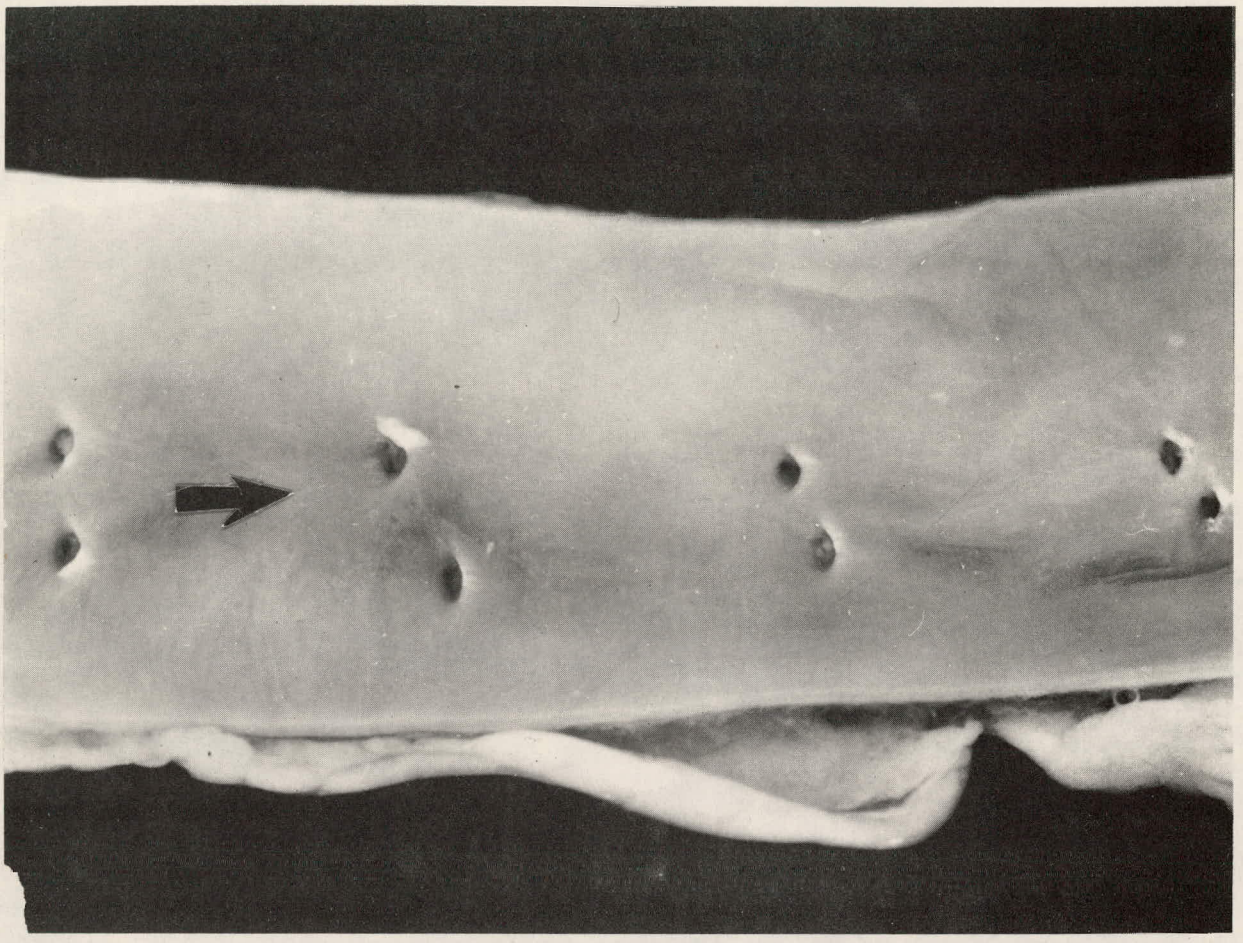
Number	I/E	Position
135	47.5, 14.2	Prebend
135	59.8, 11.8	Bend
139	54.9, 13.7	Bend
139	48.4, 15.4	Post bend
125	42.7, 16.4	Post bend
105	32.9, 14.3	Post bend

I/E = A therosclerosis, where I = intimal material area,
E = total arterial cross section

Atherosclerosis at Bifurcation

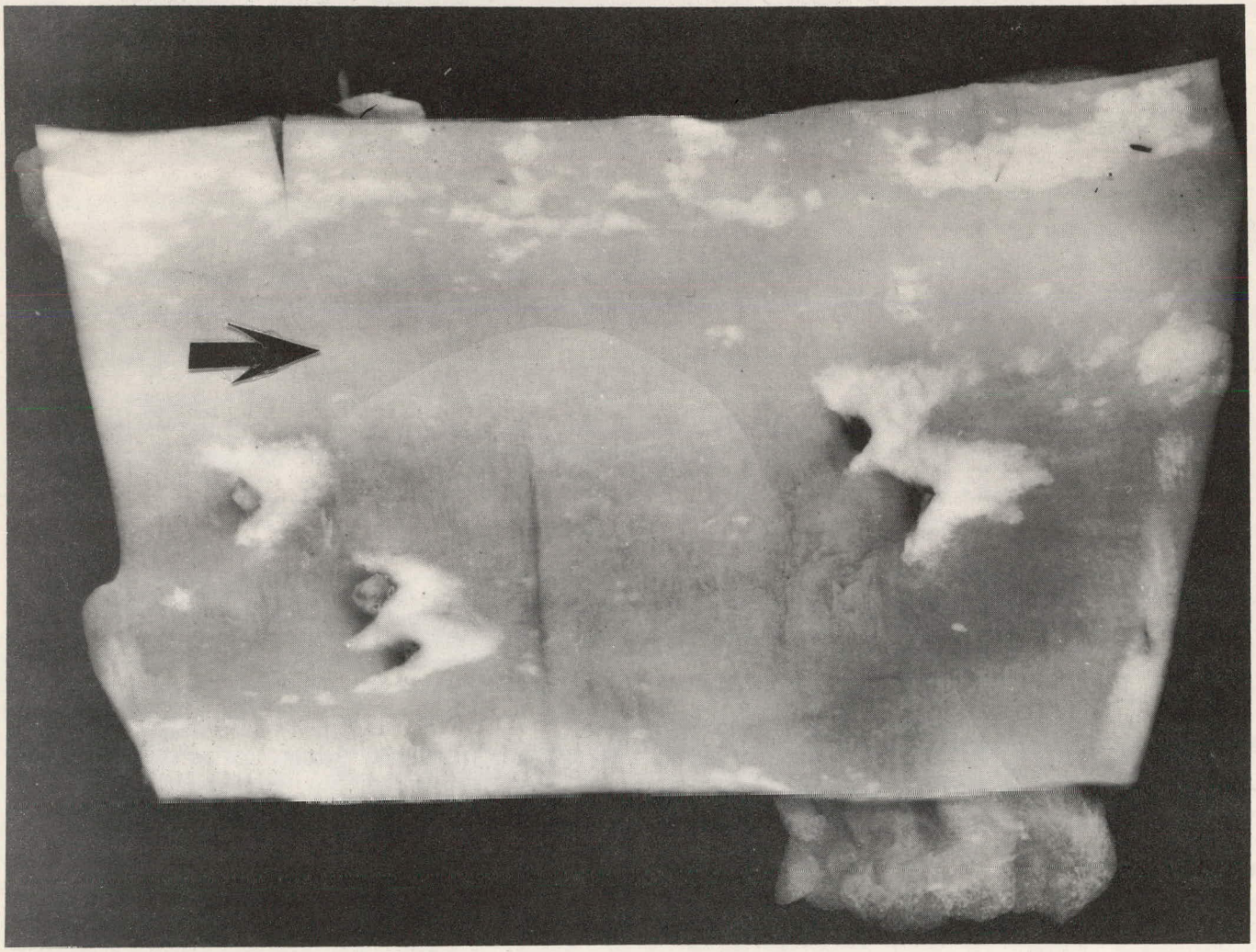
The plaques along any artery are not randomly distributed, as is frequently reported in the literature. They seem to be located according to certain hemodynamic rules. The plaques are particularly frequent at the bifurcation. Figure 14 shows a typical early phase of atherosclerosis which was formed at the branching point of an aorta in a cholesterol-fed rabbit. Figure 15 shows another aorta, which exhibits a comparatively advanced stage of atherosclerosis. The plaque is also particularly prominent at the branching point of the artery. In both figures it is evident that the plaque formed at precisely the same location with respect to flow.

In order to pursue this further, two types of approach are employed: (a) experimental atherosclerosis in an animal with artificial constriction or bending of the artery, (b) flow model. Preliminary experiments seem to indicate that the mode of flow--especially turbulence--may be responsible for such highly localized plaques.



ZN-2323

Fig. 14. Early phase of experimental atherosclerosis of rabbit aorta. Note plaque forming at each branching point. Flow direction from left to right, as indicated by arrow. x5.



ZN-2324

Fig. 15. Advanced phase of experimental atherosclerosis of rabbit aorta. Heavy plaques formed at each branching point. Note the typical shape of plaques in advanced sclerosis. Flow direction from left to right as indicated by arrow. x5.

CORONARY BLOOD FLOW AS A DETERMINANT OF CARDIAC PERFORMANCE

Marvin Bacaner

The manner and extent to which the coronary circulation affects myocardial contractility and diastolic distensibility is being explored and, within limits, quantitated.

It has been previously reported that heart failure induced by pulmonary artery constriction was fundamentally metabolic, a consequence of decreased coronary blood flow (UCRL-8705, p. 40). Whereas release of the pulmonary artery constriction did not restore cardiac working capacity once failure had been established, marked functional improvement could be consistently obtained by increasing coronary blood flow. This was accomplished by using a simple veno-arterial shunting procedure while pulmonary artery constriction was sustained. [1]

After a period of veno-arterial shunting, some of the animals acquired a capacity to sustain good cardiac function even when the shunt was discontinued and despite the continued presence of the previously disabling pulmonary-artery constriction. This "accommodation phenomenon" was regarded as a metabolic readjustment akin to shifting into metabolic "low gear," and demonstrated a remarkable capacity of the heart to recover after temporary assistance has improved the metabolic milieu.

These data and observations suggest that the coronary circulation, by its influence upon myocardial metabolism, is profoundly and intimately involved in the regulation of the heart's performance. During the course of a few strokes, if not on a stroke-to-stroke basis, the coronary blood flow has been shown to influence the diastolic size and the strength of contraction, as well as adaptive mechanisms to increased work loads. Therefore, a simple "Starling relationship" clearly cannot alone explain the control of in vivo cardiac performance. Indeed, the data tend to contradict what would be predicted from Starling's size-work relationship.

We are presently investigating alternative schemata based on the notion that metabolic processes ultimately determine the length and contractile properties of the myocardial fibrils.

PRODUCTION OF NORMAL-LIVED ERYTHROCYTES WITH ERYTHROPOIETIN

Donald C. Van Dyke and Nathaniel I. Berlin

Erythrocytes produced in rats under the stimulus of human urinary erythropoietin have a normal survival time in the circulation. This and other evidence suggest that erythropoietin administration results in the production of normal erythrocytes. This evidence is compatible with the concept that erythropoietin is part of the normal mechanism controlling erythropoiesis.

(The report of which this is the summary has been submitted under this same title for publication in Proceedings of the Society for Experimental Biology and Medicine.)

