Project #20026 - Correlation between Biological and Chemical Effect of X-irradiation with Varying Wave Lengths

George V. Taplin, M.D., James S. Grevior, M.D. and Philip Noyes

From results obtained at the recent atomic bomb tests in Nevada with shielding experiments on the chloroform-dye dosimeters, it was shown that the soft component of the gamma spectrum from atomic air bursts is completely absorbed by approximately 0.1 mm of Pb or its equivalent. This indicates that the mean energy of this soft component is about 70 kev or less.

Therefore, to assess the probable biological significance of this low energy component, a series of 50 albino mice was exposed to an LD_{50} dose (550 r) of 70 KV X-irradiation, filtered with 1 mm Al to determine the relative lethality of this soft beam.

Previous studies (Report UCLA-76) showed that the same dose (550 r) of higher energy beams of X-rays was considerably more lethal to this strain of mice (see Table VII). The most probable explanation for this difference in biological effect is the decreased penetrability of the soft beam with resultant lessening of injury to the bone marrow and other vital organs.

Table VII

Comparison of Lethality of X-ray Beams of Varying Energy on Albino Mice

Group No.	Radiation Energies	Dosage in r	No. of Mice	Percent Total Mortality in 14 Day*
1	75 KV, 5 ma	550	50	2.0
2	100 KV, 15 ma	550	87	60.0
3	150 KV, 15 ma	550	88	68.0
4	250 KV, 15 ma (soft)	550	99	72.0
5	250 KV (hard)	550	89	71.0

Notes:
1. Albino mice CF1, weighing 20-25 gm, were used.
2. Animals were radiated just inside the circumference of the field, each animal being located an equal distance from its center.
3. Filtration factors: Group 1 -- 1 mm Al.
   Group 2-4 -- 0.46 mm Cu plus 1.0 mm Al.
   Group 5 -- 0.46 mm Cu, 1.0 mm Al, plus .53 mm Pb.
4. Dosage measurements were made inside the individual animal containers, with the Victoreen r meter and appropriate Kimble chambers.

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Radioisotopes in Pharmaceutical and Medical Studies

By D. L. TABERN, J. D. TAYLOR, and G. I. GLEASON

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Radioisotopes in Pharmaceutical and Medical Studies—I

NUCLEONICS takes pleasure in offering this first in a series of three articles that bring together a fund of both published and unpublished information to which workers in the field of isotopic medicine can relate their own research

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USES FOR RADIOISOTOPES in biology and medicine fall into three broad categories. They can be employed: (1) to investigate by the tracer technique not only the metabolism of drugs and natural products but also the complex processes of a biological nature; (2) to assist in the diagnosis of disease; and (3) to aid in the treatment of disease.

The degree to which isotopes assist in the solution of biologic problems rests largely upon the skill of the investigator. Unquestionably, isotopes are being used today in problems that could be answered equally well by older methods. It is also true, however, that cleverly designed isotopic experiments have supplied crucial data obtainable in no other way.

When we enter the fields of diagnosis and therapy, we automatically encounter an added restriction—namely, that the techniques must be applicable to human beings. Among the hundreds of isotopes known, one can find almost every conceivable range of radiation, half-life, and biological affinity. In addition, the skill of the chemist has contributed the possibility of combining certain isotopes in a wide variety of structural and chemical combinations.

These still largely unsolved problems can most effectively be illustrated and discussed in this series of articles in the appropriate sections. The topics to be covered are as follows:

1. *Uses of Iodine-131*
2. *Uses of Phosphorus-32*
3. *Tumor Localization*
4. *Other Diagnostic Agents*
5. *Tumor Therapy*
6. *Barbiturates and Thiobarbiturates**
7. *Sulfur Amino Acids**
8. *The Dynamic State**
9. *Membrane Permeability**
10. *Antigens and Antibodies**
11. *Fat Metabolism**
12. *Thyroid and Antithyroid**
13. *Metabolic Studies with Phosphorus-32**
14. *Isotopes as an Analytical Tool**
15. *Mechanical Tracing**
16. *Toxicity of Carbon-14**
17. *Drug Metabolism*†

Selection of these topics and references for this paper, while admittedly quite arbitrary, was based upon a desire to demonstrate the significant contributions which radioisotope studies have made to medical progress, while still remaining within the scope of the title of this paper.

To conserve space, rather than name all the individuals involved, reference

* Will be published in Part II of this paper to appear in the December NUCLEONICS.
† Will be published in Part III of this paper to appear in the January NUCLEONICS.

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THE ABBOTT ISOTOPE PROGRAM . . .

In an effort to see how their organization could make worth-while contributions in the field of radiobiology and isotopic medicine, the scientific staff of Abbott Laboratories set out to prove three things to themselves and their company: *First*, that they could profitably use certain isotopic techniques and materials to gain a better understanding of the physiology and pharmacology of the drugs Abbott is now preparing; *second*, that, if the research clinician is to use isotopic drugs on human beings, they could make such drugs available to him in suitable form, doubly checked for purity, activity content, sterility, etc.; and *third*, that they could aid in research to augment the pitifully few useful radioactive preparations for diagnosis and therapy available today.

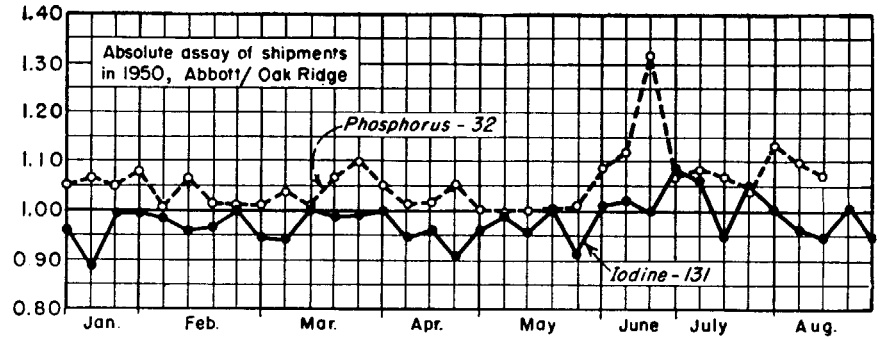
Although Abbott's executives knew that the supplying of isotopes for research and therapy might remain a "labor of love" for some time to come, they endorsed the program built on these three points and agreed to operate it as a research project.

Nominal service charges were set for preparing and distributing such supplies in the hope that increasing usage would some day make the work self-supporting. Laboratories were set up and equipped, and personnel given special training at Oak Ridge and elsewhere.

The Abbott staff looks upon the project as a cooperative venture, their part being to develop and supply materials and act as a clearing house for new information, ideas, and equipment. It was on this basis that much of the information presented in this series of papers was made available to them.

They early realized that a prosaic but nonetheless essential service to be rendered by a group such as theirs was the supplying of pharmaceutically correct solutions of such simple but widely used isotopes as P^{32}, I^{131}, etc. Materials coming from Oak Ridge are not sterile, have not been tested for pyrogens, and vary greatly in specific activity from batch to batch. To identify, dilute accurately, buffer if necessary, assay, and sterilize such solutions prior to use necessitates the maintenance of a "hot laboratory" (separated physically from the assay and therapy rooms) with specialized personnel. If such an operation is carried out only occasionally, it is obviously not economical for the infrequent user of isotopes to maintain such a facility. Abbott's hot-lab isotope processing for such a user, therefore, can be a real service to him.

Abbott, as a secondary distributor under arrangement with the Atomic Energy Commission, orders as a single shipment each week the amount it estimates as



necessary to cover requests on hand and still have a moderate reserve. Thus far, the law of averages has worked and it has been possible for them to ship on sometimes as little as an hour's notice supplies urgently needed by authorized hospital groups. Special delivery to the airport and the use of air express and air freight permits next-morning receipt almost anywhere in the country. Small shipments, which vary from 25-30 millicuries to as few as the 2–3 needed for a single case, have resulted in the development of compact, hermetically sealed, shielded containers.

The contents being sterile and of known and relatively constant composition, the unit may be taken directly to the point of administration and all or part of the contents withdrawn by a syringe without opening the container or removing it from the lead shield. The container may then be simply set aside in a safe place until decay has brought any residual activity to the level recommended by the AEC for ordinary disposal. The request may specify a given dosage to be present on the expected day of administration, still further decreasing possibilities of error. Abbott has set up their own absolute-assay procedures on the basis of three different methods, quite independent of Oak Ridge. The chart above shows a comparison of Abbott assays with those stated by the Isotopes Division at Oak Ridge. The agreement is excellent, but the obvious exceptions only prove that slips can happen even with the best of care and that, for greatest safety, Oak Ridge assays should be checked again before such materials are used clinically.

Abbott acknowledges its obligation to collaborating groups to assist in assay procedures and to attempt, especially in cases of unusual isotopes such as Au^{198}, to keep a running check on values observed and the doses used by such groups.

A simple method for absolute beta counting that does away with the necessity for absolute standards has been devised by G. I. Gleason of the Abbott staff, and publication of it will be made soon.

has frequently been made to groups working on the indicated projects. For the same reason, only the more recent references in a series are given.

Space has also necessitated the elimination of at least four subjects which otherwise would have been covered, namely, first, the effect of radiation on biological systems; second, the treatment of radiation damage; third, metabolic studies involving carbohydrates and the lower fatty acids; and fourth, studies on photosynthesis. The third of these has just been admirably treated, especially from the standpoint of biologic mechanisms by Bernhard (*1*).

Within the past few months, a number of studies have been reported dealing with isotopes in dental problems. These will be the subject of a communication by another author in a subsequent issue of NUCLEONICS.

For a discussion of the production

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and availability of isotopes, the reader is referred, in particular, to a report by Aebersold (*2*).

As we shall see, isotopic studies have firmly established the concept of the "metabolic pool," through which passes, at one time or another, a large portion of any given element (such as carbon, nitrogen, or sulfur) taking an active part in metabolism. Isotopes have, in turn, aided in unraveling the complex problems thus presented. A given lot of isotopic atoms, administered in the form of a given compound at a given time, is clearly distinguishable from all other atoms as long as it remains in the body. On the other hand, certain elements and compounds do not enter such a pool but are instead rapidly eliminated in their original form or as degradation products that are often capable of identification by isotopic means.

1. Uses of Iodine-131

Iodine-131 is the most widely used isotope and the one that has achieved the most practical success in actual clinical use. It has betas of 0.315 and 0.6 Mev, and gammas of 0.638, 0.636, 0.283, and 0.08 Mev. Radiation effects within the thyroid are predominantly due to the beta particles, but the gammas have the advantage of allowing measurements outside the body.

The most satisfactory vehicle for "dispensing" seems to be a slightly alkaline solution of essentially carrier-free material containing a trace of sulfite as a reducing agent. All glassware used in handling should be pretreated with cold iodide and thoroughly washed.

Iodine is being used extensively today for both the diagnosis and therapy of thyroid diseases. With tracer doses of 25–100 μc, hyperthyroids usually take up over 50% in 24 hours as contrasted with 10–20% for normals. These measurements can be made readily by means of a counter placed on the neck of the patient over the thyroid. The trend is toward going farther and studying actual conversion to protein-bound thyroxine, wherein hypothyroids give 6–10% conversion, hyperthyroids give over 50%, and thyrotoxics over 90%.

Jaffe and Ottoman have presented an excellent report (*3*) on the use of tracer doses, usually 100 μc, as a diagnostic test for suspected hyperthyroidism. For this purpose, the usual basal metabolism test was only 67% accurate, and failed badly in certain classes of patients. Determination of blood iodine (I^{131}) was better—76% accurate; and the simplest test of all, thyroid uptake, was 91% accurate, with 5% of the remainder being placed by the observed data in a questionable group. They conclude, "We advise radioiodine uptake studies, when available, for all patients with clinically suspected hyperthyroidism, regardless of the type of treatment to be used."

For therapy of hyperthyroidism, larger amounts (3–5 mc) are needed which give an irradiation of some 15,000 equivalent roentgens (er) per gram of thyroid tissue. Clarke (*4, 5*) at the University of Chicago lists recommendations for I^{131} over surgery as: (1) recurrent hyperthyroidism, (2) presence of other pathology making the patient a poor surgical risk, (3) propylthiouracil failures, (4) individuals over 45, and (5) people who want it.

Gordon and Albright (*6*) have reported on the first 120 cases treated at the University of Wisconsin; 24-hour uptake was taken as a measure of potential success. Doses were purposely kept small (average 3 mc) on the theory that subsequent doses could readily be given as and if found necessary. Not one of the patients failed to respond, and one half were returned to normal thyroid status by a single dose. Three cases became hypothyroid but were readily controlled by thyroid administration. Contrary to some earlier reports, both nodular and diffuse goiters responded. They say, "We consider I^{131} the treatment of choice for toxic goiter, excepting only goiter during pregnancy, large goiters with mechanical obstructions, and hard glands involving danger of malignancy."

Feitelberg *et al.* of Mt. Sinai Hospital in New York have just published an equally encouraging report covering over 300 patients studied during a two-and-a-half year period (*7*). The average dose was 8.2 mc and the maximum 11. In the average patient, subjective improvement was noted in two to three weeks, and objective improvement in four to eight. Patients not well by then received a second dose; a few required a third. Radiation sickness was not observed. He says, "Because of the simplicity of the method, the absence of important complications, and the excellence of the results, it is our opinion that the use of internal radiation with I^{131} is the treatment of choice in diffuse toxic goiter."

Feitelberg also has proposed the charting of the supposed thyroid area by means of a directional tube. Used before and after partial thyroidectomy, it gives a reasonably clear picture of the part of the functional area still remaining and its present location (*8*). See Fig. 1. With improvements in counting methods, great strides in this direction can be anticipated (*9*).

Unfortunately, only 15–25% of thyroid tumors take up iodine in sufficient quantity to be of value for radiation effects. This number may be increased by the use of suitable thyroid stimulants, by the removal of most of the "normal" thyroid, etc. Trunnell has suggested 40,000 er per gm of tissue or a total of 166 mc for a 100-gm cancer (45% excretion) (*10*). Even such doses (if properly distributed as divided doses of 30–50 mc) seem within the safe range. A number of successful cases have been recorded, and a number are now undergoing treatment with material being supplied by us under a special arrangement.

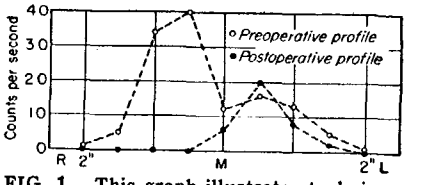

FIG. 1. This graph illustrates technique of plotting thyroid position with a directional counter (*8*)

Seidlin *et al.*, in a progress report on the use of I^{131} therapy in carcinoma of the thyroid, speak of 12 treated patients. Of these, 7 were still living after 6 years, 3 were greatly improved, and 2 moderately improved. Total doses were large (200–1,000 mc), and no mention is made of serious side effects (*11, 12*).