EFFECTIVENESS OF HUMAN URINARY ERYTHROPOIETIN IN PRIMATES

Donald C. Van Dyke

In an attempt to determine how much erythropoietin would be required for therapeutic trial in human beings, we have given three daily doses of collodion-adsorbed human urinary erythropoietin intravenously to cynamolgous monkeys over a 2-week period. The total circulating red cell volume before, during, and after treatment was determined by the P^{32}-labeled red cell method. In each case the red cell volume continued to increase for several days beyond the time of the last injection. The highest point reached after each treatment period is presented in Table XV. If one is willing to compare these responses with those obtained in rats after subcutaneous injection followed by determination of blood volume on the 15th day, then the response in terms of dose per kg body weight is similar (Fig. 16). The dose required to produce a 50% increase in total circulating red cells in either rats or monkeys is approximately 8.75 mg/kg of body weight. On this same basis, a 70-kg man would require 656 mg/day (or 4,600 cobalt units*) to produce a 50% increase in red cell volume, or roughly 1/2 g/day of human urinary erythropoietin. Since it takes one month to collect 1 g from one very active patient, then it would take the total collection from one such patient over a 7-month period to obtain enough human urinary erythropoietin to expect to produce a 50% increase in total circulating red cells in a normal human recipient. Since a 25% increase requires only 1/3 that dose in rats, one might be able to produce a significant increase in a normal human subject with 200 mg/day (1,400 cobalt units) for 14 days, or the total output of a single active patient for 2 or 3 months.

*Human urinary erythropoietin used for the published dose-response curves was equivalent to approximately 7 cobalt units per mg.

Polycythemia in Normal Monkeys after 14 Daily Injections of Human Urinary Erythropoietin

Daily dose	*Body weight*	*Increase in total circulating red-cell volume*
90 mg equivalents	6.6 kg	62 %
42	2.0	80
21	2.0	55

MU-19030

Table XV

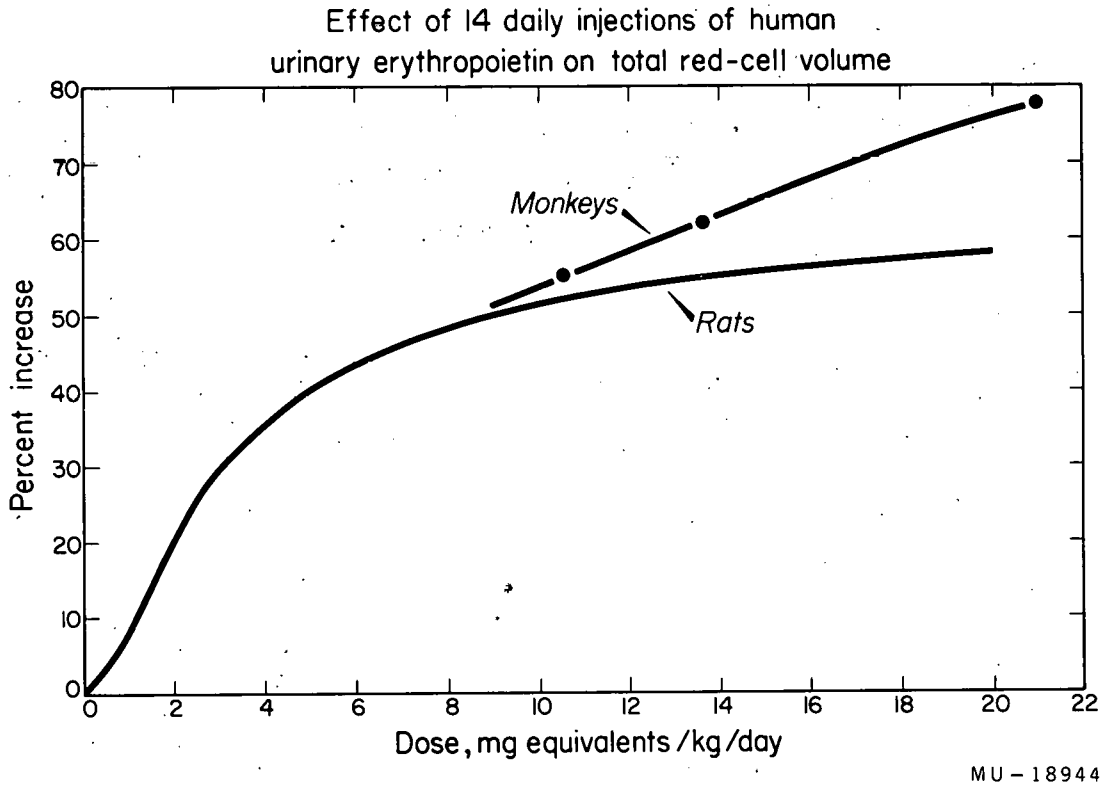


Fig. 16. Effect of 14 daily injections of human urinary erythropoietin on total red-cell volume.

These estimates must be considered to be minimum, since the cynamol monkeys used are more responsive to hypoxia than man. The increase in total circulating red cell volume of one of the same monkeys used in the erythropoietin assay at a simulated altitude of 15,000 feet was 35% greater than expected in man (Fig. 17).

ERYTHROPOIETIC HORMONE

Howard C. Mel

A biophysical study of human urinary erythropoietic hormone is under way, using high-energy alpha-particle bombardment from the 60-inch cyclotron and the heavy-ion linear accelerator. The data taken thus far indicate an exponential relationship between inactivation and dose. Associated electrophoretic and spectrophotometric investigations indicate systematic changes in molecular properties with radiation dose, even at doses at which biological activity is substantially preserved. An estimate of molecular size will be made as a result of this work.

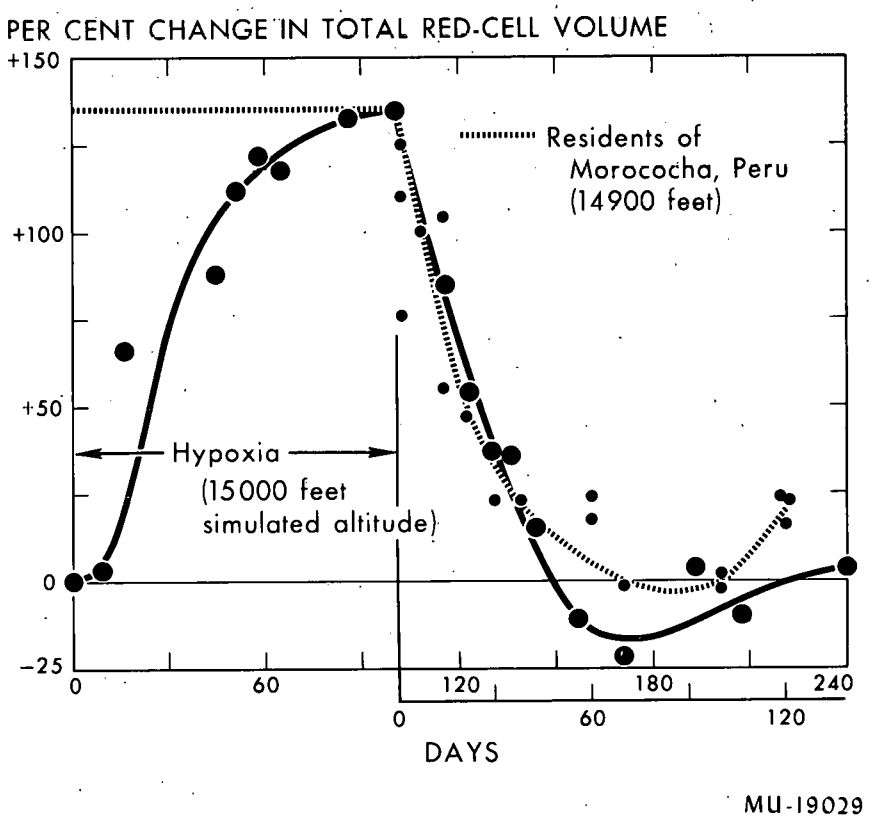


Fig. 17. Red-cell volume changes in a monkey during and following hypoxia.

ISOLATION AND ESTIMATION OF SERUM ORGANICALLY BOUND IODINE

Effects of the Removal of Ions from Serum Proteins

Gilles La Roche and Charles Oakes

In the preparation of serum prior to estimations of protein-bound iodine (PBI), the primary concern is to remove iodide from the protein fraction. Most of the fractionation procedures pay little attention to the serum proteins and involve their denaturation by precipitation or extraction. Several workers have used ion-exchange resins in estimating PBI^{131}, and we have adapted this method to isolate serum proteins and obtain routine values for protein-bound iodine-127 (PBI^{127}).

In this study, care has been taken to prevent the denaturation of serum proteins, and evidence is furnished to indicate possible effects. The dual object of preventing denaturation was to insure that none of the organically bound iodine was lost and that further concentration or fractionation of iodinated products could be performed without the production of artifacts. With this in mind two methods appeared particularly suitable: one by passing serum over an anion-exchange resin and the other by dialysis against double-distilled water. After either passage over resin or dialysis, and after restoration of the ionic concentration with 0.85% NaCl, the various serum preparations were tested for possible protein denaturation by paper electrophoresis and antigen-antibody reaction. The results were compared with those obtained with fresh serum. In addition, the effectiveness of iodide removal was tested by microdeterminations of iodine.

A Clinical Application for the Determination of Protein-Bound Iodine

Gilles La Roche, Ann Coxworth, and Dorothy Carpenter

The diagnostic use of iodine-131 has become very popular in recent years. Radioactive iodine uptake and PBI^{131} levels in plasma are easy to determine and can in many cases give an indication of thyroid activity. The speed and facility with which these tests can be carried out tends to lead some investigators to use them in combination with, or in preference to, the chemical determination of protein-bound iodine (PBI).

However, certain observations indicate that the use of I^{131} may give a less accurate diagnosis of thyroid condition than do the chemical methods. For example, Silver, Yohalem, and Newburger found that in a group of 113 euthyroid patients, 33 had elevated I^{131} uptakes and normal PBI^{131} levels, 51 had normal uptakes but elevated PBI^{131} levels, and 29 had both high uptakes and high PBI^{131}.[1] Of these two latter groups, the great majority of the patients had been previously cured of hyperthyroidism.

Another study, by Werner and Block, showed marked discrepancies between the distribution of iodine in human serum when estimated by I^{131} and by I^{127}.[2] They determined the distribution of both the radioactive and stable serum iodine on the same paper chromatogram. It was found that whereas the I^{131} was present either organically bound or as iodide (even 10 days after labeling), the I^{127} measurements showed that the circulating iodine was organically bound, with virtually none existing as free iodide.

Such studies indicate that the chemical determination of PBI is still a useful diagnostic procedure. In view of this and in the hope of being able to fractionate the iodinated products of the plasma without denaturing the circulating protein, we devised a new method.

This procedure, which expedites the entire determination, combines an ion-exchange fractionation (used for PBI^{131} estimates) with the simplified microiodine method.[3] Thus, the serum is passed through an anion-exchange resin to remove the iodide and the chemical determination is carried out on the effluent. In order to verify the dependability of this procedure, the values obtained were correlated by PBI estimates that were carried out through the well-known precipitation by addition of 5% trichloroacetic acid.

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[1] Sidney C. Werner, and Richard J. Block, Discrepancy Between the Distribution of Iodine in Human Serum When Estimated by Iodine-131 and Iodine-127, Nature 183, 406.

[2] Solomon Silver, Stephen B. Yohalem, and Robert A. Newburger, Pitfalls in Diagnostic Use of Radioactive Iodine, J. Am. Med. Assoc. 159, 1 (1955).

[3] Oscar Bodansky, Richard S. Benua, and Gina Pennacchia, A Rapid Procedure for Determination of Total and Protein-Bound Iodine in Serum, Am. J. Clin. Path. 30, 375 (1958).

THE EFFECT OF PARATHORMONE ON CITRATE PRODUCTION BY THE ISOLATED PERFUSED HIND LIMB OF THE RAT

John C. Schooley and Efraim Otero

An understanding of the various factors that regulate calcification and control the movements of calcium between the body fluids and bone may ultimately lead to a rational basis for removing radioactive bone-seeking elements from the body. The present experiments are concerned with the role of citric acid in bone physiology and its relationship to calcium metabolism.

In recent years there has been considerable interest in the possible role of citrate in the action of parathyroid hormone. The direct effect of the hormone upon bone, parallel to its well-known phosphaturetic action, has been established by several investigators.[1-3] Because of the difficulty in explaining transfer of Ca from bone to an already supersaturated serum[4,5] in terms of a simple CaxP solubility product, and also because of the complex physicochemical structure of the bone crystals, the necessity of an "active solubilizer" for bone calcium has been postulated by several authors.[6-8]

Citrate has been implicated as the solubilizer by the following experimental and clinical findings: (a) citrate increases the solubility of hydroxy-apatite and other poorly soluble Ca salts, presumably through the formation of an undissociated Ca-citrate complex; (b) most of the body citrate exists in bone and cannot be eluted from it by water;[9] (c) vitamin D increases serum and urine citrate levels in rachitic rats and infants.[10] In hypoparathyroidism, reduced concentrations of serum citrate are found together with the hypocalcemia; on the other hand, hypercitricemia associated with hyperparathyroidism has been often described. Parathyroid extract administered to rats increases serum citrate levels, particularly in parathyroidectomized animals.[11]

Experiments in the intact dog by Neuman's group have shown increased levels of citrate in the spongiosal blood flowing from a hole drilled in the femoral epiphysis, soon after the injection of 100 to 1000 units of parathyroid extract into the animal.[12] These authors have discussed the difficulty of directly testing the hypothesis of increased production of citrate by the cellular osseous elements after parathyroid administration.

[1] N. A. Barnicot, J. Anat. 82, 233 (1958).
[2] H. Y. Chang, Anat. Record 111, 23 (1951).
[3] P. J. Gaillard, Acta Physiol. et Pharmacol. Neerl. 7, 142 (1958).
[4] G. J. Levinskas and W. F. Neuman, J. Phys. Chem. 59, 164 (1955).
[5] Strates, Neuman, and Levinskas, J. Phys. Chem. 61, 279 (1957).
[6] F. C. McLean, J. Periodontol. 25, 176 (1954).
[7] H. Harrison, Am. J. Med. 20, 1 (1956).
[8] W. F. Neuman and M. W. Neuman, The Chemical Dynamics of Bone Mineral (University of Chicago Press, Chicago, 1958).
[9] F. Dickens, Biochem. J. 35, 1011 (1941).
[10] H. Harrison and H. C. Harrison, Yale J. Biol. and Med. 24, 273 (1952).
[11] M. L'Heureux and G. Roth, Proc. Soc. Exptl. Biol. Med. 84, 7 (1953).
[12] Firschein, Neuman, Martin, and Mulryan, Recent Progress in Hormone Research 15, 427 (1959).

We have studied the production of citrate by isolated rat bone, using the hind-limb perfusion technique. Female rats, weighing 200 to 300 g, have been used. The limb was perfused, with blood pooled from donor animals, by means of a pump-oxygenator system. Blood samples were taken before and at intervals after the addition of 100 units of parathryoid extract (Lilly) to the perfusing blood. Citrate was determined for each sample,[13] as well as P and Ca. At the end of the perfusion the citrate content of the bone was determined, with the bone from the nonperfused limb used as a control. Initial experiments had shown that main concentrations of citrate in the limb were to be found in blood and bone, the muscle content being almost negligible.

Four hind-limb perfusions using parathyroid extract have been performed as well as two controls. The limbs perfused with parathyroid extract have shown moderate increase in the venous blood citrate levels during the first hour after the addition of the hormone. Phosphorus levels have also been shown to increase. When no parathyroid extract was administered (control experiments) either no increase or a slight decrease in venous blood citrate was observed. No detectable differences in the levels of bone citrate were observed between the perfused and the nonperfused limbs. These findings would favor the hypothesis of an active cellular production of citrate by bone as a major effect of the parathyroid hormone, and are in agreement with Neuman's observations in the intact dog.

Experiments are contemplated to determine the effects of citrate production, in the perfused isolated hind limb, of (a) various doses of parathyroid extract, (b) formol-inactivated parathryoid extract, which lacks calcium-mobilizing activity, and (c) vitamin D. In addition, the discrimination between radioactive calcium and strontium as well as the clearance of these isotopes by the perfused bone will be determined.

[13] W. Hess and A. White, J. Dental Research 34, 462 (1955).

TECHNIQUES AND EQUIPMENT FOR MEASUREMENT AND ANALYSIS

WHOLE-BODY HUMAN COUNTER

Thornton Sargent III and Howard G. Parker

Construction of temporary space for the whole-body human counter in Donner Laboratory is well under way and will be completed soon. Part of this space will be available for other counting requiring low background. It is expected that this location, an excavation on the first floor of the new wing of Donner, will give better protection from the beam of the 60-inch cyclotron than previously available in other locations within Donner.

The 100-channel analyzer with its associated amplifier, data-readout equipment, and small NaI(Tl) crystal have been in operation for some time. Small temporary shields of lead bricks have been used in the study of the spectra of various isotopes, and for evaluating several cases of possible internal radioisotope poisoning.

The first case involved a cyclotron crew, one of whom had become contaminated while dismantling a Pu^{239} target after bombardment. The contaminating activity was found to be fission products between 30 and 90 days old, and the possibility of internal contamination in the exposed personnel was eliminated.

The second accident involved a spill of large amounts of Cm^{244} and Cm^{246}. As expected, the equipment was unable to detect gamma activity from samples with an alpha count of 2×10^4 cpm, because of the extreme rarity of gamma emissions by these isotopes. Hence there was no possibility of detecting activity in humans except in very large amounts. This accident was studied by alpha-counting of urine and fecal samples of the potentially exposed persons.

Two accidents involving possibly contaminated wounds, one with I^{131} and one with natural thorium, were found to involve no internal contamination.

Another accident involved a wound received inside a glove box from a piece of glass believed to contain isotopes of bromine. Although the wound was found to be uncontaminated, the glass was found to contain, in addition to small amounts of Br^{77}, about 1 μC of 127-day Se^{75}. The experiment had involved cyclotron bombardment of arsenic, and the presence of Se^{75} was not anticipated by the experimenter. This incident served to demonstrate the value of the equipment in detection and identification of unknown isotopes, of vital importance in cases of internal contamination.

An analysis of 22-Mev neutron-irradiated materials was performed in connection with evaluation of potential health hazards in the 88-inch cyclotron presently under construction, and is reported elsewhere.

A Russian report of an activity of 1-hour half life induced in an aluminum nose cone fired into the Van Allen belt prompted a study of isotopes produced by proton bombardment of aluminum in the 184-inch cyclotron.

A positron emitter with a half life of 98 minutes was detected in considerable abundance, and was attributed to 112-minute F^{18}. The measured half life was probably shortened by the presence of 20-min C^{11} or 9.4-min Mg^{27}. Also present were 15-hr Na^{24} and 21.2-hr Mg^{28}. All these isotopes have been previously reported from high-energy particle reactions in aluminum, and probably the 1-hour half life reported by the Russians was primarily F^{18}.

Planning and fabrication of the various components of the whole-body counter--such as crystal mount, suspension, the steel room itself, the chair for the subject, etc.--are proceeding with the expectation that all will be completed and ready for assembly when the construction of the required building space is completed.

Completion of this facility will not only mean that it is constantly available in case of accidents involving radionuclide poisoning, but will also permit research projects now awaiting its operation. These include body composition by K^{40}, iron kinetics by Fe^{59}, and ceruloplasmin study with Cu^{64}.

SCINTILLATION AND POSITRON CAMERAS

Hal O. Anger

A new scintillation camera employing a 6-inch-diameter crystal is nearing completion. It will be used primarily for obtaining gamma-ray pictures of the thyroid gland after the ingestion of iodine-131. The larger crystal should result in clearer pictures for a given dosage of I^{131}, or pictures of the same clarity for a smaller dose.

A time-lapse motion picture camera has been attached to the scintillation and positron cameras to permit time-lapse studies of the distribution of isotopes in vivo. This will permit continuous studies of the metabolism of various compounds in organs such as the liver, brain, thryoid, etc. Also, it may aid in the identification of liver abscesses as well as tumors of the brain and liver, to mention only a few potential uses.

Various positron-emitting isotopes will be produced at the 60-inch cyclotron to demonstrate clinical uses of the positron camera. Iodine-124 will be produced and incorporated into rose bengal to obtain better pictures of the liver. Fluorine-18 will also be produced, and its possible use for tagging compounds of biological and clinical interest will be investigated.

FREE-FLOW ELECTROPHORESIS

Howard C. Mel

Electrophoretic migration characteristics necessary for rapid continuous concentration and separation of protein and cellular samples have been demonstrated and some preliminary electrophoretic fractionation made. The apparatus used incorporates horizontal free-boundary flow streams with a vertical potential gradient. Stability is provided mechanically, by laminar flow by density gradients, and by a hydrodynamically unified collection system (see J. Chem. Phys. 31, 559 (1959)). Enzymatic activity and viability have been shown to be preserved during the processing. A start has been made on defining the permissible operating regions as a function of the numerous variables involved--i. e., field strength, current density, flow rate, density gradient, sample concentration, etc. A new version of the apparatus permitted operation with dry samples having free boundaries more than twice as long as those used previously, with correspondingly increased capacity. Investigation and experimentation continue both on basic design characteristics of the apparatus and on further possibilities for biological separations.

INFRARED SPECTROMETRY AND LIPID CHEMISTRY

N. Keith Freeman and Gary J. Nelson

Further procedural details of the infrared micromethod for serum lipid analysis have been worked out, including a modification of the extraction method. It is now possible to make the spectrophotometric measurements by using the total lipid extract from 0.05 ml of serum. By calculation from the infrared spectrum, values are obtained for total lipids, total cholesterol, total phospholipids, and total esterified fatty acids. When results obtained from the micromethod are compared with analyses on 1.0-ml serum samples, they are found to be 10 to 20% higher in certain components. These discrepancies are believed to arise from calibration differences between the two spectrometers used for macro and micro analysis. Recalibration is being undertaken.

The chromatographic and infrared methods developed for the analysis of serum phospholipids have been applied to lipoprotein fractions isolated from serum by ultracentrifugal techniques. In five serum samples, determinations of lecithin, cephalins, and sphingomyelin have been made for each of the major lipoprotein classes HDL-2, HDL-3, S_f0-20, and S_f20-400. Some preliminary data on the fatty acid compositions of these phospholipids have been obtained by gas chromatography. The detailed results are to be given in a separate report. In general, the significant findings are: (a) The phospholipids of S_f0-20 lipoproteins contain the highest proportion of sphingomyelin (20 to 25%); the high-density lipoproteins (HDL) contain the least (10 to 15%). (b) There are significant differences in the fatty acid composition for the three phospholipid types, but in a given serum the composition for each particular type is similar for all three lipoprotein classes. These studies complement work in the general field of fat metabolism. The observed differences in fatty acid composition of the phospholipide classes may allow further work utilizing C^{14}-labeled or tritiated fatty acids.

GAS-PHASE CHROMATOGRAPHY EMPLOYING A STRONTIUM-90 RADIATION DETECTOR

Frank Upham, Alex Nichols, and Frank Lindgren

During the last few years gas-phase chromatography has been applied successfully to the analysis of biologically occurring long-chain fatty acids. One of the limitations of the early gas-phase chromatographic systems was the lack of adequate sensitivity. Frequently in the study of serum fatty acids only small quantities of fatty acids may be available, especially when the serum lipids are partitioned into different lipoprotein or chemical fractions.

With the development by Lovelock of the ionization detector system using argon for the carrier gas,[1] sufficient sensitivity became available for analysis of extremely small fatty acid methyl ester samples. Our apparatus, in operation since March 1959, utilizes a similar detection system but incorporates a modification in the design of the chamber. A stainless steel dowel 3/16 in. in diameter is our positive electrode. It is positioned so that the hemispherical tip is centered approximately 3 mm beneath the chamber inlet opening. This change in geometry effects a considerable reduction in the size of the detection volume of the chamber and also allows a lower operating voltage between the chamber wall and the probe. Detection of the chromatographed methyl ester component as it enters the detection chamber is as follows:

Metastable argon atoms result from the interaction of argon and the β particles emerging from the Sr^{90} source contained within the chamber. These short-lived metastable argon atoms ionize a small but relatively constant fraction of the methyl ester molecules as they pass through the sensitive volume. This total ionization current, detected and recorded as a function of time, is a measure of the number of moles of fatty acid methyl ester that have passed through the chamber.