Blumgart *et al.* (*13*) have obtained favorable results in patients suffering from intractable angina pectoris by rendering them hypothyroid by means of moderate-to-large doses of I^{131}. They say that hypothyroidism induced by radioactive iodine promises to accomplish worth-while improvement through medical means without the inevitable risk and complications of surgical intervention.

Successful treatment of a metastatic lesion in the lung which had shown I^{131} uptake has recently been reported by Freedberg *et al.* (*14*).

Several excellent surveys covering

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the therapeutic uses of iodine have recently been published (*15–31*).

The exact forms in which iodine exists during and after its thyroid uptake have been the subject of much study. Leblond and Gross, for instance, have used radioautography to show that at one hour the element is present mostly in the epithelium, at 24 hours it is in the "colloid" having passed through a "free" thyroglobulin stage (*32*). Taurog and Chaikoff (*33*) believe that the circulating hormone in the normal animal consists of thyroxine bound loosely to plasma protein. The influence of internal irradiation of the gland has been carefully studied (*34*).

The effect of halides, thiocyanates, and propylthiouracil upon the distribution and metabolism of radioiodine has recently been reported by Williams *et al.* with particular reference to clinical applications (*35*).

Goldberg and Chaikoff have shown that even doses of I^{131} as large as 300–800 μc produced no direct effect on the pituitary, even though the thyroid tissue remained atypical for many months (*36*). It was also demonstrated (*37*) that the parathyroid remained undamaged except for some fibrosis resulting from very high dosages.

The Mayo group under Watts (*38*) has studied lymphocytes from persons treated with I^{131} and do not find a significant change in the number or frequency of refractile granules therein.

Hertz (*39*) recently summarized the evidence for and against hazards to the patient from the use of I^{131}. The evidence seems to be overwhelming that the fears once held by some are rapidly being shown to be without foundation.

Very recently Hamilton and the California group found that the cyclotron-produced pure alpha emitter, astatine-211, causes the same type of thyroid tissue destruction (*40*). Since At^{211} radiation has a range only 1/40 that of the beta of iodine, there can be no effect on adjacent tissue, but the nature of its radiation and the short half-life (7.5 hr) contrive to make it of no therapeutic value at present.

While not of direct interest in medicine, reference may be made to a new method wherein short-lived free radicals (from organic reactions) are allowed to combine with I^{131}, and the resulting alkyl iodides, after dilution with carrier, are separated physically and assayed by counting (*41*).

2. Uses of Phosphorus-32

Phosphorus-32 is the element of second greatest medical interest. It is a pure beta emitter (1.69 Mev) of 14-day half-life. Abbott supplies it as a sterile, buffered solution of sodium phosphate with a small amount of carrier added.

After administration, P^{32} rapidly disappears from the blood and is taken up by certain tissues and cells. It is deposited in large amounts in bone and bone marrow, and in somewhat smaller quantities in the liver, spleen, and lymph nodes—hence its use in leukemia and polycythemia. The isotope is incorporated rapidly in both circulating erythrocytes and leukocytes; later the concentration in the erythrocytes decreases sharply, while that in the leukocytes goes down much more slowly (*42*).

A second characteristic is its incorporation in rapidly metabolizing tissues; thus neoplastic tissues take up amounts greater than normal, a fact which has been made use of in the localization of certain tumors, particularly those of the brain, where access may be had to the tumor area (*43*). Roswit, Sorrentino and Yalow have applied P^{32} to

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the study of suspected malignancies of the testicles (*44*). Unfortunately, localization in bone tumors is not sufficient to achieve therapeutic results through irradiation.

However, Hertz has studied the effect of sodium acid phosphate, testosterone, and estrogens on the uptake of P^{32} by human metastatic cancer (*45*). Encouraging results were secured.

Since the pioneering work of Lawrence and associates at the University of California (*46*), P^{32} has been subjected to careful and extensive clinical trial and its place has been reasonably well established. As Karnofsky has emphasized (*47*), it has not proved consistently useful in Hodgkins disease and lymphosarcoma, and is without value in acute leukemia.

It is, on the other hand, the treatment of choice in polycythemia vera (*48*). It produces marked symptomatic relief, a remission in the abnormal hematological picture and a reduction in the size of an enlarged liver and spleen. It frequently restores the patient to a reasonably normal, useful life. In a recent report, the statement is made that this treatment is just as successful, and the prognosis for the patient just as favorable, as in the treatment of diabetes with insulin.

For intravenous administration, the usual single dose is 3–7 mc; this alone is said to produce satisfactory remission in 25% of patients. Hall and Watkins (*49*) believe that a second dose should not be given before an interim of eight weeks, after which second and third injections may be given provided the blood picture does not fall below certain stated standards.

The usual duration of remission after an effective single dose is 6–12 months. Groups at the Mayo Clinic and Washington University (*50*) found satisfactory remission in 85% and partial remission in the other 15% or 84 cases; 62% of these remissions lasted more than 6 months, 25% lasted more than 1 year, and 10% lasted more than 10 years. If the polycythemia vera reoccurs, remission can be induced a second time by more P^{32}. Whether P^{32} gives a longer survival period than X-ray or other experimental measures is difficult to say definitely. It seems significant to us that a summary of various reports showed 32 of 36 patients survived more than 5 years and 29 more than 10 years.

The chief advantages of P^{32} are: (1) the ease of administration, (2) the absence of radiation sickness and other toxicity, and (3) the simplicity with which the dose can be controlled. Its disadvantages are the possibility of bone marrow damage by overdosage, and the possible slightly greater incidence of acute leukemia.

The possibility of overdosage certainly would seem to be less important now that adequate and relatively simple assay procedures have been established. When P^{32} is given intravenously to normal persons, 10–25% is excreted in the first 7 days.

P^{32} has also been used in leukemia and allied diseases (*51–54*). See Fig. 2. Smaller doses are employed (5–7 mc) in order to have maximal depressant effects on leukopoiesis and minimal effect of the formation of erythrocytes and platelets. One Chicago group employs an initial dose of 5–7 mc and weekly sustaining doses of about 2.5 mc, as determined by excretion studies (*55*). This dosage has been found to give maximum therapeutic effect with a minimum of pronounced blood picture disturbance.

Oral rather than intravenous administration may be employed, in which instance at least 25% is lost in the stool and 75% may be assumed to be absorbed.

In chronic (but not acute myelogenous) leukemia, remission has been induced in a high proportion of patients;

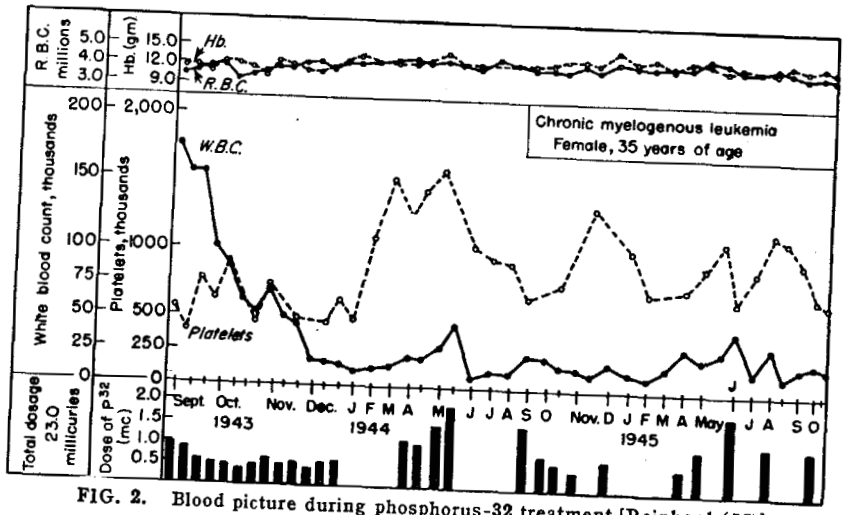

FIG. 2. Blood picture during phosphorus-32 treatment [Reinhard (*55*)]

reports indicate a duration of remission, and a prolongation of life at least equal to roentgen-ray therapy. For an excellent statement of the physiology of P^{32} use, see Friedell, Storaasli, and Marshak (*56, 57*).

There have been several reports regarding multiple myeloma. The most recent is that of Lawrence and Wasserman (*58*), who used P^{32} in 11 cases, P^{32} and Sr^{89} in 9, and colloidal yttrium-90 in 1. Results were not markedly better than those from X-ray or stilbamidine. Combination therapy seems worthy of further trial. Stilbamidine-C^{14} does not localize sufficiently to indicate its use in therapy.

Low-Beer has pioneered the use of P^{32} absorbed on blotting paper of known dimensions and dried, for application directly to the skin. After preliminary experimental studies, he treated about 400 skin lesions including carcinoma, hyperkeratosis, warts, and hemangiomas. Over 90 % of the lesions disappeared (*59*).

The Friedell group in Chicago has employed P^{32} and its rate of deposition in a given extremity for determining the probable response of that patient to sympathectomy (*60, 61*).

The use of P^{32} to label red blood cells to determine blood volumes is discussed in Section 4 of this article and its application in the localization of tumors in Section 3.

P^{32}-tagged leukocytes have been used in a study of the fate of white cells (*62*). The authors conclude that the process is more than a mere foreign body filtration by the reticuloendothelial system, as evidenced by the pronounced role played by the lungs.

To encourage the use of this isotope without removing necessary safeguards, the AEC, on page 3 of Supplement No. 3 (Feb., 1950) of their Isotopes Catalog and Price List No. 3, states that they will authorize the use of P^{32} by a physician in good standing provided that: (1) he has a suitable survey instrument, (2) he has been certified as qualified by a university medical school or hospital, and (3) his solutions be reassayed before use by the medical school or by a radioisotope supplier customarily engaged in making such measurements.

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3. Tumor Localization

There is no need to recount here the many efforts that have been made to secure materials that would localize in tumor tissue to permit exploration or therapy.

Selverstone, Solomon and Sweet (*43*) recently reported on deposition of P^{32} in brain tumors. Since P^{32} is a beta emitter whose effective range in tissue is of the order of a few centimeters, it was necessary to devise a minature Geiger counter that could be inserted directly into the brain tissue. Good differentials between normal and pathologic areas were obtained and the method holds promise as a mechanical aid to the neurosurgeon. Localization of P^{32} has also been found in mammary and testicular tumors (*44*). The Selverstone group has also stated orally that the potassium (K^{42}) ion is similarly localized in brain lesions.

Most investigators have preferred to try more complex organic derivatives. Moore (*63*) of the University of Minnesota tried fluorescein in a wide variety of carcinomas and sarcomas, using as a criterion of uptake fluorescence under ultraviolet light. Less than half of abdominal tumors gave satisfactory uptake but, in lesions of the central nervous system, accuracy in both a positive and negative sense was of the order of 90 %. He then incorporated iodine into this molecule and it was at this point that active cooperation with Abbott Laboratories was begun. I^{131} has the advantage over P^{32} in that it permits measurements to be made outside the skull and thus quite independent of operation procedures. The diiodo derivative proved less toxic than the tetraiodo and has since been used exclusively (*64*).

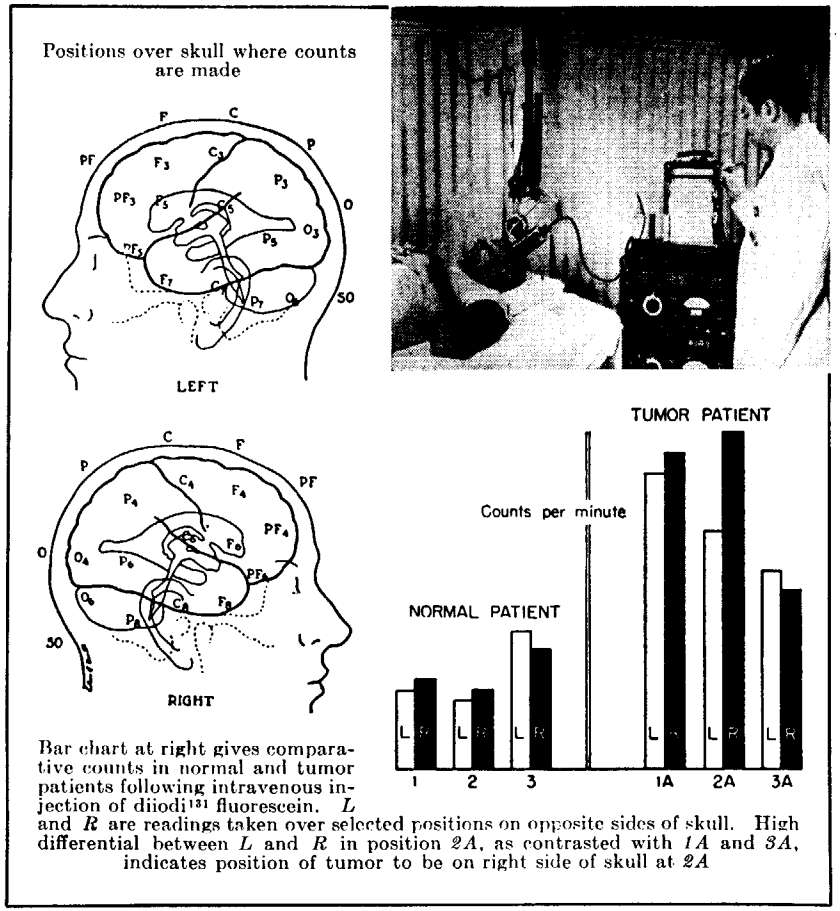
The factor of concentration is only moderate—some eight to ten fold in favorable cases. The mass of the tumor is small in comparison with the immediately adjacent normal tissue. Geiger tubes are neither particularly directional nor gamma-sensitive; even at best, readings represent the summation of the tumor and those portions of the brain beyond it. Greater directional effect can be secured by the use of a narrow opening in a heavy lead shield, but the counts are thereby so decreased that the time necessary to record a statistically significant number of counts exceeds the time which may be devoted to any given location, and there is actually danger of overlooking tumors—due to small variations in positioning of the counter tube.

We early found that the dye made by the original method was a mixture and contained a small amount of labile halogen. We have devised a new preparation yielding what appears to be essentially pure diiodo-I^{131}-fluorescein. This material has been used by practically all investigators during the past year. The LD-50 in mice is greater than 250 mg/kg; the sodium salt is contained in a solution of *p*H 7, and has no labile iodine.

The concentration of activity in the thyroid following administration is less than that from the customary and perfectly harmless tracer dose of iodine. Excretion is through the urine and feces, and the compound's biological life is 2–3 days.

Localization in the tumor seems to proceed more rapidly than with the original dye, possibly due to the absence of the preferentially absorbed fluorescein. Specific activity does not seem to be critical, but we have found a convenient value to be from 10–15 mc per gm of dye.

Recently Davis, Martin, Ashkenazy, LeRoy, and Fields reported before the American Neurological Society more



Bar chart at right gives comparative counts in normal and tumor patients following intravenous injection of diiodi[131] fluorescein. *L* and *R* are readings taken over selected positions on opposite sides of skull. High differential between *L* and *R* in position *2A*, as contrasted with *1A* and *3A*, indicates position of tumor to be on right side of skull at *2A*

FIG. 3. Use of I^{131}-tagged diiodofluorescein for tumor diagnosis

than 200 consecutive cases thus localized with an accuracy of 94–95%. See preliminary report (*65*). (Work at Hines Veterans Administration Hospital and at Northwestern University since then has raised the total to over 350.) The formal paper is to appear shortly in the *Journal of the American Medical Association.*

Their method which has been adopted in principle by most other users is briefly as follows: A solution containing 1 to 1.2 mc of I^{131} activity (about 1 cm^3) is given intravenously. "Counts" from the brain area become significant within 15–30 minutes, and are most pertinent from then on up to 1½–2 hours. Probably due to general edema, the actual concentration in a diseased brain usually is higher than in a normal brain and it approaches 1000–1200 cpm measured externally (1-inch tube opening). See Fig. 3. At or just before this time, measurements are made at some 32 predetermined locations around the skull. Corresponding areas on opposite sides are measured next to each other and compared. The differential between such corresponding areas, one normal, and one containing a tumor, will be 80–200 cpm. Differentials of less than 50 cpm are of doubtful significance. During the next hour or so, all values decline gradually, but in most instances a differential persists.

Since differences in technique and equipment may cause significant variations, it is essential that each group run a number of "normal" cases to establish the "normal" counts for the various positions about the skull. Marked variations from these are, of themselves, of primary diagnostic value in establishing the existence of physiologic abnormality. The use of fewer positions for counting is likewise to be avoided; in fact, suspected areas must be rechecked several times during the observation. Such care not only befits the serious nature of the lesion being studied, but has occasionally brought to light unsuspected multiple tumors. Obviously, with mid-line tumors, comparison of opposite sides does not suffice and one must rely on an accurate "mapping" of the suspected areas.

The Davis group states that the position and size of the tumor can be quite definitely defined and an estimate made of its malignancy. In their work, such accuracy and success is possible because the isotopologist and neurosurgeon work together during each individual test; they can thus recheck suggestive values before concentration changes have taken place; the same team is present during the operation, and has the opportunity to recheck the examination on the patient after operation, and again if recurrence or incomplete removal is suspected.

Thoma, using a similar procedure, has reported favorably on some 60 cases of brain tumors, 20 of which have been verified (*66*).

Tumors of the upper spinal cord have been similarly located by the Davis group, but with difficulty because of the stray radiation from the liver.

LeRoy, Tweedy, and Ashkenazy have submitted a paper to the *Journal of Laboratory and Clinical Medicine* concerning the health physics aspects of the use of diiodo-I^{131}-fluorescein for diagnostic studies. They conclude that in doses of 1000–1100 μc given intravenously, it can be used safely for diagnostic purposes. They believe there is no significant radiation hazard to the patient or to the institution. While there is a potential radiation hazard in the case of attendants of incontinent patients, in practice, significant contamination has not been observed (*67*).

This, then, it would seem, is a very useful, accurate, convenient, and painless diagnostic test—one which will grow in value as increasingly sensitive detection equipment becomes available.

Myers (*68*) has diazotized and coupled sulfanilic acid and 2,6-diiodosulfanilic acids with varied intermediates to form both acidic and basic dyes. In all, some 70 compounds were tested on four different tumors growing in mice. There was definite localization, as compared with blood levels, in some 18 members of the series.

Gallium, a bone-seeking element, has been extensively studied by Dudley *et al.* at the Naval Research Hospital. It is unquestionably taken up by bone tumors, but the diagnostic and therapeutic possibilities remain to be evaluated (*69–72*).

4. Other Diagnostic Agents

The clinical importance of determining blood volumes accurately is becoming well recognized. The azo dye T1824 (Evans Blue) has been extensively studied, but has several obvious disadvantages, notably that repeated

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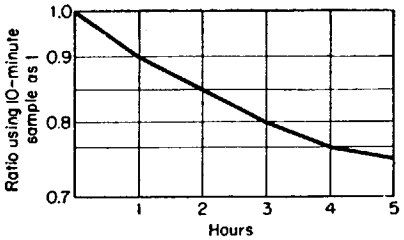

FIG. 4. Graph of iodinated serum albumin disappearance from the blood (*84*)

determinations cannot be made in the same individual over a short period of time.

By means of radioisotopes, easier and more accurate methods have been evolved. In 1941, P. Hahn *et al.* described a method for determining red-cell volume by means of radioactive-iron-tagged red cells (*73*). They found that the iron, given orally to dogs, became incorporated in their erythrocytes and that the tag remained as long as the cell existed intact. Almost simultaneously L. Hahn and Hevesy obtained P^{32}-tagged red cells by orally injecting this isotope into a rabbit, and Hevesy found that incubation of the cells in a test tube accomplished the same purpose (*74*, *75*). Nylin used this as a method of studying blood volumes (*76*). Since that time numerous papers describing modifications of the method have appeared (*77–80*).

Based upon the original observations of Fine and Seligman (*81*, *82*), Krieger, Storaasli, Friedell *et al.* (*83*) have proposed a method involving I^{131}-tagged human serum albumin. Being derived from a constituent of plasma by methods which do not appreciably alter its properties, the tagged serum would seem to satisfy all biological requirements for such a diagnostic reagent.

The procedure is indeed simple, no incubation being required. An amount of the tagged albumin, sterile, in standardized solution (as supplied by Abbott) corresponding to 30–50 μc, is diluted to 20 cm³ with saline. An aliquot is reserved for dilution with serum to serve as a standard. The remainder is given intravenously, and a sample, or samples, is withdrawn from the opposite area between 10 and 30 minutes later. The sample is centrifuged (15-30 minutes) and the activity of 1–2 cm³ of the plasma determined by the usual Geiger counter methods or by a gamma ionization chamber. Either liquid counting, or evaporation, (as in NaI^{131} assays) may be used. From this value and the hematocrit, blood volume can be calculated as follows: (*Counts Std.* × *Vol. Injected* × *Dil. of Std.*) ÷ (*Counts in Sample/cm³*) = *Plasma Protein; Plasma Volume* ÷ (100 − *Hematocrit*) = *Blood Volume.*

Storaasli, Krieger, *et al.* compared the three methods carefully on ten dogs. They state, "The iodinated protein method permitted accurate determinations of plasma volumes for a much longer period after injection than did the other two methods. It was simple and direct. It permitted the calculations of the volume within a few minutes after samples were obtained. It appeared to have a decided advantage over blood volume estimation with P^{32}-tagged red cells and the T1824 dye method."

The same group has reported (*84*) on extending the work to 76 human subjects, in 15 of whom a direct comparison was made with the T1824 method. It was found that at the end of one hour more than 90% of the injected tagged protein was still present in the blood; some 78% remained at the end of four hours. (See Fig. 4; this curve is quite characteristic within ±5%).

Some of the tagged protein that is removed finds its way into the lymph; 8% to 12% of the activity is found in the urine in 24 hours (*85*). Some finds its way to the thyroid, but in mice, at least, this did not exceed 5% of the injected dose. The latter effect is, of course, quite insignificant and can readily be prevented by the administration of inactive iodide.

Crispell, Porter, and Nieset have reported using an essentially similar material but only in the amount of 12 μc per determination (*86*). By withdrawing a blood sample just before injecting a new dose, they found that the amount remaining in the blood at that time could be accurately determined; this enabled them to make repeated determinations on the same patient. Repeated serial determinations in the same patients gave values which checked within 2%. Combining the iodo plasma albumin and the P^{32}-tagged red cells method, enables one to make a *direct* measurement of total blood volumes.

Optimal time for the taking of samples seemed to be at 10–25 minutes. Surface radiation over the thyroid and liver areas ranged from 0.01 to 0.15 mr, and had practically disappeared after 24 hours. They found up to 60% of the iodide to appear in the urine during 24 hours. Krieger *et al.* found that some of the tagged human serum albumin is found in the lymph (of dogs), the amount at the end of 10 minutes being negligible but increasing moderately over the 12 minutes of their observation (*85*).

There are other pertinent references regarding the use of tagged blood constituents (*87–89*).

Gray and Sterling have reported on the Cr^{51}-tagging of red cells for red cell volume determinations on dogs (*90*). When 7–26 μc of tagged dichromate were allowed to stand with 50 cm³ of heparinized blood, practically all became attached to the cells and only 1% remained in the plasma. After reinjection of the red cells, samples were removed as usual, dried over night, ground and counted on planchets. Corrections were applied to offset the softness of the X-rays self-absorption. The advantage of the method is the tightness of the bonding, no chromium being lost from the cells during the first-day post injection; the loss at three days was only 15%.

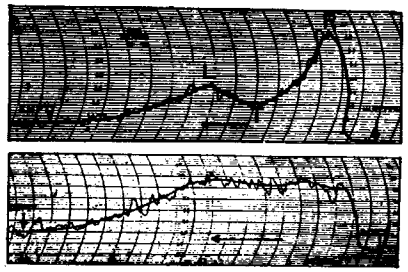

FIG. 5. Radiocardiography (*94*)

Somewhat older, dating back to 1942, is the use of Na^{24} for determining circulation time. In the method of Smith and Quimby, 60–200 μc of $Na^{24}Cl$ were injected at any predetermined site, and the time necessary for the appearance of activity at the sole of the foot was noted (*91*). The arm-to-foot circulation time varied from 15–90 seconds, with normals coming in the 45–55-second range. More interesting clinically was the build-up of counts in any remote area. For instance scleroderma and Raynaud's disease uniformly gave curves falling below normal. Kety and Cooper have proposed the "sodium clearance constant," a measure of local circulation, as a clinically useful determination (*92*, *93*).

During the past year, Prinzmetal has developed a modification known as "radiocardiography" (*94*). A well-shielded, directional, gamma-sensitive tube is placed directly over the heart of the patient, and 40–300 μc of Na^{24} are given intravenously, as rapidly as possible. In Fig. 5, the peak *R* in the curve drawn by the output of the Geiger tube represents the flow of the still relatively concentrated "slug" of activity through the *right* side of the

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heart. About 5–6 seconds later, a second peak L appears which is the flow through the *left* chamber of the heart. Because of intermediate dilution, this is obviously not as sharp or as high. If either side of the heart has lost some of its pumping power, there is a greatly increased passage time, and even fusion as in the lower radiocardiograph in Fig. 5. Thyrotoxicosis shows up as a more rapid circulation.

A formidable disadvantage is the 14.8-hour half-life of Na^{24}, but in spite of this, Prinzmetal reported on 400 cases in which it was used to differentiate heart disease from conditions simulating cardiac insufficiency, and we understand this program is continuing.

Forbes and Perley (*95*) have determined the total body content of sodium, and found the normal adult value to be 41–43 milliequivalents per kg body weight, with higher values being found in young children. The method has been applied by the British to the venous flow during labor (*96*). Levitt and Gandino (*97*) have made simultaneous use of Na^{24}, K^{42}, and D_2O along with inulin excretion to determine the average intracellular cation concentration. The value reported was 115 milliequivalents per liter for K and 35 for Na.

Layton and Frankel (*98*) have attempted to diagnose cartilagenous tumors by treatment with a nutrient solution containing S^{35}-tagged sodium sulfate. They conclude that in adult human tissues there is a differential fixation, which may be used as a rapid confirmatory test.

The use of S^{35}-tagged methionine will be discussed in Part II, Section 7, to be published next month.

5. Tumor Therapy

Prior to some ten years ago, the administration of radiation to a given tissue was largely of interest to the physicist, the source of irradiation being X-rays from radium or one of its daughter elements. Today, the problem is becoming a chemical one. Which of the some 750 known chemical isotopes best supplies the radiation (alpha, beta, or gamma) required with a minimum of side effects and danger? How may that isotope be most effectively induced to localize so as to yield the desired therapeutic effects?*

The range of X-rays or gamma rays is measured in feet so that it is physically impossible to localize their effects entirely in any given area. The alpha particle, on the other hand, has a range in tissue of fractions of a millimeter and 100% dissipation of energy is secured solely at the site of deposition. Alpha emitters are found primarily among the toxic bone-seeking metals like radium and plutonium and therefore have no practical value. A reasonable compromise is a beta emitter whose particles have a mean free path of from one millimeter to a centimeter or two. This constitutes a complete reversal of technique because, to avoid the necrosis from single sources of radium or radon (needles), the radiologist has purposely screened out these betas and depended upon the much less efficient gamma. Actually, even though it is not used for therapeutic purposes, a gamma component is sometimes convenient in that it permits following the localization or measurement from outside the body, as we saw in the case of iodine.

If the isotope is to be used in medicine, it must also meet these additional exacting requirements: (*A*) It must be nontoxic in the doses required, and be free from toxic impurities or daughter elements. (*B*) It must not localize strongly in areas other than those to be treated. (*C*) It must have a short half-life so that radiation will not persist beyond the desired period. The same result is achieved if its biological half-life (the period it remains in the body) is brief. (*D*) On the other hand, its half-life must be sufficient to permit conversion to useful forms and must outlast transportation time to the site of administration. The maximum desirable hah-life may be taken as 10–15 days and the workable minimum 12 hours. (*E*) It must be capable of intensive and economical preparation if it is to achieve therapeutic usefulness. Today that means production in a nuclear reactor.

Hahn, Sheppard, *et al.* recently reviewed 450 different isotopes and came to the conclusion that only 5 meet the highest standard of requirements (*100*). If one is willing to make certain allowances, this number can be increased to 22. At first quite startling, but nevertheless preponderately accurate, is their statement that had radium been discovered recently and evaluated in the light of the above requirements, it would never have been used in the treatment of a single tumor.

Table 1 shows some of the more eligible isotopes. In addition, Mn^{52}, Cu^{64}, Zn^{72}, As^{76}, Kr^{91}, Sr^{91}, Y^{90}, Ag^{106}, Ag^{111}, Cd^{115}, Sn^{121}, Sb^{121}, Sb^{127}, Xe^{133}, W^{187}, and Bi^{206} offer good possibilities when their biological properties are better understood.

The University of California group has done much pioneering on the distribution of particulate colloids, initially chromic phosphate (*101*), and more recently on the distribution of solutions of zirconium, columbium, and yttrium (*102–104*). They have shown that the biological distribution depends upon the nature of the particles. Zirconium particles of relatively large size show rapid disappearance from the blood stream (half-life, ½ minute) and are deposited primarily in the liver and spleen. Colloids of intermediate particle size disappear more slowly (20-80 minutes) and are deposited primarily in the bone marrow and spleen. Once deposited, no significant distribution takes place over a 2–4 weeks period, and body excretion is slow.