Chromatography is achieved with a straight 52-in. glass column, 6 mm in inside diameter, terminating in a drawn capillary tip (of 1 mm i.d.) which just barely projects into the chamber itself. Initially the column contains 30% by weight liquid phase (LAC 2R-728) and 70% (mesh 48-65) chromosorb. Prior to actual usage the column is conditioned (or prebled) within an ancillary thermal chamber for 4 weeks at approximately 195°C; 25% of the liquid phase (by weight) is bled from the column. Such a conditioned column yielded the following calibration data.

The data in Fig. 18 illustrate the degree of linearity of response for the individual components, methyl palmitate and methyl arachidonate, respectively. These data are for operating conditions of 193°C, 80 to 85 mg/min flow rate; methyl palmitate retention time of 7 to 8 min, and column efficiency of approximately 1200 theoretical plates (calculated for methyl palmitate).

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[1] J. E. Lovelock, J. Chrom. 1, 35 (1958).

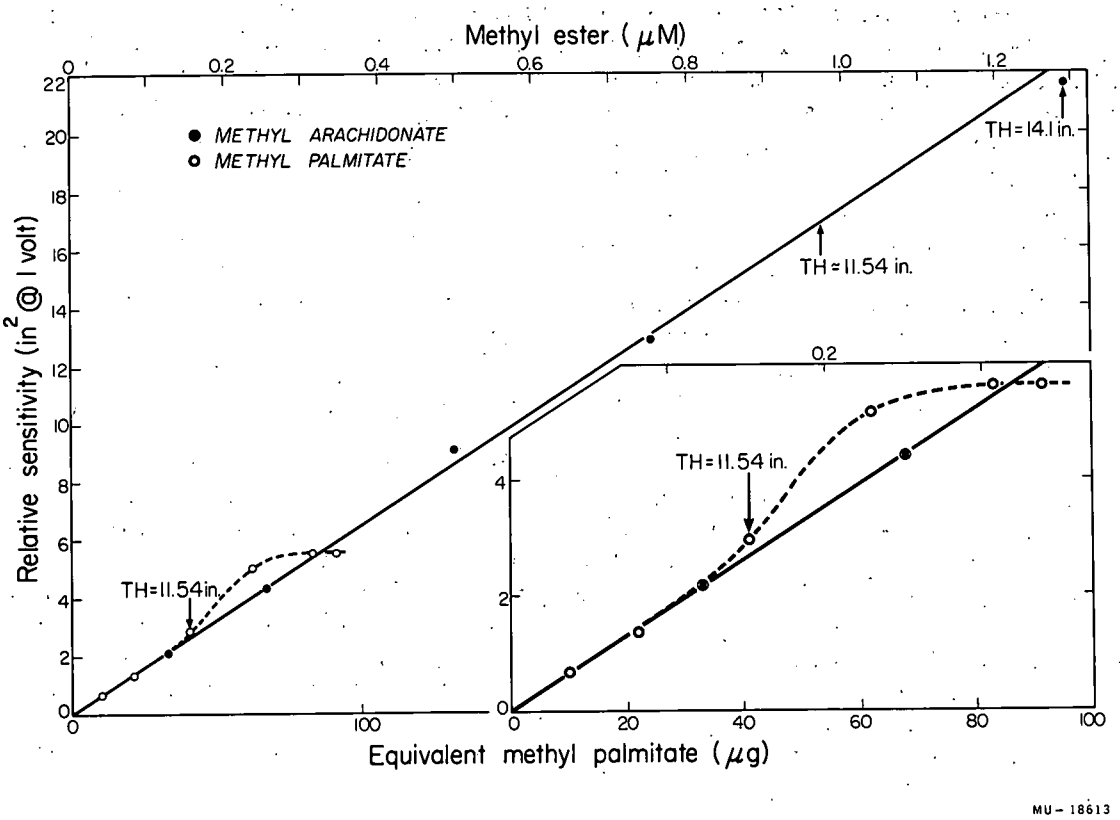

Fig. 18. Response of chromatographie equipment to methyl palmitate and methyl arachidonate at different concentrations.

Each methyl ester studied has its own characteristic range of linearity of response. Departures from linearity appeared whenever ionization currents in the detector rose above 1.0×10^{-8} amp. For methyl palmitate the response curve was found to depart from linearity at sample injections above 30μg. However, for methyl arachidonate departures from linearity were only above 250 μg. Because of the much longer elution time of methyl arachidonate and its subsequent diffusion, an equivalent maximum concentration for detection in the chamber (expressed as a triangle height) for methyl arachidonate occurs at approximately 250 μg, whereas a similar peak for concentration in the chamber occurs at only 30 μg for methyl palmitate. For mixtures of methyl esters, as found in various serum lipid fractions, a range of sample mass of from 1 to 200 micrograms allows complete methyl ester analysis.

The optimum detector properties are affected markedly by its surface properties and the geometry of the collecting electrode. Therefore, the characteristics of each detector--including those with only small differences in design--are different, as has been pointed out by Farquhar.[2]

The great detection sensitivity of this device as well as its sensitivity to flow rate requires that there be maximum stability in the carrier gas flow rate. Gas leaks, especially in the inlet gas system, are particularly detrimental, resulting in harmful base-line drifts and sporadic fluctuations. The inlet system, which includes the injection assembly, must be gastight. Our injection assembly (see Fig. 19) consists of a firmly compressed 1/16-in.-thick silicone rubber disk through which a hypodermic needle may be inserted and removed without gas leakage. Thus, aside from the disturbance in flow rate resulting from the injected sample itself (and its sudden vaporization) there is minimal disturbance of the chromatographic analysis so far as the inlet gas system is involved.

Table XVI gives typical results obtained on a sample containing a mixture of seven methyl esters. Results covering the sample range of 1-100μg reveal adequate accuracy for normal fatty acid methyl ester analysis. In the calculations yielding the above data, small correction factors based both on elution time and on triangle heights have been applied.

It is expected that this instrument will allow full investigation of the fatty acid content of various serum lipid and lipoprotein fractions obtained from persons in states of health and disease. Such studies may include disturbed metabolic states such as those resulting from radiation exposure.

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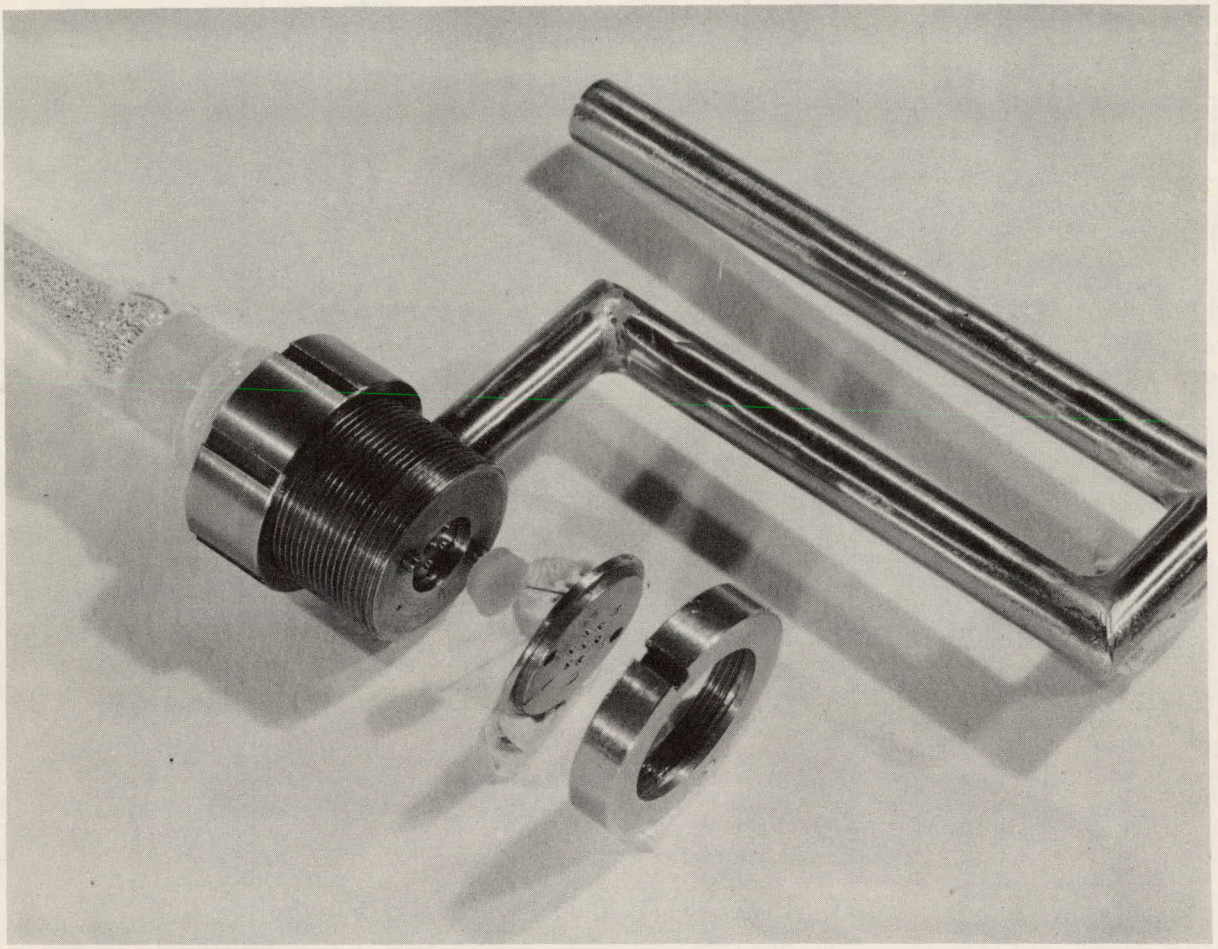
[2] John W. Farquhar, William Insule, Jr., Paul Rosen, Wilhelm Stoffel, and Edward H. Ahrens, Jr., Nutr. Rev. 12, 1 (1959).



ZN-2318

Fig. 19. Injection assembly for chromatography apparatus.

Table XVI

Calibration of chromatographic apparatus with six methyl ester components

All compositions expressed as mole % of total mixture analyzed. The methyl esters were obtained from the Hormel Institute and each component was corrected for its impurities.

	Total mass of sample (μg)						
	1	10	20	50	100	Average	Actual composition of methyl ester mixture
Volume of injection (ml) (in normal hexane)	0.0025	0.01	0.02	0.005	0.01	-	-
16:0 (methyl palmitate)	24^{1}_{-}	26^{0}_{-}	26^{3}_{-}	26^{9}_{-}	26^{4}_{-}	25^{9}_{-}	26^{9}_{-}
18:0 (methyl stearate)	16^{9}_{-}	16^{0}_{-}	16^{4}_{-}	16^{5}_{-}	16^{8}_{-}	16^{5}_{-}	16^{5}_{-}
18:1 (methyl oleate)	21^{2}_{-}	20^{1}_{-}	20^{7}_{-}	20^{9}_{-}	21^{9}_{-}	21^{0}_{-}	20^{4}_{-}
18:2 (methyl linoleate)	18^{4}_{-}	16^{6}_{-}	16^{2}_{-}	16^{2}_{-}	16^{1}_{-}	16^{7}_{-}	17^{3}_{-}
18:3 (methyl linoleate)	5^{2}_{-}	4^{2}_{-}	4^{7}_{-}	5^{2}_{-}	4^{9}_{-}	4^{8}_{-}	4^{7}_{-}
20:4 (methyl arachidonate)	14^{1}_{-}	17^{2}_{-}	15^{8}_{-}	14^{2}_{-}	14^{0}_{-}	15^{1}_{-}	14^{2}_{-}

UCRL-8988

IN VIVO ESTIMATION OF BLOOD FLOW TO THE GASTROINTESTINAL TRACT)

Marvin Bacaner and Myron Pollycove

The lack of a method for measuring blood flow to the gastrointestinal (GI) tract leaves a void in understanding the physiological consequences of normal circulatory function and pathological disturbances of circulation. A simple technique has been devised to assess circulation in accessible areas of the GI tract (colon, rectum, esophagus, and stomach).

A suitably designed Geiger-Müller probe is inserted into the organ. After intravenous injection of a pure beta-emitting radioisotope, its concentration in the region of the probe is recorded. The short range of the beta particle virtually restricts the monitored field to the surrounding gut. To aid interpretation of the curves obtained from the gut probe, cardiac output and liver blood flow are simultaneously determined. The derived curves reveal several aspects of gut circulation: (a) circulation and transit times; (b) magnitude of existing blood pool and turnover of blood in the organ.

It is possible to characterize circulatory patterns (curve shapes) for the dog colon under normal conditions and during procedures which decrease or increase circulation: (a) vascular occlusion; (b) hemorrhage; (c) saline infusion; (d) autonomic stimulation and autonomic drugs. This technique should be useful in studying normal GI physiology, vascular disease of the gut, and vascular effects of autonomic nerve stimulation and drug administration.

RADIOLOGICAL CHEMISTRY

Warren M. Garrison in charge

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

The term "radiological chemistry" is used here to designate the study of the chemical action of ionizing radiation upon matter in general and upon systems of biological interest in particular. Heretofore, this section of the Biology and Medicine Report has appeared under the subheading "Radiation Chemistry". Briefly, our work involves (a) the study of indirect and direct actions of radiation on chemical bonds and groups in the simpler organic molecules, and (b) the parallel study of the corresponding reactions as they occur in more complex biological systems.

RADIATION-INDUCED OXIDATION OF AQUEOUS GLYCINE ANHYDRIDE–OXYGEN SOLUTION

The diketopiperazines are among the simplest chemical models that can be employed in the study of radiation-induced reaction involving the peptide bond. On the basis of work published some years ago by Dale,[1] it has been generally assumed that the peptide bond in diketopiperazines, protein, etc., is relatively resistant to the indirect action of radiation in aqueous solution. Recently, however, we have reported studies[2,3] consistent with the idea that (a) the peptide bond is an important locus of indirect action in the radiolysis of aqueous protein systems, and (b) the net reaction for cleavage of the peptide C-N bond in oxygenated solution can be represented in terms of the formation of amide and carbonyl groups according to

$$\sim CO\text{-}HN\text{-}CH(R)\sim + O_2 + H_2O \longrightarrow \sim CONH_2 + RCO\sim + H_2O_2 . \quad (1)$$

It was decided, therefore, that the radiation chemistry of the diketopiperazines in aqueous solution should be re-examined from this standpoint. Data on glycine anhydride, the simplest derivative, are presented here.

Glycine anhydride (Nutritional Biochemicals, Lot No. 9994) was recrystallized twice from water to obtain a product containing less than $\sim 10^{-3}$ mole percent "free" glycine as determined by the ninhydrin method. The irradiations were made with Co^{60} γ rays on 10-ml volumes of oxygenated 0.1 M glycine anhydride solution. A standard dose of 1.1×10^{20} ev/ml was used in all exposures. Immediately after irradiation an aliquot was made alkaline to phenolphthalein and distilled to dryness in vacuo. "Free" ammonia in the distillate was determined by the Nessler procedure. A second aliquot was made 1 N in hydrochloric acid and hydrolyzed in vacuo for 60 minutes. The hydrolyzate was then analyzed (a) for glyoxylic acid (and formaldehyde) by use of 2,4-dinitrophenylhydrazine, and (b) for "total" ammonia by use of Nessler's reagent as above. Analytical procedures were those developed in earlier studies.[2-4] Appropriate control and blank runs were made. The following 100-ev yields were obtained: $G(NH_3) = 2.1$, $G(CH_3CONH_2) = 3.0$, $G(CHOCOOH) = 0.43$, $G(CH_2O) = 0.06$.

Yield data obtained at pH 5 and pH 1 agreed within the over-all experimental error, which was less than ± 5%.

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[1] Dale, Davies, and Gilbert, The Kinetics and Specificities of Deamination of Organic Compounds, Biochem. J. 45, 93 (1949).

[2] M. E. Jayko and W. M. Garrison, Formation of)C = 0 Bonds in the Radiation-Induced Oxidation of Protein in Aqueous Solution, Nature 181, 413 (1958).

[3] W. Bennett and W. M. Garrison, Production of Amide Groups and Ammonia in the Radiolysis of Aqueous Solutions of Protein, Nature 183, 889 (1959).

[4] Warren M. Garrison and others, in Biology and Medicine Semiannual Report, UCRL-8705, May 1959, pp. 14-21.

These results are in general agreement with our earlier observations. The simplest mechanism proposed for cleavage of the C-N bond by indirect action in oxygenated solution involves the intermediate production of the imino derivatives,

$$\sim CO\text{-}NH\text{-}CH_2 \sim + OH \longrightarrow \sim CO\text{-}NH\text{-}CH \sim + H_2O, \quad (2)$$

$$\sim CO\text{-}NH\text{-}CH \sim + O_2 \longrightarrow \sim CO\text{-}N{=}CH \sim + HO_2 . \quad (3)$$

Hydrolysis of -CO-N=CH- yields the observed products. It has been emphasized, however, that this sequence merely represents the simplest path, and that equivalent reaction schemes involving the formation of organic peroxy radicals in Step (3) could be formulated.[5] The data reported above for oxygenated glycine anhydride solutions indicate that the latter mechanism may predominate. If amide and glyoxylic acid were formed only through hydrolysis of the imino intermediate, they would appear in about equal yield. The fact that the amide yield is found to be so much greater than the yield of glyoxylic acid (both at pH 1 and pH 5) suggests that organic peroxides are involved, leading to reactions of the type

$$\sim NH\text{-}\underset{\displaystyle OOH}{\underset{|}{CH}} \sim \longrightarrow \sim NH_2 + COOH .$$

Experiments now in progress will be extended to provide additional information on the role of peroxides in the irradiation of oxygenated diketopiperazine solutions.

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[5] W. M. Garrison, Indirect Action of Ionizing Radiation on Some Organic Compounds Containing the C-N Bond, UCRL-8719, April 1959.

RADIATION-INDUCED OXIDATION OF AQUEOUS PROTEIN-OXYGEN SYSTEMS

It is now well established that introduction of $>C=O$ bonds represents a principal chemical modification of protein molecules that have been subjected to the indirect action of radiation in oxygenated solution. Current studies are being directed primarily to problems related to (a) the identification of specific reaction sites that give rise to $>C=O$ bonds, and (b) the measurement of the relative importance of these reaction sites under various experimental conditions. The experimental approach to this phase of the study is based on detailed analytical studies of the various product fractions that are liberated on complete hydrolysis of the modified protein.

Although the peptide bond has been identified as one reaction site, there has remained the question of whether or not other sites are preferentially attacked before the peptide bond becomes involved. Experiments just concluded indicate that the peptide bond must be included among the loci of initial reaction, at least in the gelatin system. Yield data for "free" ammonia and amide groups from γ-irradiated gelatin solutions are shown in Fig. 20 as a function of dose over the range 5×10^{18} to 2×10^{19} ev/ml. Figure 21 shows corresponding data for α-keto glutaric acid, the most easily determined component of the α-keto acid product fraction. Experimental methods employed in obtaining the data of Figs. 20 and 21 were those described in earlier reports. It is seen that (a) formation of the indicated products is strictly proportional to dose, and (b) the extrapolated curve passes through the origin. The first experimental point on each curve corresponds to the formation from water of one OH radical per protein molecule in solution. Data leading to a conclusion similar to the above have been obtained with β-lactoglobulin which has a single N- terminal amino acid, viz. leucine. Chromatographs of the α-keto acid products isolated from the hydrolyzate as the 2, 4-dinitrophenylhydrazine derivatives show essentially identical product spectra over the same dose range. No preferential oxidation of leucine to α-keto isocaproic acid could be detected.

Considerable attention has been given to various analytical problems involved in the identification and quantitative determination of carbonyl products. As mentioned elsewhere, determination of α-keto acid products as the corresponding 2, 4-dinitrophenylhydrazones has a number of limitations. A principal difficulty arises from the fact that each α-keto acid hydrazone derivative can appear on the chromatogram in both the cis and trans forms.[6] These forms have different R_f values, and may vary in relative amount depending on methods of hydrazone preparation, etc. Hence, the complete separation and quantitative determination of a mixture of α-keto acid hydrazones is accomplished with some difficulty. We have found, however, that most of the analytical problems can be eliminated by application of ion-exchange chromatography. An additional advantage of the modified procedure is that it also

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

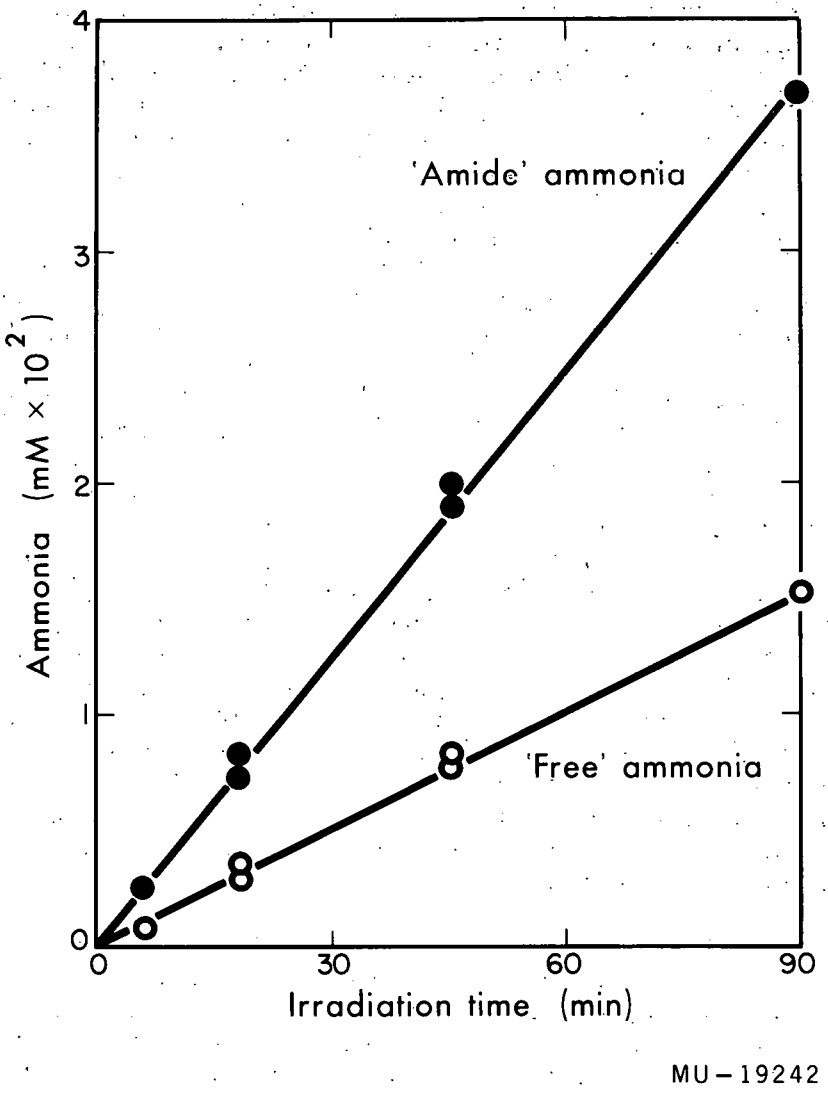


Fig. 20. Production of "amide" ammonia and "free" ammonia as a function of dosage.