In an effort to make use of the favorable radiation characteristics of P^{32}, Allen *et al.* tried injecting sodium phosphate directly into mouse adenocarcinoma (*105*). The material was rapidly absorbed, and there was no tumor regression. Insoluble chromic phos-

TABLE 1
Therapeutically Useful Isotopes

Isotope	*Half-life*	*Beta*	*Gamma*	*For*
Na^{24}	14.8 hr	1.39	2.75	Total body irradiation
P^{32}	14.3 d	1.71	....	Treatment of leukemia
K^{42}	12.4 hr	3.6	1.5	Total body irradiation
I^{131}	8.0 d	0.60	0.37	Thyroid studies
Br^{82}	34.0 hr	0.46	1.3	Brominated dyes
Au^{198}	2.7 d	0.97	0.44	Tumor injections

* For organ dosage determinations in the use of radioactive isotopes, see the very excellent article of Marinelli (*99*).

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phate, of particle size about 1 micron, was therefore tried and, for technical reasons, injections were best made into tissues surrounding the tumor. Lesions smaller than 2 cm in size responded best (as would be expected from the 0.7-cm range of the 1.7-Mev betas) and showed permanent regression. Animals with larger tumors died from associated toxemia. In other areas, except in the atrophied regional lymph nodes, only traces of P^{32} were found.

Regressions were noted in other types of tumors as well, but melanosarcoma and fibrosarcoma required much larger amounts of radiation. There was no microscopic evidence of distant parenchymatous damage.

To Hahn and his group must go cluch of the credit for the intensive minical application of radiocolloids (*106*). They established that colloidal ferric hydroxide, given by vein, was distributed in the reticulo-endothelial system roughly in proportion to the phagocytic activity, with maximum concentration in the spleen. Manganese dioxide did not deposit in the spleen, the manganese tending to accumulate in the pancreas, possibly following liberation in an ionic form.

With the advent of the nuclear reactor, the economics of isotope therapy became important. In the reactor, Au^{197} has an unusually high neutron-capture efficiency (100 barns, as compared with 0.01 barns for sulfur) and is converted by an (n,γ) reaction to Au^{198} with a half-life of 2.7 days. The latter means not only that 10–12 days of pile irradiation suffices for the production of maximum activity, but that for all practical purposes its effect in the body is gone after 2–3 weeks.

For over two years, we have been able to work with Hahn and supply gold colloid to him. The service Abbott Laboratories renders in connection with this gold colloid is typical of the service this company can render hospitals and other institutions that do not have "hot" labs and are otherwise unequipped to handle very-high-activity samples.

We have irradiations carried out both at Oak Ridge and in the reactor at Argonne National Laboratory. Argonne is about 1½ hours' travel time from our laboratories. The freshly withdrawn samples, when they are returned to us, are heavily shielded and contain up to 750 mc of gold activity, an activity certainly not to be tolerated in an ordinary hospital laboratory (250 mc gives maximum permissible exposure of 10 mr at 6–7 feet from the sample). By the time our processing is completed and shipments are made some 20 hours later, about 250 mc of activity remain. This activity can be shielded in a 30-lb lead "pot" which is much more adaptable to shipment by air than would be the much heavier shielding required around samples freshly withdrawn from the reactor.

We have made extensive studies of the method of colloid production originally suggested by Hahn, that is, reduction of $AuCl_3$ in alkaline solution by ascorbic acid in the presence of gelatin. As a result, our colloid is heat sterilized, sterile, pyrogen free, stable to 10% NaCl solution, to pectin, and other colloids. Electron microscope measurements indicate surprisingly uniform particle size (0.001–0.003 micron) with only a few larger particles. Activities of 15–20 mc/cm³ or more are readily produced and can be increased to 25–30 when desired.

Goldie and Hahn (*107*) have studied the effects of two radioisotopes, Au^{198} and I^{131}, on sarcoma-37 cells growing vigorously in the peritoneal fluid of mice. Doses were small (of the order of 0.2 to 0.4 mc). With I^{131}, evidence of damage to the tumor cells was detected largely by their inability to grow when injected into other animals. With higher dosages, actual disintegration of the tumor cells was noted. Even this is quite surprising since the readily diffusible iodide had completely disappeared after 24–48 hours.

With colloidal Au^{198}, on the other hand, (given 2 to 5 days after inoculation) tests on three consecutive days after treatment showed the absence of tumor cells. In controls, tumor cells were numerous and showed high proportion of mitoses. Again nonviability of the altered cells was demonstrated by their inability to multiply or even to survive after their transfer to new mice.

On injection, the ultramicroscopic colloidal gold particles mixed freely with the peritoneal fluid. Later they condensed into dot-like particles in the cytoplasm of the macrophages. The macrophages containing the gold maintained their structural and functional integrity, while lymphocytes and polymorphonuclears are partially and temporarily destroyed by the radiation effect.

The conclusion is drawn that the sarcoma-37 tumor cells are highly sensitive to small amounts of radiation well tolerated by the mice themselves while macrophages showed very high resistance. Thus the complete destruction of free tumor cells in the peritoneal cavity may be described as a selective radiotherapeutic effect of the radioactive colloidal gold.

Hahn's clinical work has been about equally divided between the treatment of lymphoid-microphagic diseases and tumor therapy by direct infiltration (*108*). His group has used some 15,000 mc (the equivalent of 15 gm of radium) in giving 620 treatments to 268 patients.

The method of direct infiltration of the tissue to be irradiated represents a considerable "come-down" from the theoretical ideal of selective localization following intravenous administration. To us as chemists, however, it represents a more realistic view of the current situation. The more we use isotopic tracers, the more convinced we become of the complexity of biological processes and the fact that at least some of almost everything thus administered becomes dispersed throughout the body. To subject "innocent" normal tissues to the radiation attendant upon the localization of this range of activities is almost out of the question.

This point becomes increasingly important as evidence increases that we may have to revise our ideas of the amount of isotopic radiation required to destroy tumor cells completely. Clinically, doses of 50–150 mc are common. The optimal amount appears to be of the order of 0.6 mc of gold per gram of tissue.

Hahn has recently gone as high as 200,000 cr, in which range the contribution of the gamma rays plays a significant part in the radiation of the regional lymphatics (*108*). Obviously, the time over which the dose is acting is important, and 1 roentgen given over a week or so is less lethal than the same energy given over a few minutes. On the other side of the ledger, there seems little question but that avoidance of injury to adjacent tissue will permit much larger doses with no reduction in safety.

The question is often asked why, if the tumor can be reached for injection, surgery is not performed. The oncologist and the surgeon frequently find tumors where extensive scar tissue or the condition of the patient preclude operative procedures or the use of X-ray. Then, too, if a tumor has been removed, stray cells may remain that travel along lymph channels and start new tumors. The implantation in the operative area of a radioactive material which will help to destroy these cells before they become established, is the goal toward which several collaborating groups are working, with,

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FIG. 6. (Left) Untreated control mouse with two large transplanted sarcomas. (Right) Same mouse with depilitated scars that remained after complete regression of treated tumors

it seems, a reasonable chance of success.

The second big question is how well does such a colloid remain in the tumor. Again animal experimentation, as well as clinical trial, is supplying the answer. See Fig. 6.

Recently, Williams of the Medical College of Virginia reported before the Southern Medical Association the results of his studies (*109*). Using a semipermeable cellophane bag, he found not more than 4% of the gold as we supplied it dialyzes over several days. Mice and rats readily tolerated 3 and 7 cm^3 of an equivalent "gold" colloid, showing absence of "gold" toxicity. Following intraperitoneal, intracardiac, intrasplenic, and intratumoral injections, the loss of radiogold through the urine and stools was less than 0.1% during the first week, and none thereafter. In other words, 99% of the active gold is retained within the tissue indefinitely.

Four types of mass tumors (usually 1–1.5 cm in diameter) were injected with small doses of colloid. With 22 relatively solid carcinomas, 15 retained 95–100%, 5 retained 90–95% and 2 retained 85–90%.

Injection of 200–500 μc of colloid into sarcomas-180 and 37 gave regressions in 21 of 31 tumors—67%. Local infiltration and systematic administration of nitrogen mustards gave 42% regression; combination of the two gave 82% regression.

Another recent report is that of Sherman *et al.* of Washington University, St. Louis (*110*). They have taken the colloid we supplied and added pectin as a further suspending agent. Using interstitial injections, transplanted squamous-cell carcinoma in mice could be safely and completely "cured" by 200–300 μc per cm^3 of tumor tissue, with high survival rates. Injected into the paracervical tissue of rabbits, gold entered the lymphatic system and reached primary and secondary lymph nodes where definite radiation effects were noted. Introduced into the uterine horns of rabbits, radiation effects extended to the ovaries.

Sherman has also reported on the use of colloidal gold in the treatment of cervical cancer. The doses were purposely held below the presumed optimal therapeutic level so that possible toxic effects could be evaluated. None were found, and it was possible to demonstrate active uptake and transport along lymphatic channels. It may, therefore, offer a valuable adjunct to surgical removal, by decreasing the possibility of the development of metastases (*111*).

Clarke and LeRoy likewise have reported that colloidal gold injected into tissues remains fixed at the site of injection (*112*). A 50,000-er dose has been found effective without harmful local or systemic reactions. When the gold is given intravenously, some 80% goes to the liver.

We have recently received a personal report from Dr. J. H. Müller of Zurich. In spite of the difficulties of transatlantic transportation he has used our gold colloid in 6 cases of inoperable ovarian malignancies. Administration was by intraperitoneal injection of 100–150 mc, the patient being mechanically rotated at frequent intervals.

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The results have been in his words "decidedly positive," notably in the stoppage of ascitic fluid production. No apparent damage has resulted to surrounding tissues. There was only nominal excretion of activity in the urine; no side reactions were noted.

It is to be emphasized that there is every advantage in removing all large tumor masses. Several gold treatments are commonly employed, but the management of each case is an individual problem, and no fixed rules can be laid down. We have recently made a number of shipments to Latin American countries for use on tumors.

Dr. D. W. Smithers of London reported upon his experiences with gold before the recent International Congress on Radiology. The abstract of his paper is in part as follows: "Intraperitoneal injections of colloidal gold were given to five patients, once shortly before death so as to observe its distribution and four times as treatment for carcinomatosis peritoneii. One of these patients, a woman of 41, was treated with a radical mastectomy for carcinoma of the breast in January, 1947. She remained well until January of this year when she had a rapid onset of abdominal swelling and was found to have ascites and an ovarian tumor; the whole peritoneal surface was studded with secondaries. Both ovaries were removed and an intraperitoneal injection of 95.5 millicuries of colloidal gold-198 in 1000 cm^3 of saline was given shortly afterwards. She had some flatulence and discomfort after food for a few weeks but has otherwise remained very well for six months since her treatment, had no return of ascites. She and the three others since treated are being followed with interest. Dr. Müller of Zurich has been using a similar technique and is reading a paper on this subject to the congress this week. We have measured the gamma-ray dose received by an ionization chamber swallowed before treatment and passing through the intestine during the period of activity of the gold. This has proved to be an interesting observation, the physics department having predicted the gamma-ray dose that would be measured with remarkable accuracy."

Müller pioneered the use of Zn^{63}; this element in pectin has a remarkable affinity for the pulmonary capillaries, as much as 40 mc having been given to a single patient with no activity demonstrable in the urine (*113*).

Müller has also employed simple solutions of Co^{60} salts (and enough NaI to render them radio-opaque) in flexible "balloons" for the irradiation of bladder tumors (*114*). An English group, Wallace, Walton and Sinclair (*115*), has used Na^{24} for irradiation of the bladder in carcinoma; 200 mc in 150 cm^3 of Na^{24} solution were used to give gamma-ray doses of 2,000-3,000 r. All of the ten cases showed some evidence of regression.

Haik has discussed the advantages of a pure beta emitter in the treatment of ophthalmological lesions (*116*).

Since arsenic has long played an important role in medicinal chemistry, it seems quite appropriate to mention the work of Jacobson and his group at the University of Chicago on As^{76} as a source of both radiation and chemotherapeutic effects. Cacodylic acid was pile-bombarded and the As isolated as the trichloride (*117*). Patients treated included 24 with tumors, 2 with polycythemia, and 1 with metastatic carcinoma. They concluded that in view of the considerable difficulties in handling, this isotope offered no particular advantages (*118, 119*).

A theoretically interesting modification is to localize an element like U^{235} in an organ such as the liver or spleen, and then to subject the area to slow neutron bombardment. The nuclear disintegration thus induced produces

greater radiation effects than beta radiation itself due to the fission recoils (*120*).

Radioactive colloidal gold has been shown to produce uniform and consistent remissions in chronic leukemia in humans, both in the lymphogenous and myelogenous forms. In these types it sometimes possesses the advantage of longer term palliation (*121*).

The group at the Southern Research Institute have shown that administration of C^{14}-labeled $NaHCO_3$, and C^{14}-labeled 2,6-diaminopurine have no effect on the pattern of deaths from leukemia in mice (*122*).

The many experiments enumerated above certainly indicate the potential value of individual radioisotopes in the radiation therapy of tumors.

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Radioisotopes in Pharmaceutical and Medical Studies—II

In this second of a series of three articles, reference is made to both published and unpublished information on tagged barbiturates, amino acids, and other compounds of interest in metabolism studies. Antigens, antithyroid drugs, membrane permeability, and C^{14} toxicity are also discussed

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IN THIS SECOND of a series of three articles, discussion of a wide range of topics is offered to add to the picture of how radioisotopes are being put to use in biology and medicine today.

A brief introduction to the subject of isotopic medicine and an explanation of Abbott Laboratories' "isotope program" for giving assistance to researchers in this field were contained in the first article in this series, which also took up at length the following topics: 1. *Uses of Iodine-131;* 2. *Uses of Phosphorus-32;* 3. *Tumor Localization;* 4. *Other Diagnostic Agents;* and 5. *Tumor Therapy.* In continuation, the topics to be discussed below are: 6. *Barbiturates and Thiobarbiturates;* 7. *Sulfur Amino Acids;* 8. *The Dynamic State.* 9. *Membrane Permeability;* 10. *Antigens and Antibodies;* 11. *Fat Metabolism;* 12. *Thyroid and Antithyroid Drugs;* 13. *Metabolic Studies with Phosphorus-32;* 14. *Isotopes as an Analytical Tool;* 15. *Mechanical Tracing;* and 16. *Toxicity of Carbon-14.*

The numbering of figures and bibliography references is continued from that used in Part I.*

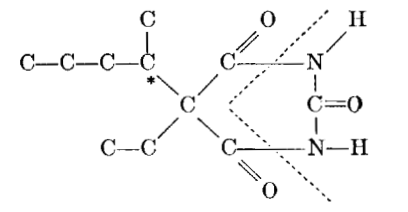
* Part I appeared in the November, 1950, NUCLEONICS, page 3. Part III, which includes discussion of the additional subject of *Drug Metabolism* and conclusions to be drawn from the three-part paper as a whole, will be published in the January NUCLEONICS.