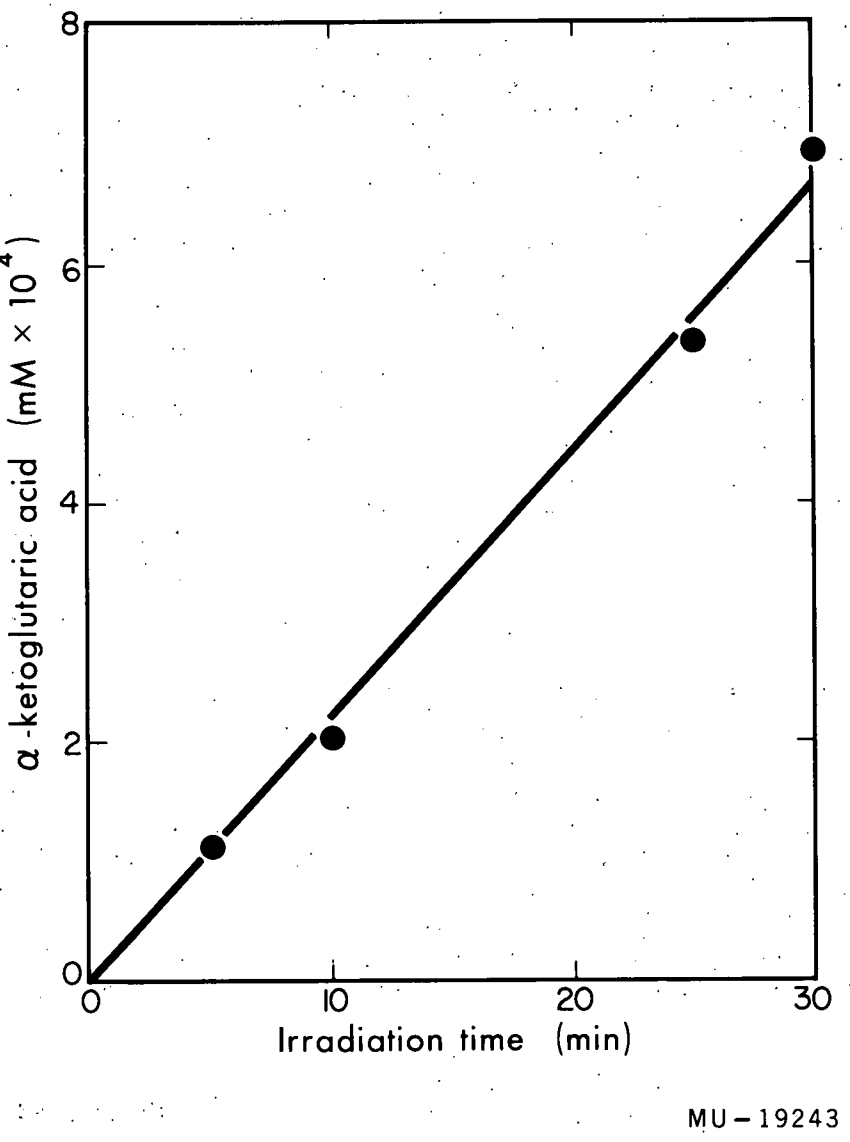


Fig. 21. Production of α-keto glutaric acid as a function of dosage.

provides a rather straightforward method for the determination of other carbonyl products. In brief, this procedure is as follows: The protein hydrolyzate after neutralization is added to the top of a Dowex-50 ion-exchange column in the acid form. The α-keto acids, together with other nitrogen-free products present in the hydrolyzate, are then washed through with a small volume of water (Fraction I). The nitrogenous material (Fraction II) remains on the column. Fraction I is treated with 2, 4-dinitrophenylhydrazine reagent, and the resultant hydrazone mixture is hydrogenated to form the corresponding amino acids. These are then chromatographed on a second column and assayed by use of the ninhydrin procedure. Carbonyl-containing products present in Fraction II have not yet been studied in any detail.

It is to be emphasized that our reference to amide and carbonyl groups as products of petide-bond cleavage are based on chemical analysis made after conventional acid hydrolysis of the irradiated (protein) system. It is possible, of course, that the initial products of radiation-induced oxidation at the peptide bond (e. g., the imino and (or) peroxy derivatives) remain as such in the irradiated solution for some time if hydrolysis is not intentionally brought about. The question of molecular weight changes in irradiated protein solutions will be treated in later reports from this viewpoint.

RADICAL-COMBINATION REACTIONS IN THE RADIOLYSIS OF AQUEOUS PROTEIN SYSTEMS

Previous work at this Laboratory has shown that irradiation of oxygen-free protein solutions containing added C^{14}-labeled solutes results in a chemical incorporation of C^{14} activity into the protein moiety.[4] With added C^{14}-labeled acetic acid, incorporation occurs via radical-combination reaction involving $CH_2C^{14}OOH$ and radical species derived from the protein molecule. Chemical study of these C^{14}-labeled polypeptides and their hydrolysis products provides a source of detailed information on reactive sites of the parent macromolecule.

In the preceding Semiannual Report[4] we presented some detailed studies of radiation-induced reaction between $CH_3C^{14}OOH$ and the proteins, pepsin, gelatin, and β-lactoglobulin. Nondialyzable C^{14}-labeled products formed by helium-ion irradiation of the oxygen-free solutions were hydrolyzed to mixtures of constituent compounds and then chromatographed on Dowex-50. Each of the proteins gave a series of C^{14}-labeled nitrogen-containing products, and one of the C^{14}-activity peaks of each chromatograph was subsequently shown to contain C^{14}-labeled aspartic acid as a principal component.

The formation of aspartic acid in these systems may be represented as one path of a general type of radical-combination reaction,

$$\underset{\text{I}}{\sim NH\text{-}\dot{C}(R)\text{-}CO\sim} + CH_2C^{14}OOH \longrightarrow \underset{\text{II}}{\overset{CH_2C^{14}OOH}{\overset{|}{NH\text{-}C(R)\text{-}CO\sim}}} ,$$

where I represents radical sites formed by removal of H from C-H bonds

along the peptide chain. Hydrolysis of the modified proteins, II, would then yield C^{14}-labeled aspartic acid if R is H, and a series of α-substituted aspartic acid derivatives in all other cases. The relative yields of these products would be expected to be strongly dependent on steric factors related to protein configuration. Reaction at the glycine and alanine residues would, of course, give the two simplest structures for the reaction site of Type I. We have obtained some information on the relative yields of C^{14}-labeled aspartic and methyl aspartic acids from β-lactoglobulin which contains glycine and alanine residue percentages of 2.35 and 8.45 respectively. Earlier experiments showed that this pair of amino acids (aspartic and methyl aspartic) was not separated by our standard Dowex-50 procedure. A reasonably satisfactory separation has since been achieved by using a Dowex-1 column (acetate form) and acetic acid in progressively increasing concentration (0 to $\sim$1 N) as the eluting agent. Figure 22 shows a separation on Dowex-1 of authentic C^{14}-labeled aspartic acid and titratable amounts of α-methyl aspartic acid. Figure 23 shows a chromatograph of titratable amounts of authentic aspartic and methyl aspartic acids with C^{14} activity that had been isolated as peak A in a preliminary separation on Dowex-50 (See Fig. 25). Although the resolution obtained in Fig. 23 is not complete, it is sufficient to show that the yield of C^{14}-labeled methyl aspartic acid cannot be more than 5% of the yield of C^{14}-labeled aspartic acid even though the ratio of alanine residue to glycine residue in β-lactoglobulin is almost 4. An aliquot of C^{14} activity identified with the methyl aspartic titer peak of Fig. 23 was treated with nitrous acid and co-chromatographed with authentic citramalic acid; the identification was confirmed.

A parallel approach to the study of reaction sites in their protein systems does not necessarily require identification of each individual C^{14}-labeled amino acid derivative. Information on positions of CH_2COOH substitution relative to specific types of bonds can be obtained through application of certain of the conventional reagents of protein and amino acid chemistry. For example, the reactions of nitrous acid, ninhydrin, etc. on C^{14}-labeled products both before and after hydrolysis lead to well-defined chemical reactions that can be followed by measurements of C^{14} activity. Our preliminary experiments along this line are based on the fact that, in reactions with nitrous acid, (a) α-amino acids are deaminated to yield nitrogen and an organic acid derivative, which is usually the corresponding hydroxy acid, and (b) imino acids are either unchanged or, like secondary amines, form unstable N-nitroso compounds from which the imino compound is readily reformed on hydrolysis. A sample of hydrolyzed C^{14}-labeled product from β-lactoglobulin plus added alanine, proline, and imino diacetic acid $[NH(CH_2COOH)_2]$ in titratable amounts was treated with nitrous acid by the method of Hamilton and Ortiz.[7] After addition of ammonium sulfamate to eliminate excess nitrous acid, the reaction mixture was evacuated to dryness, added to a Dowex-50 column, and chromatographed according to our standard procedure. Figure 24 shows an elution curve for the nitrous-acid-treated sample, and Fig. 25 shows the corresponding curve for an equal aliquot of the untreated control hydrolyzate. The titration data of Fig. 24 confirm that the recovery of iminoacetic acid and proline and the removal of alanine were essentially quantitative. Comparison of the counting data of Figs. 24 and 25 gives an estimate of the total C^{14}-activity incorporated into the protein

[7] Paul B. Hamilton and Priscilla J. Ortiz, J. Biol. Chem. 187, 733-742 (1950).

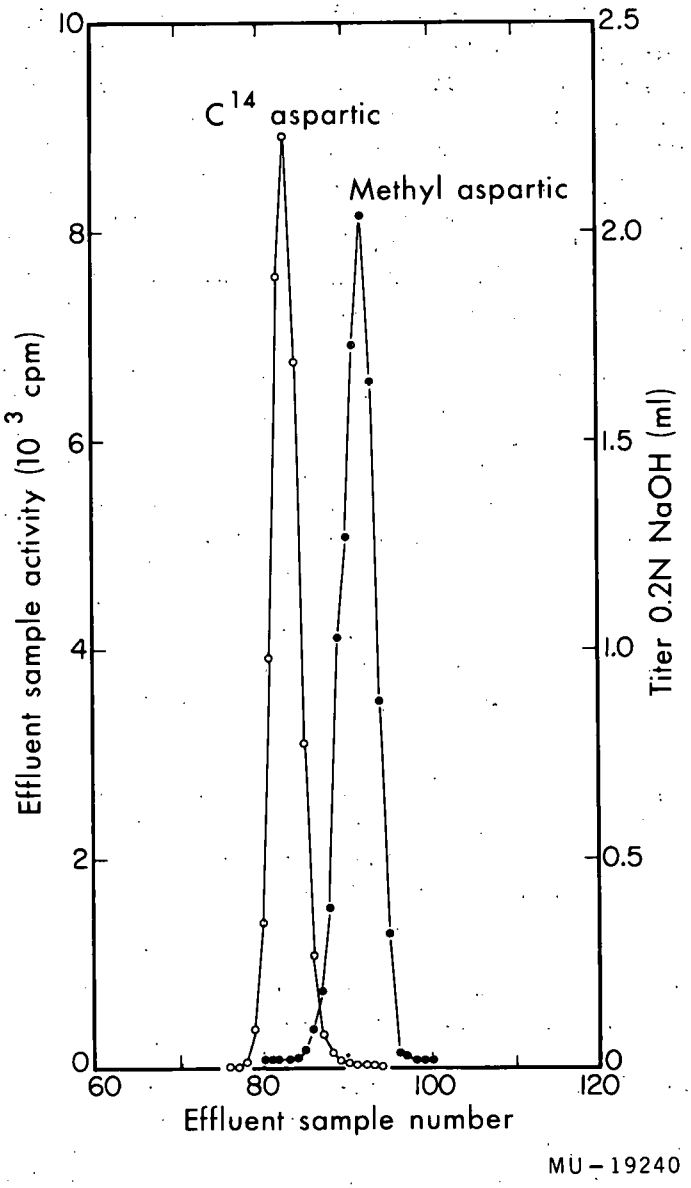


Fig. 22. Elution curve of authentic C^{14}-labeled aspartic acid with α-methyl aspartic.

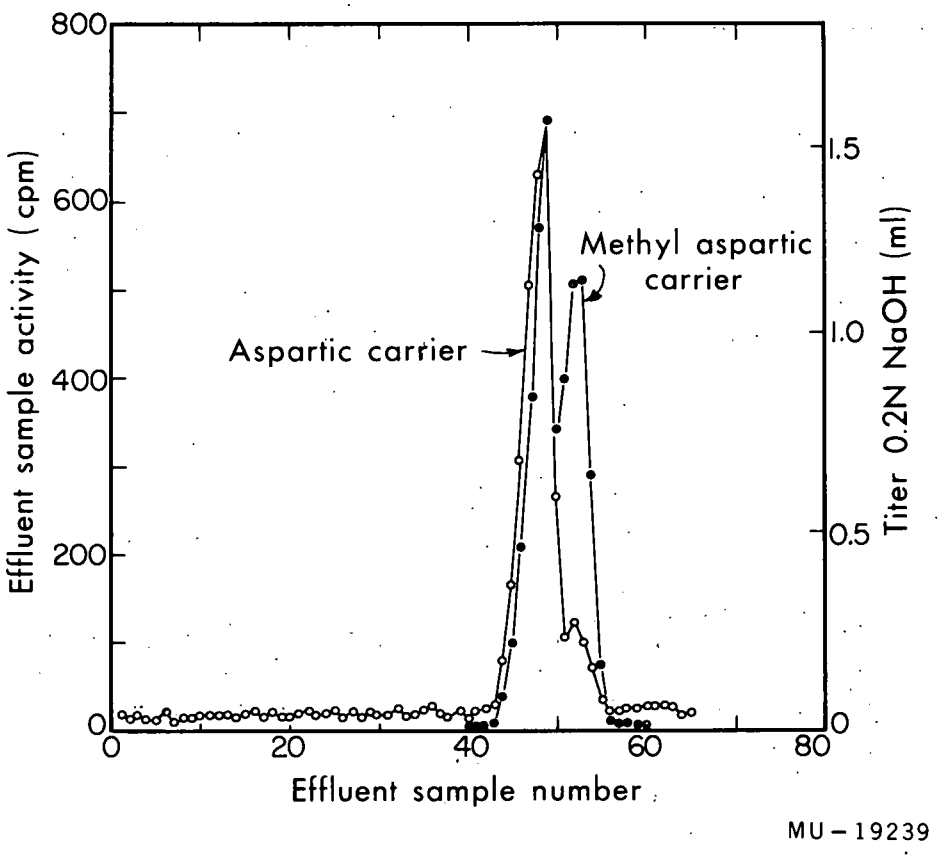


Fig. 23. Elution curve of authentic aspartic and α-methyl aspartic acids with peak A of Fig. 6.

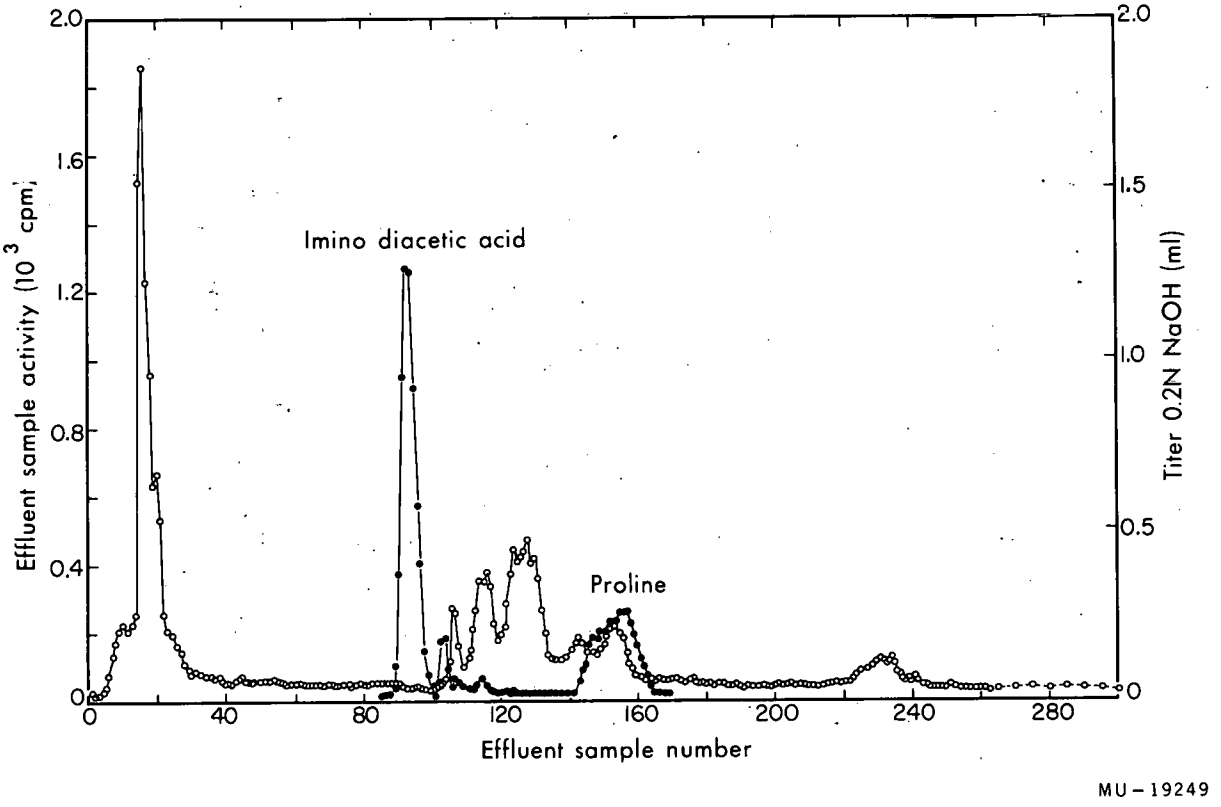


Fig. 24. Elution curve of β-lactoglobulin-CH_2COOH derivatives after nitrous acid treatment.

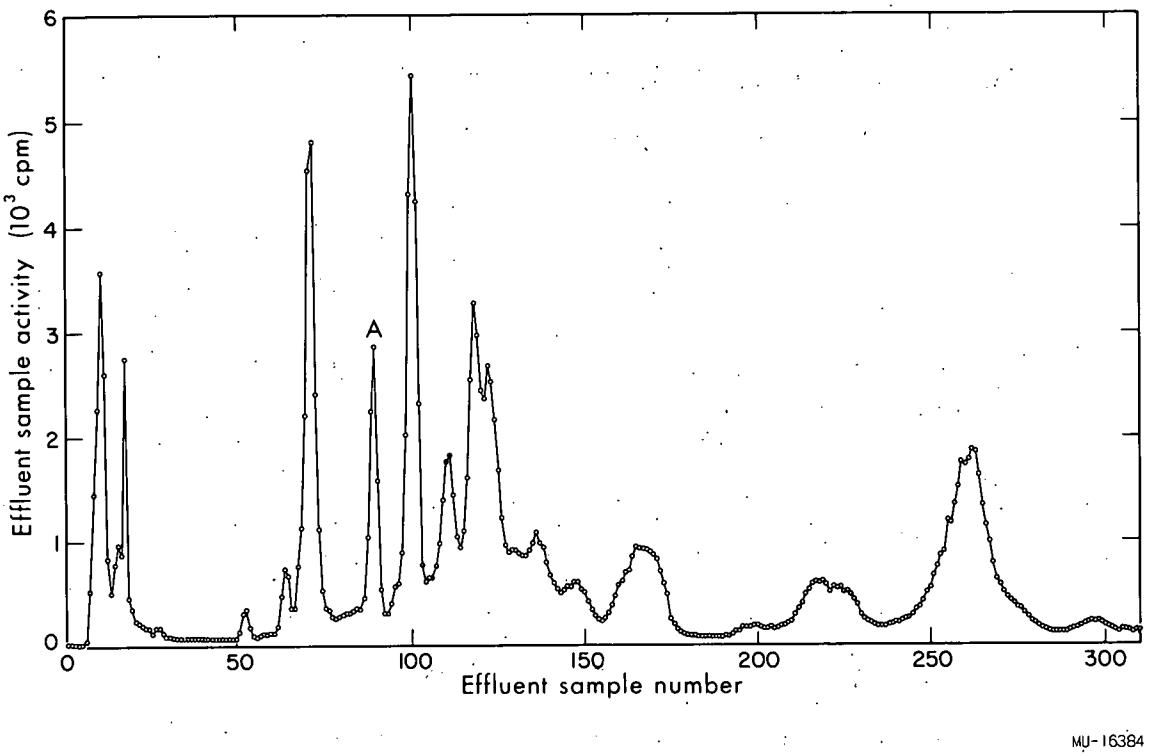
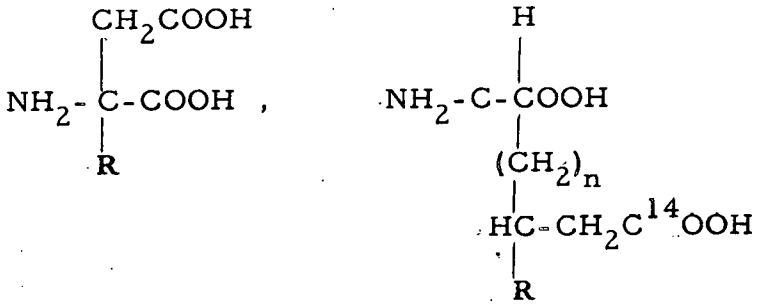


Fig. 25. Elution curve of β-lactoglobulin-CH_2COOH derivatives.

through carbon bonding of $CH_2C^{14}OOH$, either on the peptide bond or on the side chain, to give amino acids of the type

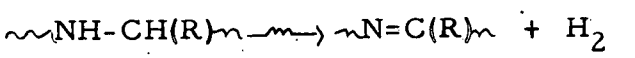


II III

The side chain R may contain a primary amino group but not a secondary or imino-acid-type configuration.

IRRADIATION OF PEPTIDES IN THE SOLID STATE

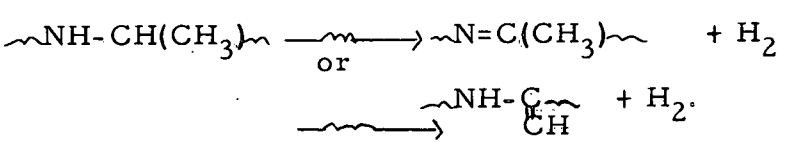
The radiation-induced dehydrogenation reaction



has been discussed[4] as one source of the carbonyl function found to be associated with irradiated protein (solid, evacuated) after dissolution in oxygen-free water. Preliminary studies of the direct action of γ radiation on glycine anhydride as a model peptide have been previously referred to.[4]

Hydrolysis of the irradiated glycine anhydride solid liberates both ammonia and glyoxylic acid, but in low yields: $G(NH_3) \simeq 0.2$, $G(CHOCOOH) \simeq 0.1$. Small amounts of formaldehyde and glyoxal are also present ($G \leq 0.01$). Chromatographic analysis of the higher-molecular-weight products shows that the combined yield of diaminosuccinic acid, aminosuccinic acid, iminoacetic acid, and succinic acid cannot be greater than ~ 0.1. Of particular interest is the finding that water is the single major product. Hydrogen and small amounts of carbon dioxide are also formed.