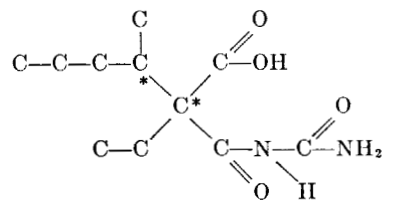
6. Barbiturates and Thiobarbiturates

Our knowledge of the metabolic fate of the barbiturates and thiobarbiturates has benefited much from isotopic studies. Even before C^{14} became popular as a label, we had tagged nembutal with N^{15}. The first publication by Van Dyke, Scudi, and Tabern (*123*) showed that when given orally in therapeutic ranges, 60% of the total N^{15} was excreted in the first 24 hours, but that less than 8% was found in urinary ammonia or urea fractions.

Within the past few months Maynert and Van Dyke have synthesized the expected ring breakdown products, the malonuric acid, the acetyl urea, the acetamid, and the malonamid, and applied the test of isotopic dilution to the urine containing the actual metabolic products (*124*). The latter three were definitely absent; at first it seemed that the malonuric contained the N^{15}, but conversion to derivatives proved that it was there simply as an impurity.

There was one more possibility—while nembutal contains but one asymmetric carbon, opening of the ring can give rise to two, with the theoretical existence of two racemic forms. Only one was secured synthetically, but the body could have made the other:





On the other hand, amytal, having no asymmetric carbon, could give rise to compounds having but one racemic form. Labeled amytal was made by the Lilly group. Here, too, none of the labeling could be found in the expected breakdown products.

The answer would seem to lie in the isolation by the Columbia group of a metabolite which is apparently 5-ethyl-5-(3-hydroxyl-1-methyl butyl) barbituric acid. We understand that the amytal analog has also been secured (*125*). Such side chain oxidation had been reported many years ago by the Germans, but only when unsaturation existed there. The products from amytal are found to be excreted at less than one half the rate for nembutal, an observation in harmony with the therapeutic duration of action of the two drugs.

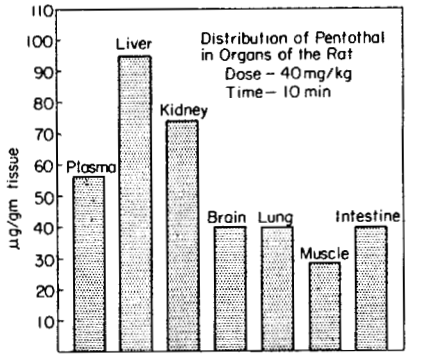


FIG. 7. Pentothal distribution in organs (*129*)

In barbital tagged by us and studied by the Columbia group, there seemed to be no destruction at all. Apparently 3 and probably 4 carbons are necessary before side chain oxidation takes place. Specific experiments were carried out to check the old observations of Keeser that barbital is specifically localized in the diencephalon of dogs and rabbits (*126*). There seemed to be no such localization.

Nembutal, C^{14}-labeled in the urea portion, has been prepared by the Los Alamos Organic Group. No significant amount of labeled $C^{14}O_2$ was eliminated. By chromatography, they showed the presence of five products in the urine but their nature was not further studied (*127*). Kahn has also reported work in this field (*128*).

Left unanswered was the most intriguing question of all: Why does the simple replacement of the oxygen of nembutal by sulfur to give the well-known intravenous anesthetic pentothal cause so marked a change in the duration of action and other properties? For more than a year now, we have been using S^{35}-labeled pentothal in an effort to answer some of these metabolic questions. See Fig. 7.

We found that, as the figure shows,

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pentothal was, like barbital, reasonably randomly distributed throughout the body, with the usual somewhat higher concentration in the liver and kidneys (*129*). See also report of Bollman *et al.* (*130*). Certainly there was no preference for the central nervous system, and the disappearance from the blood stream was too prompt to be accounted for by metabolism alone. For instance, in the rat, most of the activity is excreted in the first 24 hours, with a total of 86 % being accounted for in urine and feces at the end of 4 days. During 24 hours, 26 % was as inorganic sulfate, 6 % as ethereal sulfate, 39 % as neutral sulfur, and 9 % as chloroform extractable. This alone makes improbable a ring breakdown to thiourea, since that product gives rise to little sulfate.

The results from some experiments with monkeys are qualitatively similar, with the $CHCl_3$ extractables going up to 14 %.

The chloroform extractable fraction was at first classified as unchanged pentothal on the basis of its ultraviolet absorption, but since the characteristic band at 286 is due to the ring structure, identification was uncertain. The New York University group has supplied another missing link—the isolation of "pentothal carboxylic acid," in which one of the side chain methyls has been oxidized to an acid (*131*). It absorbs at the same ultraviolet wavelength as pentothal, but has a slightly different extinction coefficient. This is obviously reminiscent of the work of Fieser on the napthaquinone antimalarials (*132*). Following large doses, man excretes 10–25 % of pentothal in this form. We have injected radioactive pentothal in monkeys and extracted the corresponding tagged compound which has been used, in turn, for a study of its metabolism.

In an effort to resolve the highly complex mixture present in the urine, we used filter-paper chromatography.

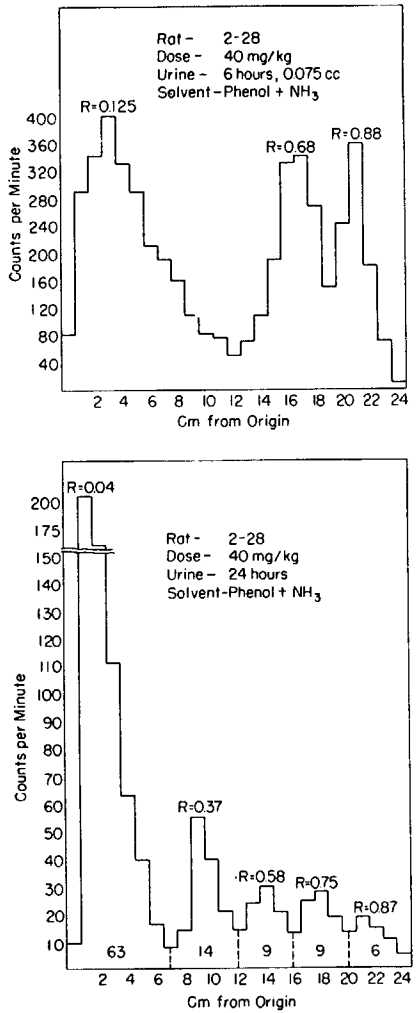

FIG. 8. Pentothal distribution in rat urine after 6 hours and after 24 hours, as determined by paper chromatograms. *R* denotes the R_f value, namely, the distance traveled by the individual band, expressed in terms of per cent of the total distance traveled by the solvent

[Work in this field has also been done by Tomarelli (*133*)]. We were fortunate in having five radioactive compounds on hand for comparison. They were thiourea, methionine, sulfate, sulfide and thiocyanate, and their R_f values on our paper chromatograms are as follows:

	Phenol Ammonia	*Collidine*	*77 % Alcohol*
Thiourea	0.60	0.70	0.62
Methionine	0.79	0.38	0.49
Sulfate	0.05	0.05	0.05
Thiocyanate	0.22	0.84	0.77

The R_f values on sulfide are purposely omitted from the table because in each case several bands were figured. Sulfide is oxidized by air, giving some sulfate, and there is evidence that it also reacts with phenol and possibly with collidine; to present all of this data here would unnecessarily complicate the picture.

The number and relative amounts of the degradation products from pentothal varied with the species and the time after injection. For instance, in one fraction of monkey urine at least nine radioactive compounds were found, four of them extractable by chloroform. One of the latter is unchanged pentothal, and another probably the pentothal carboxylic acid described by Brodie (*131*).

Pentothal distribution in rat urine, as determined by paper chromatography, is shown in Fig. 8. Results too extensive to be quoted here led us to believe that there must be at least two or probably three modes of destruction possible in the body: (1) side chain oxidation, and (2) enzymatic removal of the sulfur.

A third route for which there is admittedly no direct evidence may well be the one the old-time organic chemist would expect, namely, the splitting of the ring with the ultimate liberation of H_2S and NH_3 in line with the in-vitro experiments of Gad (*134*). The fact that the injection of "hot" sulfide gives a chromatographic picture resembling parts of that formed after pentothal makes such a mechanism seem reasonable.

By way of explaining the previously mentioned discrepancy between removal from the blood and destruction, Mark and associates have recently called attention to the role of body fat as a depot, while Goldbaum has stressed the relatively firm combination (first observed by Richards) between pentothal and certain body proteins (*135*, *136*).

Perhaps this "buffering" action of fat stores on pentothal blood concentrations, plus the obvious multiple alternate routes of destruction and elimination, are in part responsible for pentothal's practical safety, even with a known disturbed body function.

7. Sulfur Amino Acids

We have developed a simple method of tagging thiourea, the prime ingredient of the thiobarbiturates. This method consistently gives us a very pure material in a yield of 85 %. Specific activities of not less than 12–25 mc/gm have been ample to date, but samples of higher activity can be made as needed.

Thiourea seemed a suitable intermediate for methionine and homocystine, and recently has been adapted for use in the preparation for cystine as well. Several very successful runs have been made starting with 25–30 mc of S^{35} in each case. We have found that, by proper coordination of requests from a number of sources and operation at this level of activity (with the recovery of all activity in the residues), it is possible to make supplies available at a fraction of previous costs, and greatly

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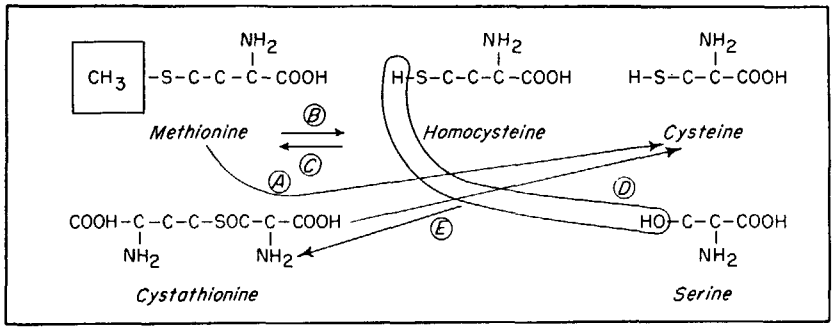

below cost of similar C^{14} compounds.

Since only one optical form of most amino acids is utilized by the body, resolution of synthetic samples becomes a real problem. Wood and Gutman have employed a most ingenious method (*137*). If we take, for instance, active benzyl-*d,l*-cysteine and mix it with inactive *l*-benzyl cysteine and repeatedly recrystallize, there is an exchange that results in the formation to a considerable measure of free *l*-active *l*-benzyl cysteine which comes out (in somewhat diluted form) during the purification.

While we are concerned with these sulfur-containing amino acids, it would seem quite appropriate to review briefly what the Cornell and California biochemical groups (and others) have contributed in recent years to our knowledge of the metabolism of these acids.

In 1931, Jackson and Block observed that methionine could replace cystine in the diet of growing animals (*138*). Tarver and Schmidt proved the reality of the biological conversion by feeding labeled methionine and isolating labeled cystine from tissues (*139*). This was recently confirmed for the cystinuric human by Reed *et al.* (*140*).

An inspection of the formulas in the diagram above shows that to do this by straightforward methods would require the apparent removal of one carbon atom from the chain without touching the S or the carboxyl at the ends (*A*).

It has been shown also by isotopic studies that the methyl group of methionine is labile, and that not only can methionine be demethylated to homocysteine (*B*), but that, for the formation of methionine (*C*), the body can utilize them ethyl groups of choline or betaine (*141*). The term "transmethylation" has been applied to these metabolic reactions, and it now appears, incidentally, that B-12 plays a part in the process. To explain the methionine-cysteine conversion more logically, the intervention of cystathionine was evoked, the assumption being that it was formed from homocysteine and amino acrylic acid, and that it, in turn, reduced to cysteine (*D*).

In testing this hypothesis, du Vigneaud and his group shrewdly tried the (*l-l-*) isomer of cystathionine, and found that it (but not the other isomers) could serve as a source of cysteine for body growth. By another ingenious experiment, this same group found that the splitting (*D*) could be carried out by a liver enzyme. The same enzyme went farther and formed labeled cystathionine from homocysteine and serine (*E*) in an over-all yield (*E–D*) of 60%.

Recently Stekol, Weiss, and Weiss fed C^{14}-methyl-labeled methionine and glycine C^{14}-labeled on the amino carbon (*142*). Both showed up in cysteine (isolated as *p*-brom-phenyl mercapturic

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acid), presumably through the serine as just indicated.

Again by tracer techniques, but in this case with stable isotopes, the S and the C atoms of methionine were tagged, and it was fed to rats. It was shown that the resultant cysteine from the hair had taken up the sulfur but not the carbon, again clearly indicating the mechanism of the reaction.

The relationship of methyl donating ability to chemical structure has been studied by Maw and du Vigneaud. Dimethyl propiothetin was found to be highly active but S-methyl thioglycollic acid, and several simpler thetin compounds were found quite inactive (*143*).

The methyl group of methionine can give rise to formate and can serve as the source of the beta-carbon of serine (*144*). The methyl group of choline also gives rise to formate, as does the beta-carbon of serine. It has been confirmed that the reverse procedure occurs and that formate is reduced, in some unknown manner, to become the methyl group of methionine.

Along the same line, but still somewhat unexpected, is the report of Mackenzie that formaldehyde is a naturally occurring compound in the animal organism (*145*). It was isolated by several methods following the incubation of C^{14}-labeled sarcosine and liver slices or homogenates. Formic acid and CO_2 were also produced, the former appearing in the urine. Mackenzie has indicated that the methyl group of methionine can become part of sarcosine and part of the adenine of nucleic acids and the porphyrin of hemoglobin as well. Obviously, methyl groups are a highly essential link in metabolic processes.

Further support comes from Siegel and Lafaye (*146*), who find that C^{14} from labeled formaldehyde was converted (by rat liver homogenate) into the beta-carbon of serine more rapidly than from formate and glycerol.

The part that living tissues play in the actual build-up of labile methyl groups has received further confirmation in a unique experiment carried on jointly by the Columbia group and the Lobund Laboratory of Notre Dame. Two rats maintained in a sterile condition were fed D_2O for 23 days and at the conclusion of the experiment, the body choline was isolated. Data showed conclusively that methyl groups had been synthesized by the germ-free animals (*147*). In this same report, information on the influence of B-12 factors on methyl transfers and the role of possible precursors is brought up to date.

A paper by Kinsel and others of a large group at Berkeley represents a decided step forward in the use of isotopes as diagnostic research tools (*148*). They used tracer doses of S^{35}-labeled methionine in the study of protein metabolism in the human and report that, in addition to being safe to use in doses of 25–100 μc, S^{35} has many of the advantages of C^{14} and is free from the serious disadvantage of the 5,000-year half-life of the latter. Metabolically, too, it has certain specific advantages.

Their findings indicate, first of all, that the incorporation of S^{35} into plasma proteins, and its subsequent disappearance and excretion follows, in normals, a typical picture. In patients with chronic liver disease, with idiopathic hypoproteinemia and with Cushings syndrome, significant deviations from normal were observed. (Other S^{35} studies will be discussed in Part III under the subject of *Drug Metabolism.*)

Reference should be made to the protective action of cysteine against radiation effects (*149*).

The demonstration of the ready accomplishment by the body of seemingly complicated reactions (complicated at least for the organic chemist) is but a simple example of the concept which isotopes have done so much to establish. By way of emphasis we would like to

quote in the following section two paragraphs from the Schoenheimer monograph on the subject of "Dynamic State of Body Constituents" (*150*).

8. The Dynamic State

"The large and complex molecules and their component units, fatty acids, amino acids, and nucleic acids, are constantly involved in rapid chemical reactions. Ester, peptide, and other linkages open; the fragments thereby liberated merge with those derived from the other large molecules, and with those absorbed from the intestinal tract, to form a metabolic pool of components indistinguishable as to origin. These liberated molecules are again subject to numerous processes. Fatty acids are dehydrogenated, hydrogenated, degraded or elongated, and thereby continually interconverted. While some individual molecules of these acids are completely degraded, other individuals of the same chemical species are steadily formed from entirely different substances, notably from carbohydrate. Similar reactions occur among the split products of the proteins. The free amino acids are deaminated, and the nitrogen liberated is transferred to other previously deaminated molecules to form new amino acids. Part of the pool of newly formed small molecules constantly re-enters vacant places in the large molecules to restore the fats, the proteins, and the nucleoproteins. Some of the small molecules involved in these regeneration reactions constitute intermediate steps in the formation of excretory products. Part of the nitrogen in mammals travels via the amidine groups of arginine to be either excreted as urea or transferred to glycine to form guanidoacetic acid, creatine, and creatinine. In birds, the nitrogen on its way to excretion does not take the form of amidine groups, but may, for short periods, be present in the purine groups of nucleic acids. Components of an animal are rapidly degraded into specific molecular groups, which may wander from one place to another. The chemical reactions must be balanced so delicately that, through regeneration, the body components remain constant in total amount and in structure. This constancy is not to be taken as an indication that the structural matter of the living organism is inactive and takes little part in metabolism.

"All regeneration reactions must be enzymatic in nature. The large molecules, such as the fats and the proteins, are, under the influence of lytic enzymes, constantly degraded to their constituent fragments. These changes are balanced by synthetic processes which must be coupled to other chemical reactions, such as oxidation . . ."

9. Membrane Permeability

In these days when studies of so-called capillary permeability and fragility are so much in the literature with reference to the actions of rutin, "vitamin P," vitamin C, and now of the steroid hormones like cortisone, we may well consider recent publications from the Carnegie Institute and Tulane.

If Na^{24} is given intravenously and proper determinations made of its rate of disappearance, it becomes mathematically possible to show that 60% of the sodium of the plasma leaves the plasma each minute to be replaced by the sodium from the extravascular space (*151*). The corresponding percentage for Cl (Cl^{38}) is approximately the same, while that for water is higher, 140% per minute. To interpret these figures in somewhat more dramatic terms, a quantity of water equivalent to the entire weight of the individual is lost from and then replaced into the plasma every 20 minutes. It has been calculated that 50 pounds of salt is thus transferred across the capillary membranes every day.

Iron on the other hand is lost at less than 1% per minute, presumably because it exists there as a globulinate of quite different particle size. The question as to the relation of such permeability to the type of organ, to the condition of the test animal, and to various drug actions would seem to be a very fruitful field for research with isotopes. A good start has already been made; it has been noted that, in the dog, the disappearance curve is not simple but consists of several components. The reason seems to be that in certain tissues like muscle, lung, intestine and liver, there is rapid transfer; in others like skin, bone, tendon, and brain, the rate is relatively slow.

The Quimby group has studied the effect of drugs on diffusion of Na^{24} from blood vessels (*152*). Histamine increased such transfer, but papverine was without action.

Both Na^{34} and K^{42} have been used to study the flow of these ions across the gastric mucosa (frog). The transport of the two from the secretory to the nutrient side was similar, but in the opposite direction, the K moved only 5% as fast. The addition of histamine reversibly increased K transport as much as 10% (*153, 154*).

It has been shown by C^{14}-tagged plasma (made by lysine feeding of anemic and hypoproteinemic dogs) that carbon is in rapid exchange with the ascitic fluid (*155*).

In passing, we should mention the practical use made of isotopic iron in a pharmaceutical problem during the war. Fe^{59}-tagged hemoglobin was "grown" in the donor dog. Blood was then removed and subjected to the various proposed stages of processing. Later it was injected into a recipient animal, and the survival time of the red cells was determined. Survival of 70% was taken as fair and 90% as excellent. Further studies are in progress.

10. Antigens and Antibodies

No discussion relating to the use of isotopes in medical and pharmaceutical problems could possibly ignore current studies on the antigen-antibody mechanism. It has been mentioned earlier that albumins and globulins may be tagged with iodine without affecting their normal behavior. They can also be tagged with S^{35}, C^{14}, or P^{32}, though much less conveniently (*156*).

Crampton and Haurowitz (*157*) injected rabbits with iodine-tagged antigens and studied the subsequent distribution of activity in the homogenized liver of the animal. The bulk of the deposited antigen was found in the mitochondria fraction, from which fact the conclusion is drawn that these particles are involved in the formation of antibodies.

One theory of anaphylactic shock states that the antigen combines with antibodies fixed to the cells, while the humoral theory maintains that the reaction occurs with antibodies in the circulating blood. By using I^{131}-tagged bovine gamma globulin as an antigen in guinea pigs, Warren and Dixon were able to locate significant antigen concentrations during such shock (*158*). Only the lung and the liver were involved, and since in the latter organ the

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histological distribution was the same in sensitized and non-sensitized animals, it was presumably not related to the degree of sensitivity. The sensitized lung, on the other hand, showed twice the affinity of the controls. This increased amount of antigen complex was found in the edematous bronchial fibrous tissue, and the edema appeared to be the principal factor of bronchial obstruction. Labeled crystalline egg albumin behaves similarly. Knox has compared such isotopic tests with quantitative precipitin tests and found good agreement (*159*). Further studies on this subject have been made by Garvey and Korngold (*160, 161*).

It has been emphasized earlier that we know of no *chemical*, except iodine, with a sufficiently specific and firm localization in living tissue to be of practical value against tumor cells. Several investigations have shown that exposure of tumor tissue to its homologous antisera before transplanting destroyed its ability to propagate. It seemed, therefore, that, by tagging, it might be possible to demonstrate some sort of localization and reaction between the malignant tissue and antisera.

Pressman, of the Sloan-Kettering Institute for Cancer Research, has made basic and important contributions in this field (*162, 163*). An antiserum was prepared against egg ovalbumin, iodinated by tracer amounts of I^{131}. By suitable experiments it was demonstrated that it retained its specificity. In a similar manner, "anti rat kidney serum" (which is known to produce physiological changes in the structure of the kidney) was prepared and tagged; upon giving to rats, a definite accumulation of activity was found in the kidney. Tagged antiovalbumin, given under the same conditions, did not show up in the kidney. It was further found that the biological half-life of such activity in the kidney is 3 to 10 times that of the small activity picked up by other organs, which is taken to mean that the antibody is attached to the cells in such a way that tissue enzymes cannot metabolize it. In fact, tenacity of retention (in the glomerulus) of this combination even exceeds that of iodine in the thyroid (*164*).

A very ingenious method was reported for the protection of the specifically active region of the antibody during iodination. This consisted in reacting with the benzoate ion or the benzene arsonate ion. The protecting "hapten" could be subsequently removed by dialysis. For further studies on the precipitin reaction and antibody, see Banks *et al.* (*165*).

Butement, using I^{131}-tagged globulins, found that the quantity of agglutinin for precipitation corresponds roughly to formation of a unimolecular layer of globulin on the bacterial surface (*166*).

11. Fat Metabolism

A discussion of the field of carbohydrate and lower fatty acid metabolism is beyond the scope of this paper, but in view of current nutritional research interest in the use of fat emulsions for caloric feeding, we should like to mention some contributions which isotopic studies have made in this field.

Lerner *et al.* have made very fine emulsions from C^{14}-labeled synthetic palmatin (*167*). Given intravenously (intramuscular absorption is too slow), active $C^{14}O_2$ appeared in the expired air within 1 hour; 36 to 60% of the dose had been thus eliminated within 24 hours. Between 6 and 8% appeared in fat deposits. Furthermore, a large percentage of the labeled fatty acids in the liver and intestines had been utilized for incorporation into phospholipids.

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The Boston group used $C^{14}OOH$-labeled trilaurin and found that in 4.5 hours 76% had been exhaled as $C^{14}O_2$, possibly because lauric acid derivatives (12 carbons) are not measurably deposited in adipose tissue (*168*).

Stanley and Thannhauser fed patients an iodized oil (*169*). During the first 24 hours, 50 to 78% of the activity was either collected in the thyroid or excreted in the urine. In fact, its destruction curve paralleled destruction of the C^{14} analog, indicating that iodide liberation results from, or parallels, metabolism for the normal use in the animal. Patients with idiopathic hyper lipemia utilize lipids much more slowly. Rutenburg, Seligman and Fine also found absorption from tissue was slow and was not appreciably increased by hyaluronidase (*170*). They found that more than half of the activity present in the circulation after 24 hours was in the lipid fraction, and 20% as inorganic iodide. Apparently there is storage in lung, liver and spleen, with gradual release over several days. We have supplied such iodized oils and emulsions as tools for use in several quite unrelated problems.

The California group employed C^{14}-palmitic acid and tripalmatin to study fat transfer in the rat (*171*). Within 19–24 hours, some 90% had been absorbed. Of this amount, 70–92% was recovered as the acid from the thoracic duct lymph and 69–84% from the intestinal lymph. These figures, obviously, do not completely rule out alternative modes of transfer.

12. Thyroid and Antithyroid Drugs

Isotopes have made a definite and valuable contribution in another field, the antithyroid drugs. Thiouracil and its methyl and propyl homologs were introduced on the basis of simple biological experiments wherein the animal growth rate, or the thyroid weight, was taken as a criterion of suppression of thyroid activity. It was obvious, however, that serious species differences were present.

In this connection, Astwood and his group began experiments on human beings—an expedient made possible by the nontoxic nature of tracer doses of I^{131} (*172*). A standard dose of activity was given and the exact thyroid uptake curve for that patient was determined. When its slope was established, a definite dose of the drug under test was given; if active, the "pickup" decreased or stopped entirely for a longer or shorter period of time. It was found that not propyl thiouracil but mercapto imidazole is the most active compound, and apparently the latter is now in clinical trial (*173–176*). See Fig. 9 on the next page for some test results.

13. Metabolic Studies with Phosphorus-32

Phosphorus plays such an important part in so many biological processes and the properties of P^{32} are so favorable for experimentation that an extensive (and sometimes confusing) literature has developed. Since Kamen's book "Radioactive Tracers in Biology" (*177*) has an excellent chapter devoted to the details of reported experiments, and Hevesy (*178*) and Chaikoff (*179*) have written excellent reviews, only a few remarks directly bearing on our subject will be made here.

P^{32} is absorbed to a high degree when injected and to varying degrees when given orally. In addition to going

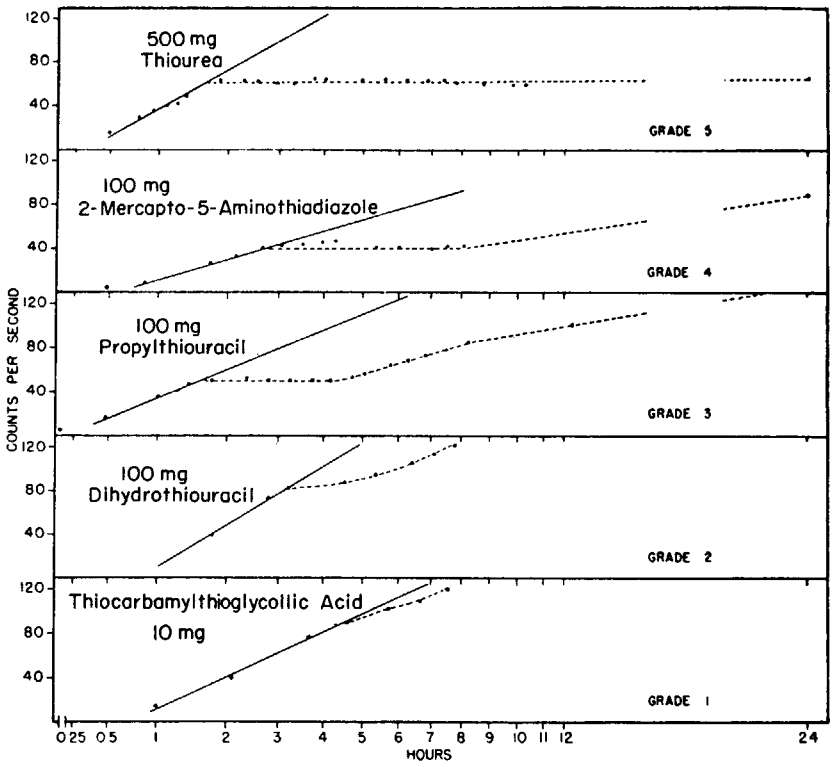

FIG. 9. Tests showing how the uptake of radioiodine by the human thyroid is influenced by the administration of "antithyroid agents" (*173*)

rapidly into extracellular space and into bone, it is metabolized into phosphate esters of the hexosephosphate type, into the nucleic acid and nucleoproteins, into phospholipids, etc. It is possible to prepare phosphorylated intermediates of varying "energy levels," enzymatically and biologically, and it is with perfection of these techniques that a great amount of current work is concerned, particularly at the biological division laboratories at Oak Ridge (*180–183*). In general, such bound phosphate compounds do not exchange in vitro with inorganic phosphate, so that any such change observed represents some metabolic process. The ability of any protein to incorporate P^{32} is a measure of its metabolic turnover. The thymus nucleoprotein has an especially rapid turnover.

In phospholipids, P^{32} holds on one side an organic base such as choline and on the other a complex alcohol, and as such it is not exchangeable. Phospholipids are normally prepared in the liver but may be synthesized also by the kidney and intestine.

Of considerable value in medicine is knowledge concerning the mechanism by which lipo-tropic substances, such as choline and betaine, prevent or relieve fatty liver. This appears to be primarily a stimulation of phospholipid turnover, and can be measured by suitable P^{32} studies (*184–187*).