A general study of these radiation-induced reactions leading to the dehydrogenation and dehydration of peptides is in progress. We are presently engaged in a detailed comparative study of a series of amino acid anhydrides. Preliminary work indicates that side-chain substitution exerts a strong influence on the reaction course. For example, hydrolysis of irradiated alanine anhydride liberates pyruvic acid in amounts corresponding to $G \simeq 0.75$. This is almost ten times the yield of glyoxylic acid from glycine anhydride. It is perhaps significant that with amino acid anhydrides containing side-chain substituents, the dehydrogenation reaction can be written



Hydrolysis of either unsaturated configuration would yield pyruvic acid.

RADIATION DETECTION AND PROTECTION

HEALTH CHEMISTRY

Nelson B. Garden

In line with the continuing philosophy of applying engineered protection rather than the hazard-evaluation approach when dealing with the health and safety aspects of radioisotope work, the following activites were carried out.

Slug Processing

Four slugs irradiated in the MTR were processed for the Cf, Bk, and Fm content. These slugs, originally containing Pu, Am, and Cm, had been packaged here (reported in an earlier semiannual report, UCRL-8261) and the planning, fabrication, assembly, and testing of the equipment to accomplish a safe and satisfactory run to meet the chemists' requirements were carried out by or under the supervision of the Health Chemistry Group, which has been participating in similar runs over the years. As anticipated in the preceding semiannual report, the radioactivity involved the usual curie level of beta-gamma activity from fission products plus the high alpha level (around 10^{13} disintegrations per second), and, in addition, shielding protection had to be combined with remote controls to care for 10^{7} neutrons per second from the spontaneous fission of Cf^{252} and Cm^{244}. In order to gain information toward solution of the last problem through empirical tests, such tests were carried out and have been reported in a paper by H. J. Browne, J. A. Kaufmann, and N. B. Garden, Investigation of Windows and Shields for Neutron Point Sources, UCRL-8770, May 1959.

It has been found that the safest and most convenient method of making an assay during this type of run is to make a neutron determination. A compact, reliable neutron counter, to be assembled within the cave area, necessitated special development and fulfilled the need.

The run was carried out in the 6-in. lead cave and the separated fractions were transferred to various 2-in. lead-shielded boxes. Again the operation was conducted in totally enclosed boxes of low-leak design, employing a recirculating ventilation system. The application of this recirculation technique was extended for this run to include the 2-in. lead-shielded boxes for the first time. No alpha activity was detected in the many off-gas samplers that continuously monitored the operations.

Decontamination at the Hilac

Health Chemistry carried out an extraordinary decontamination job following a high-level spill at the hilac on July 3.

A failure to observe engineered precautions, combined with a human error, produced serious contamination at the heavy-ion linear accelerator. Approximately 10^{11} cpm of Cm^{244} was involved. The incident has been reported in detail elsewhere.[1] The hazard of ingestion by personnel was the primary concern, but no individual received a serious exposure. Most of the equipment was successfuly decontaminated, so that the principal costs

[1] Nelson B. Garden and Carroll Dailey, High-Level Spill at the Hilac, UCRL-8919, Sept. 1959.

were the expense of decontamination work (requiring about 50-man-months) and--most serious--the loss of hilac experimental time.

Enclosures

The Enclosures Group has made a significant contribution in persuading the researchers to recognize and specify the most suitable materials (with respect to corrosion resistance, etc.) for the processing equipment to be installed in glove boxes or other enclosures. This equipment is designed by researchers in collaboration with Health Chemistry personnel, and constructed by Health Chemistry. Health Chemistry is constantly conducting tests on materials and can recommend the best stockroom items, including plastic and coated products, to have on hand for a particular application.

Researchers have developed greater appreciation of the value of eliminating dust and impurities in general through the use of multiple doors, tight boxes, and filtered inlet air. Seventy-five enclosures were issued by the Enclosure Group during this period; some were new items and others were renovated or modified. The Atomic-Beam Group has unique requirements for complex designs and special nonstandard enclosures.

Transportation

An additional work load has been put on the Transportation Group through the Atomic Beam Group's making use of the General Electric reactor at Vallecitos (approximately 45 miles from Berkeley) for the production of short-lived isotopes.

The Health Chemistry Group was called upon to apply its enclosure philosophies and techniques to change the dimensions of a 90-lb lithium hydride block and transfer it from a sealed container with one set of dimensions to a sealed container of another set of dimensions; the work was carried out in an argon atmosphere.

Equipment and Construction

As the quality and adaptability of television equipment is steadily improving, its applicability to laboratory operations increases. On the basis of studies over several years, closed-circuit television equipment has been ordered for use in remotely controlled monitoring and manipulations in connection with 60-in. cyclotron operation. This is of particular importance in target handling. Similar studies are being made for use of closed-circuit television in remote chemical operations.

Considerable work has been done in analyzing the radiological factors involved in the designs for the Chemistry Building 70 addition and for the new 88-in. cyclotron facilities. Recommendations along these lines have been made at meetings with the architects and engineers.

Decontamination and Waste Disposal

There is continued improvement in the techniques employed by the Decontamination Group. The boxes and enclosures are more and more being decontaminated without being dismantled, the box serving as its own decontamination chamber. For small parts a decontamination enclosure has been set up with peripatetic ports. From this the decontaminated items pass through a polyvinyl interchange area to a cold box for examination before removal.

The radioactive waste problem is well in hand, 853 gallons having been processed during the period. Standard procedures now permit the use of second-hand 30-gallon drums. The waste is first passed through a blend tank in a glove box, then agitated, and brought to a pH of 3 before solidification. This step eliminates the possibility of the violent reactions that have sometimes occurred.

Special Problems

During this report period the Special Problems Group fabricated 207 made-to-order radioactive sources; inspected, adjusted, and repaired 59 sources; prepared 10 standard solutions; made 243 assays for radioactivity; and tested 72 different types of materials for their adaptability for various uses in the Laboratory. A paper by Albert E. Salo and Nelson B. Garden, "Summary of Corrosion Spot-Testing Results", UCRL-8812, June 1959, was published.

Information

Dissemination of information in the field of radiological protection was made through a television presentation of the "concentrate and confine" philosophy used at Lawrence Radiation Laboratory, programmed on the series given by the American Society of Safety Engineers.

Institutions and individuals made about 25 requests for Health Chemistry drawings of equipment for control of radioactive materials during this period.

Seven members of Health Chemistry delivered 75% of the instruction in a course on radiological regulation and use, given by the University of California Engineering and Sciences Extension Division during the summer. Subjects and laboratory sessions covered by Health Chemistry included philosophy of radioisotope control, monitoring, instruments, laboratory counting, mathematical relationships in radioactive phenomena, air sampling and cleaning, environmental surveys, shielding, radioisotope handling, and waste disposal; personnel from other departments of the Radiation Laboratory and the University covered nuclear fundamentals, radiation biology, fallout, x-ray machines, radioactivity in food and water, and AEC and Federal Regulations. The special equipment required for the course was covered by an $80,000 grant from the AEC.

HEALTH PHYSICS

Burton J. Moyer

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

Beta-gamma meters	22
I D L meters	18
Juno ion chamber	20
Abacus logarithmic ion chamber	24
Recording-intensity meters	8
Victoreen proteximeter	3
Slow-neutron proportional counters	15
Fast-neutron proportional counters (portable)	11
Slow-neutron protable 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 2300

Summary of Instrument Use:

Instruments	Buildings	Number per day (approx.) for 6 months	Man-days of coverage
Electroscopes	51	40	7200
	53 and 80	17	3060
	71	15	2700
	70	15	2700
	2	10	1800
			17,460 total
Dosimeters	53 and 80	17	3060
	2	10	1800
			4,860 total
Slow-neutron Chambers	53 and 80	17	3060
	2	10	1800
			4,860 total

Cases of Monthly Exposure Exceeding 0.5 r (not including Livermore)

Monthly film expos. above (r):	184-inch area	60-inch area	Bldg. 51	Chem.	Other	Total
Individual gamma-ray exposures only						
0.5	0	17	2	5	13	37
1.0	0	4	1	0	2	7
1.5	0	0	0	0	1	1
2.0	0	0	0	0	0	0
3.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0
Individual neutron exposures only						
0.5	2	0	10	0	5	17
1.0	1	0	1	0	1	3
1.5	0	0	0	0	0	0
2.0	0	0	0	0	0	0
3.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0
Both neutron and gamma-ray exposures						
0.5	0	1	5	0	7	13
1.0	0	0	1	0	0	1
1.5	0	0	0	0	0	0
2.0	0	0	0	0	0	0
3.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0
Combined total of all neutron and gamma-ray exposures						
0.5	2	18	17	5	25	67
1.0	1	4	3	0	3	11
1.5	0	0	0	0	1	1
2.0	0	0	0	0	0	0
3.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0

RADIATION SURVEY WORK AND RESEARCH PROJECTS

1. The surveys of radiation from Building 51 have been continued and augmented by a measurement of the attenuation of the neutrons by concrete, in an attempt to determine the thickness of roof needed for the Bevatron.

2. The three sensitive integrating ionization chambers for use under adverse environmental conditions have been completed and put into operation. Presently a much improved circuit is being designed and tested which, it is hoped, will reduce the electrical leakage experienced so far.

3. Work with the 50-channel pulse-height analyzer has been continued on sensitive neutron threshold detectors.

4. Equipment has been installed in a Boeing 707 to continue measurements of the cosmic-ray neutron energy spectrum at northern latitudes.

5. Preliminary plans have been drawn for a new Health Physics office and laboratory building to accommodate all the present scattered Health Physics work.

6. A training program for AEC Radiological Physics Fellows has been organized. This will include a new course to be offered: Introduction to Health Physics No. 127.

7. Shielding calculations for the new 88-inch cyclotron have been made.

8. Shielding calculations for the long-range Bevatron development program have been made.

9. A study of the neutron energy response of the Kodak NTA dosimeter films has been completed. (Submitted to Health Physics.)

10. Equipment for measuring proton recoil track length in thick nuclear emulsions has been completed in the shops and is being tested. This equipment is designed to make use of nuclear emulsion as a neutron-energy-spectrum analyzer for stray neutron fields and for neutron sources.

11. A fast neutron-flux integrator utilizing the $Co^{59}(n, r)Co^{60}$ reaction has been designed and calibrated. This detector can measure an integrated flux as low as 10^7 n/cm^2, and can perform accurate flux integration over a period of at least 1 year.

12. Additional shielding against fast neutrons has been installed at the Bevatron between the machine itself and those areas permanently occupied by Bevatron operating personnel. The shield consists of wood 1 foot thick, and provides an attenuation factor of 4, measured in terms of the biological effect of this flux.

REPORTS AND PAPERS

1. H. Wade Patterson and Roger Wallace, A Method of Calibrating Slow-Neutron Detectors (submitted to Health Physics).

2. Lloyd D. Stephens and Alan R. Smith, Fast-Neutron Surveys Using Indium-Foil Activation (submitted to Nucleonics).

3. Wilmot N. Hess and Alan R. Smith, Measurement of Average Neutron Energies for (α, n) Neutron Sources, Ann. Physics 2, 115 (1959).

4. Wilmot N. Hess, H. Wade Patterson, Roger Wallace, and Edward L. Chupp, The Cosmic-Ray Neutron Energy Spectrum, Phys. Rev. 116, 445 (1959).

5. Wilmot N. Hess, H. Wade Patterson, and Roger W. Wallace, The Cosmic-Ray Neutron Energy Spectrum, UCRL-8657 Abstract.

6. Joseph B. McCaslin, A High-Energy Neutron Dosimeter, (submitted to Health Physics).

7. James W. Beasley, The Mean Fission-Fragment Range in Bismuth as Applied to Pulse-Type Ion Chambers, (submitted to Rev. Sci. Instr.).

8. Roger Wallace, H. Wade Patterson, and Edward L. Chupp, The Cosmic-Ray Neutron Energy Spectrum Above 40,000 Feet, (submitted to Health Physics).