Cayer and Cornatzer reason that, since plasma phospholipids are likewise made in the liver, measurements made on the newly formed plasma components should mirror the liver activity. They have such a study in progress (*188–191*).

Pihl and Bloch using C^{14}-labeled acetic acid as a precursor have shown that phospholipids (of the liver) are not especially necessary precursors of neutral fats (*192*).

The uptake and turnover of phosphorus appear to be low in all phosphorus-containing fractions of normal nerve tissue. In contrast, regenerating nerve about one week after section shows a much greater uptake in all fractions (*193*).

The University of Southern California group has used P^{32} and I^{131} in an effort to study the mechanism of virus action (*194*). When a virus (GD-7) was mixed with minced brain, the uptake of P^{32} was markedly stimulated during the period of maximal virus propagation. The virus-containing tissue had an increased ribonucleic acid P content and a decreased desoxyribonucleic picture. There was also increased incorporation of glucose fragments in the protein fraction and decreased incorporation in the lipid fraction of the injected tissue. CO_2 uptake did not seem to be influenced. P^{32} uptake has been studied by Graham (*195*).

It has been shown that phospholipids may be absorbed in various stages of hydrolysis, varying from the intact molecule to inorganic sulfate (*196*).

Of particular interest is the fact that malignant tissues take up P^{32} somewhat more rapidly than do most normal tissues, except those concerned with its metabolism. At least at first the major portion of the activity is in nucleoprotein. For instance, the Harvard group has found that the rate of P^{32} turnover by gastric carcinoma is at least 45% greater than by normal gastric mucosa. This picture was attributed to a 124% increase in turnover in protein phosphorous, a 45% increase in turnover in lipid phosphorous, but no change in acid-soluble phosphate. It should be emphasized, however, that the phosphorous total content remained normal (*197*).

The influence of hormonal inbalance offers another fertile field for research. For instance, P^{32} uptake by slices of grey matter increased 50 to 400% after hypophysectomy because of an increased rate of phosphorylation.

The paper chromatography of P^{32}-labeled phospholipids has always been difficult, and the bands observed probably do not always represent entities. O'Leary and Neuman have presented a resumé of present information (*198*).

A Russian publication reports that camphor accelerates P^{32} exchange 17–20 times in the brain and only 5 times in the muscles. This, it is stated, is the cause of camphor convulsions of the central nervous system. Tetraethyl ammonium iodide has an opposite effect; that is, it slows P^{32} exchange (*199*).

Also of considerable potential value in our field is the production of biological mutations due to chromosome breakage induced by P^{32} (*200*).

Mention should be made of the use of P^{32} in parasitology by McCoy, Downing, and Van Voorhis (*201*). They found that *Trichinella spiralis* larvae in the encysted stage exchange ions through the cyst wall and probably, therefore, carry on an active metabolism

14. Isotopes as an Analytical Tool

The extreme sensitivity of radioisotope measurements would seem to make isotopes ideal as analytical tools. As such, they seem neglected (*202*).

Tobias and the California group have made use of "activation analysis," with

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neutrons from a reactor as the activator (*208*). If an element capable of absorbing neutrons is thus irradiated, it becomes activated to a degree proportional to the time of exposure. If a set of samples is thus treated and covers the expected range of concentration, simple counting of all samples suffices to give the concentration of the unknown. Actually, of course, all other absorbing elements present will be similarily activated; thus recourse must be had to chemical methods, and absorbers must be used in counting in most practical cases. The almost unbelievable sensitivity of the procedure is evidenced by the fact that only 10 cc of blood was needed to show concentrations of gold of the order 10×10^{-6} grams in an entire human body.

This method should have interesting possibilities in the quantitative study of trace impurities that influence pharmaceutical stabilities in nutritional problems and in the labeling of pharmaceutical specialties (*204, 205*).

Thyroid iodine, and thyroid P^{32} uptake have been used as a measure of thyrotropic hormone efficiency, and of sex hormone clinical efficiency (*206, 207*).

Dorfman has made ingenious use of K^{42} for the assay of the adrenal cortical steroids (*208*). The hormone is given in oil followed one hour later by 10–200 μc of the isotope. The activity of the urine is determined six hours later to calculate the difference between the injected and excreted K^{42}. Amounts as small as 10 μg of desoxycorticosterone may be thus determined.

I^{131}-tagged fibrinogen has been used as a measure of proteolytic enzyme activity (*209*). The tagged fibrin clot, held at *p*H 6.3–8.8, was made the substrate for the enzymes plasmin and trypsin; the amount of iodine liberated into the supernatant served as a very sensitive measure of enzyme concentration.

Perhaps the most extensively studied isotope reagent is pipsyl chloride (*p*-iodo-I^{131}-phenyl sulfonyl chloride) proposed by Keston and the New York University group and elaborated upon by Udenfriend and associates at Washington University (*210–214*). By the original technique, the unknown amino acid mixture (a protein hydrolysate, for instance) was "pipsylated" in good yield (and on a milligram scale, if necessary). Known inactive pipsyl acids were added as carriers to prevent undue losses, and the resultant products isolated by partition, by chromatography, or by crystallization. From the data, a reasonable quantitative assay of the amino acids can be derived.

More recently, the use of S^{35}-labeled amino acids as "indicators" has been introduced. The radiation characteristics of the two "tags" are so different that it is easily possible to determine the quantity of each present.

After the appropriate separation procedures, the mixture of the *known* S^{35}-labeled pipsyl amino acid, A, and the I^{131}-labeled pipsyl derivative of the supposedly same amino acid, B, is chromatographed. The resultant "band" is cut into sections across the strip. If all sections of the band have the same S^{35}-I^{131} ratio, one may feel confident that but one compound is present and that acid A is the same as acid B. This promises to be a very precise tool for biochemists. We are currently engaged in perfecting the techniques, for preparing the labeled compounds required.

Raben reacted an unknown minute iodine concentration, as iodate, with an excess of I^{131} of known specific activity. The resultant I_2 was extracted with CCl_4 and counted to give the original iodate concentration (*215*).

Wieland proposes a new method for determining α-amino acids on chromatograms and ionopherograms (*216*). Cu^{64} (12.8 hours) is spread by capillarity over a ready-made chromatogram, or the paper is impregnated beforehand with copper acetyl acetone. Assay is by counts or radioautography.

15. Mechanical Tracing

One of the early uses of isotopes in pharmaceutical problems was for tablets or capsules containing a suitable radioactive salt ($Na^{24}Cl$) and covered with the enteric coating under test (*217*). Appearance of sodium in the circulation was, of course, a sign that the tablet had disintegrated.

Cyr *et al.* (*218–220*) have used isotopes in a number of other pharmaceutical problems, among which was a comparison of the absorption of NaI from 37 different ointment bases. Teeth of rats were made radioactive and the effect of various mouth washes on them was observed. Detergents containing S^{35} were prepared and the amount remaining on the skin determined.

Talbot, Quimby, and Barach have used aerosols containing Na^{24} to measure sodium retention and rate of appearance in the blood stream (*221*). The count, measured above the chest, went up immediately after inhalation, and decreased quite rapidly because of absorption of the ionized salt.

Tagged bacteria have been used to study the distribution of the organisms following injection (*222*). More recently, Goldberg and Leif have atomized suspensions of an avirulent strain of *Pasteurella pestis*, the organisms having been previously incubated with P^{32} phosphate. Retention by mice in the respiratory tree following 20 minutes' exposure to the aerosol approximated 30%, while some 85% was found to be retained by the entire body (*223*).

As reported in a European journal, circulation of the cerebrospinal fluid has been examined by the use of thorium X, following injection into the lateral ventricle. The thorium X was found useful diagnostically (*224*), but obviously better isotopes are available in this country for studies of this sort.

Radioautographic methods have been developed for determining the particle size of radioactive aerosols containing an alpha emitter (*225*).

Several groups have labeled mosquitoes by P^{32} or Sr^{89} by growing the larvae on solutions of appropriate concentrations. For whatever it may prove, the females showed twice as much radioactivity as the males. Knowing the activity per mosquito and measuring the activity per "trap" sufficed to determine the extent of migration from various areas. Silkworms injected with tagged nutrients secrete radioactive silk, whose amino acid composition is being studied (*226, 227*).

16. Toxicity of Carbon-14

When C^{14} first became available, it was classified as among the more dangerous isotopes, first, because of its 5,000 year half-life and, second, because indications were that uptake by the bone was considerable (*228*). All evidence since that time has pointed in the opposite direction. Brues, for instance, injected mice with $NaHC^{14}O_3$, and made them rebreathe the expired air for 10 hours (*229*). Less than 2% of the carbon had exchanged and 90% of this was lost within a week.

Skipper, White and Bryan found that

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more than 95% of 18 μc of sodium bicarbonate injected into a mouse was excreted in 24 hours, the major portion (94%) being lost within the first four hours by respiration (*230*). After 24 hours, the carbon apparently remained in organic compounds generally distributed in all tissues. It disappeared at a rate dependent upon the metabolic activity of the individual tissue. After 12 weeks, only 0.13% was detectable, with localization mostly in the skeletal muscle. This 18-μc dose represented 0.11 rep for the first 24 hours, and only 0.003 rep in the period 1–3 months. They have suggested 3 μc as the maximum permissible dose in man. We have noted that even the use of organic compounds does not result in much accumulation. Gould *et al* found the 4-hour cumulative excretion to be 95% for bicarbonate, 87% for acetate, and 86% for succinate (*231*).

When C^{14}-methyl-labeled glycine is injected into mice, up to 70% is lost as $C^{14}O_2$ in the first 48 hours. After 43 days, 5.5% of the injected activity is present; it has an over-all biological half-life of 10.5 days (*232*).

Govaerts (*233*) says, "From these data we may conclude that most of the C^{14} absorbed by the organism as barium carbonate is expired by the lungs as $C^{14}O_2$, and that dangers to those who work with C^{14} are not as great as expected."

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Radioisotopes in Pharmaceutical and Medical Studies—III

Studies to determine the chemical changes in living cells after administration of certain tagged drugs, amino acids, vitamins, etc., are outlined in the following discussion of drug metabolism. General conclusions to be drawn from this and the preceding articles in this series are also offered

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IN INTRODUCING the subject of *Drug Metabolism* which is the concluding section of this three-part paper,* it seems appropriate to note that, when isotopes became readily available, the belief was frequently expressed that their use would greatly simplify studies on the mechanism of drug action. This has been true in certain instances but, in general, the problems have been made more baffling and complex than expected.

For instance, as we saw in the discussion on barbiturates, simple theories postulating greater concentrations in the brain have had to be discarded in favor of much more complex and less understood ones, involving either greater sensitivity on the part of certain cells, or interference with still obscure enzyme systems. On the basis of older methods we would have been content to follow three or four breakdown products, but now to our own dismay, we find that pentothal gives rise to some nine or ten breakdown products involving but one of the atoms of the parent compound. The same is true for benadryl, nitrogen mustards, etc.

As we shall see in the following specific illustrations, a little of almost every administered compound (or its breakdown products) goes to every organ. With few exceptions, significant-to-large quantities show up in the liver, spleen, and lungs. Deciding on what concentrations are of biological interest and which are purely accidental remains a big problem.

* Part I [NUCLEONICS 7, No. 5, 3–23 (1950)] in which Sections 1–5 appeared, and Part II [NUCLEONICS 7, No. 6, 40–55 (1950)], in which Sections 6–16 appeared, contained, in addition to information on Abbott Laboratories' isotope program for giving assistance to medical researchers, discussion on the following subjects: 1. *Uses of Iodine-131*; 2. *Uses of Phosphorus-32*; 3. *Tumor Localization*; 4. *Other Diagnostic Agents*; 5. *Tumor Therapy*; 6. *Barbiturates and Thiobarbiturates*; 7. *Sulfur Amino Acids*; 8. *The Dynamic State*; 9. *Membrane Permeability*; 10. *Antigens and Antibodies*; 11. *Fat Metabolism*; 12. *Thyroid and Antithyroid Drugs*; 13. *Metabolic Studies with Phosphorus-32*; 14. *Isotopes as an Analytical Tool*; 15. *Mechanical Tracing*; and 16. *Toxicity of Carbon-14*.

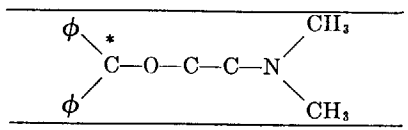
17. Drug Metabolism

As a convenient mode of classification of this very diversified information collected from many sources, we have selected ten general groups and attempted to place compounds within them either in alphabetical order or in an order as dictated by closely related properties. Reference to other compounds, which have been prepared but on which reports of use are lacking, may be found in the articles entitled "Biological Syntheses of Radioisotope-labeled Isotopes" [NUCLEONICS 7, No. 2, 26 (1950)] and "Chemical Syntheses of Radioisotope-labeled Compounds" [NUCLEONICS 7, No. 3, 49; No. 4, 44 (1950)].

I. Tagged "Drugs"

Adrenalin-C^{14}. With adrenalin beta-labeled with C^{14}, as recently reported (*234*), loss from the blood was quite rapid. Regardless of the mode of administration, the majority of the activity appeared in the urine. Later chromatograms of the urine indicated a complex pattern of metabolites.

Benadryl. Benadryl tagged with C^{14} was studied several years ago by the Parke Davis group, and a report was made that there seemed to be some localization in the lung. A more detailed paper has since appeared (*235*). Ordinary chemical methods had accounted for only 5–15% of the administered drug, and there was evidence that enzymatic degradation was leading to products not responding to the usual color tests. Labeling was on the carbon indicated:



Assays by chemical and tracer studies yielded surprisingly consistent results, with the lung tissue accounting for some 60–70% of the drug at 15 minutes. Chromatography and subsequent radioautography showed the presence of at least six different radioactive compounds in the urine, one of which was found to be unchanged benadryl. Simple hydrolysis by normal HCl for 1 hour at 100° caused the disappearance of all six bands and the appearance of a new strong band, indicating a basic similarity of structure for all six. Apparently exhalation of $C^{14}O_2$ was not studied which would seem most desirable in view of the fact that only 25–45% of the activity was accounted for in the above experiments.

Chiniofon. A purely practical metabolic problem served as our introduction into this field (*236*). The drug chiniofon (iodo-I^{131}-oxyquinoline sulfa acid) was used empirically to treat amoebic dysentery in humans without any knowledge of its absorption, or of those portions of the body in which an effective concentration could be found. A brief study showed that it was absorbed, though poorly (7–17%), from the intestine, with a resultant moderate concentration near the intestinal wall, that small amounts appeared in the blood, and that elimination in the urine was rapid, first as the unchanged drug and then as an ever increasing proportion of iodide (average 50%).

Dicoumarol. Langham has informed us that dicoumarol C^{14} labeled in the methylene group has been prepared and studied (*237*). The results clearly rule out the possibility that salicylate is the active agent produced therefrom.

Fluorophosphates. An excellent contribution to this field of drug-action literature has recently been published by the Army Chemical Center Group (*238*). The fluorophosphates represent quite a unique group of chemotherapeutic agents and are active as well as toxic in very small doses, thus necessitating high specific activities.

As usual the liver, kidney and lung take up relatively large amounts. Experiments with the analogous but inactive di-isopropyl phosphate showed that none was retained in these organs. A large part of the ultimate elimination appeared to be through the kidneys. In the principal areas of retention, it

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was found that following administration of di-isopropyl fluorophosphate, at least half of the P^{32} was "protein bound" within 10 minutes, the "reaction" being complete at the end of 4 hours. This strong binding, which can resist trichloroacetic acid and organic solvents, certainly indicates some type of chemical combination with the protein.

It seems significant that those tissues having the highest specific retention of P^{32} are those that have been shown to be especially rich in an enzyme capable of hydrolyzing DFP at the P-F bond. Obviously, however, hydrolysis must follow, and not precede, combination since the fluorine free compound is not retained. The result may be looked upon as the introduction of P^{32} and the isopropyl (but not the fluorine) into the protein molecule. In the kidney and liver, the total P^{32} remains constant for 1 hour and then gradually decreases over the next 24 hours. There is no relation between the cholinesterase activity of the organs and their ability to retain DFP-derived phosphorus.

In plasma of rabbits injected with DFP, most of the P^{32} originally present was regenerated before regeneration of cholinesterase activity had started. In erythrocytes, the elimination of P^{32} paralleled the reappearance of cholinesterase, indicating the destruction of cells whose cholinesterase was inactivated by DFP, and their replacement with new cells containing fresh active esterase.

Insulin. Insulin was tagged by coupling with the *p*-iodo-phenyl azo group (*239*). It still retained to a considerable degree its typical biological effects. It was injected into human beings in its three pharmaceutical forms —ordinary, globulin zinc, and protamine zinc. Absorption was followed by simply using an external counter tube. The rate of absorption reached its peak at the same time as the greatest reduction of blood sugar. The rates of absorption decreased in the order of the drug's forms as previously listed (*240–242*).

Mercuhydrin. The mercurial diuretic mercuhydrin ($Hg^{203-205}$) has been studied by the Tulane group (*243–244*). A series of three component "disappearance curves" were obtained. The first curve seems to be due to the mixing of the mercury in the plasma, the second to the filling of the potential mercury space, and the third reflects the elimination of the mercury.

Further use was made of the tagged material in studying its uptake following oral administration. At best, the amount of mercury excreted (and therefore presumably effective) represented 5% or less of the administered dose. At no time did levels approach those expected from the usual parenteral administration (*245*).

Methadone. The California group has prepared methadone C^{14}-labeled in the 2 position. For 1–3 hours following the subcutaneous administration of 10 mg/kg, brain levels were no higher than those of the carcass indicating no specific affinity for the central nervous system. On the other hand, certain organs such as the thyroid, liver, lungs, spleen, and the gastrointestinal tract had concentrations well above carcass levels. Though some conjugation may have taken place, excretion was complete in 24 hours, via the intestinal route and secondarily through the kidneys. It was subsequently found that the bile is the principal route of excretion, activity being found there within 10 minutes after the subcutaneous administration of 5 mg/kg. There seems to be a modification going on to a very water soluble compound still giving a methyl orange test. No $C^{14}O_2$ was recovered from the respired air indicating that oxidation does not proceed to completion (*246–249*).

Nitrogen Mustards. In a personal communication, Langham informs us that his group has prepared C^{14}-methyl nitrogen mustards. In a forthcoming publication they will emphasize the very complex nature of the metabolic products, which seem closely related to products resulting from simple chemical decomposition.

In the meantime, the Southern Research Institute has found (*122*) that the active carbon in a nitrogen mustard was distributed throughout all organs and tissues and there was no profound difference between normal and leukemic mice.

Distribution studies on the iodine analog of a nitrogen mustard [methyl-bis (-beta-iodo-ethyl-amine)] have been reported (*250*). On a molecular basis, it was more toxic than the chloro compound. The radioactive iodide ion was very rapidly formed and excreted in the urine. Evidence that the uncyclized mustard entered the tissue cells was established only for the lung.

The "one armed" analog, diethyl-beta-iodo-ethylamine, likewise rapidly cyclized, but again a small fraction penetrated the cells (*251*). Tests made on a patient with a sarcoma (of the tonsil) showed a relatively higher concentration of radioactivity in tumor, blood and muscle.

Pentothal. A discussion of the metabolism of this thiobarbiturate is to be found in Section 6.

Procaine. Howarth has studied, quite extensively, dibrom procaine as a spinal anesthetic (*252*). Following intrathecal injection, the concentration in the subarachnoid space rapidly declines at the same time that a rapid rise occurs in urine concentration. Blood levels remain persistently low. Only the spinal roots regularly show any capacity to concentrate the drug above the cerebrospinal fluid at the site of injection. Of the tissues, only kidney and liver (bile) concentrate it above the circulating blood.

The drug was found to enter the spinal cord during spinal anesthesia, though it is improbable that it is able to produce a functional cord transection. Of the routes of departure from the spinal theca, the venous system appears to be the most important.

Penicillin and Derivatives. The site and mechanism of action of penicillin has been studied simultaneously by Rowley *et al.* in England and by Johnson and Maass at the University of Wisconsin (*253–257*). Since the *S. griseus* cultures take up sulfate, a fairly simple fermentation suffices for the production of sufficient amounts for bacteriological experiments. The uptake of penicillin by bacteria increases with concentration for both resistant and nonresistant organisms. Attainment of equilibrium across the cell walls is rapid—less than 3 minutes—the concentration inside the cell being (at low concentrations) much higher than in the environment. During rapid cellular division, uptake is greatest and, at least under certain conditions, increases with the sensitivity of the strain. It reaches a peak shortly before growth is stopped.

About 0.8 unit is bound by each cubic centimeter of cells, exclusive of that penetrating by simple diffusion. This is equivalent only to about 2×10^{-9} moles of pencillin, so that the combining substance is probably of a catalytic nature rather than a metabolic. It has been further suggested that some reaction essential to cell division but not to cell respiration is inhibited.

When growth is reinstated by transfer to a fresh medium, the bulk of the antibiotic is not excreted; rather the penicillin binding component of the cells is synthesized at a more rapid rate than the other cell substances, until an excess is again present. Attempts made to prevent uptake by pretreatment with formaldehyde, CTAB, euflavine, and acid were unsuccessful, but pretreatment with inactive penicil-

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lin prevented uptake. The tightness of the binding is also attested by the fact that neither washing nor post-treatment with inactive penicillin served to dislodge activity from the treated bacteria.

When S^{35}-labeled penicillin was given by injection, almost all of the injected sulfur was eliminated in the urine within 8 hours. At the most, 30% was in the form of unchanged penicillin, thus showing extensive breakdown. With oral administration, only about half of the sulfur was recovered in the same time (*258*).

The English group has concluded that at the end of 20 minutes' contact between penicillin and *S. aureus*, all of the drug taken up is concentrated in the cytoplasm (*259*).

Reference should be made to the use of S^{35} to establish the actual presence of penicillin in the impure product secured in very poor yield in du Vigneaud's synthetic process for that antibiotic. S^{35}-tagged *d,l*-penicillamine was condensed with 2-benzyl-4-methoxymethylene-5-(4)-oxazolone. Natural benzyl penicillin was added and recrystallized. The specific activity remained constant thus indicating the presence of synthetic benzyl penicillin in the reaction product (*260*).

We have recently synthesized iodinated penicillin X having an activity of 245 mc/gm which has been used in some very interesting experiments not yet completed.

Salicylic Acid. Salicylate metabolism has been the subject of a recent study (*261*). In the dog, salicylic acid is broken down so that one-half appears in the urine as free salicylate, one-fourth as hydroxyl glycuronate, one-fifth as salicylurate, and one-tenth as gentisates. The picture was somewhat similar in patients, one-half being salicylurate, one-third glycuronates, one-tenth free salicylate, and a few per cent of gentisate. (No activity appeared in the expired CO_2). Evidence was found that the glycuronate formation could involve both the hydroxyl and carbonyl groups.

Stilbamidine. Following intravenous injections of C^{14}-labeled stilbamidine, there was no significant excretion of $C^{14}O_2$. This indicated absence of metabolic degradation in the body (*268*). During the first 30 days following injection, approximately 96% of the initial dose was excreted in urine and feces, of which approximately 70% appeared during the first four days. The highest concentration of activity in the autopsied mice was found in the kidneys, liver, heart, and intestines. Work is in progress on cancerous mice.

A note has appeared indicating that there is no significant concentration in human tumor tissue (*58*).

Sulfa Drugs. A number of studies have been directed toward the elucidation of the mechanism of the action of drugs on bacteria. Erlenmeyer's group abroad prepared sulfur labeled *p*-aminophenyl-sulfathiazole, and found that the tubercle bacillus in a synthetic medium took up the drug in an amount proportional to the concentration of the drug in the medium (*262*). This concentration was sufficient to exert a bacteriostatic effect. When 10% of serum was added to the medium, the organisms grew, in spite of the fact that they still contained a bacteriostatic amount of the drug. It looks, therefore, as if the attachment of the drug to the bacterium is not the only factor involved; possibly the serum contained some source of material which made possible an alternate source of metabolic energy (*263, 264*).

Sulfapyridine. Sulfapyridine was labeled with S^{35}—unfortunately only to the extent of 16 cpm/mg. This necessitated dosages of 2–3 gm per rabbit. No inorganic sulfate was formed at any time. Some 25–60% of the activity appeared in the urine during the first day, 10–30% the second day, and, in one animal, a small amount on the third day. There was no significant retention after three days. The usual diazo test was carried out simultaneously. The values secured were 20–25% under those from counting, suggesting the presence of a sulfanilimid in which the amino groups had been modified. If present, it was not isolated (*265*).

Sulfanilimid. Somewhat surprising in light of the findings just noted above are the observations of Klotz and Melchoir on S^{35}-labeled sulfanilimid, (*266*). *E. coli* suspensions were incubated with what would appear to be a large excess of sulfanilimid for 6 hours. At the end of this time, the cells were centrifuged and washed quickly. The binding within the cells was less than 0.2% and less than 5% could have been attached to the cell surface.

Bloch and Ray have prepared a number of tagged compounds including iodo-sulfanilimid, iodo-sulfapyridine, which do not seem to have been further studied (*267*).

Thiocyanate. Wood and Williams recently found that the thyroid gland fixes thiocyanate sulfur to a greater degree than does any other tissue protein (*269*). This ability is impaired by propylthiouracil treatment. Since the KCNS level of all tissues decreased at the same rate as the plasma level, their observations were interpreted in terms of a metabolic antagonism of thiocyanate toward iodide. Most of the activity was found in the urine as unchanged thiocyanate.

Thyroid Drugs. Several years ago, the Finks hydrolized thyroid tissue, and applied the comparatively new two-dimensional chromatography and followed it by radioautography (*270, 271*). In addition to the expected spots corresponding to thyroxine and di-iodotyrosine, a strong spot was found to be due to monoiodotyrosine, even though this constituent is considered to be present only in small amount in thyroglobulin. Control experiments seemed to show that exchange had not taken place during hydrolysis.

Taurog, Tong, and Chaikoff extended these results and confirm that monoiodotyrosine comprises approximately 15–20% of the total iodine in the thyroids of rats and chickens as contrasted with 25–30% for diiodotyrosine (*272*). Apparently thyroxine undergoes extensive decomposition during the alkaline hydrolysis with the liberation of iodine.

By filter-paper chromatography, the same authors studied the nature of the plasma iodine and found that, in addition to free iodide, only thyroxine was present, and it was loosely bound to plasma protein.

Clayton *et al.* used 3′,5′-I^{131}-thyroxine to study absorption and behavior (*273*). Breakdown was extensive; the activity in the urine was present almost entirely as iodide, but the feces, bile, etc., contained both inorganic and organic forms.

The Mayo group used not only labeled thyroxine but also labeled thyroglobulin in normal patients and in a patient with myxedema and found similar liberation of iodine with time (*274*). The picture is similar when the drug is given intravenously.

In a recent review paper, mention is made of the incorporation of I^{131} into diiodohydroxyphenyl propionic acid, phenyl dihydroxy cinnamic acid, and the ethyl ester of phenyl diiodohydroxyphenyl propionic acid, for metabolic comparison with the parent analog, diiodotyrosine (*275*).

Diiodotyrosine and phenyl dihydroxy phenyl propionic acid (priodax) behave rather similarly, though possibly the rate of decomposition of the latter into iodide is somewhat slower. It also appears to a greater extent in the liver and kidney. The "spuriously" high

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values for exogenous protein-bound iodine due to improper manipulation is stressed (*276*).

Many studies have been made on the use of iodine labeled "biologicals" for thyroxine production. For instance, the Mayo group have found iodocasein to be effective clinically (*277*).

Thiourea. The metabolism of S^{35}-labeled thiourea has been studied by Schulman and Keating (*278*). Of the activity in the urine, 98% was excreted in 48 hours; it was mostly unchanged except for 6% in the form of inorganic sulfate and 6% as ethereal sulfate. There was a 55-fold concentration of activity in the thyroid. The nature of the concentrated compound is not known, but there is good evidence that it appears as sulfate during isolation. On the other hand, neither the ingestion of sulfate or sulfide leads to thyroid concentration.

Thiouracils. The same group has completed work on thiouracil and 6-propyl thiouracil. These drugs seemingly also localize in the thyroid.

Studies with 4-methyl-2-S^{35}-thiouracil were made on cockerals (*279*). The thyroid, the feather shafts, and the pituitary contained most of the drug at 24 hours; excretion was largely complete in 96 hours.

Urethane. Urethane has been carboxyl-labeled by Bryan, Skipper, and White (*280*). Within 24 hours, 90% of the labeled carbon had been expired as $C^{14}O_2$, and 5–10% was found in the urine. Of the small amount remaining, the most was found in the blood, liver, intestines, and tumor tissue. Two mice with advanced lymphoid lukemia and two with mammary carcinoma retained much more C^{14} in all tissues after 24 hours than did normal mice. Whether this retention is due to failure to break down the urethane as rapidly as in normal mice, or whether it is due to a greater fixation of urethane or a metabolite is not known.

The Southern Research Institute group has found that fertilized sea urchin eggs, in mitotically effective concentrations, rapidly accumulated urethane in excess of the surrounding medium. It is concluded that the mitotic effect is produced by material free or loosely bound within the cell, and not to the very small part more firmly held (*122*).

Benzedrine, Sucaryl, Tetraiodophenolphthalein, and Antabus. These drugs have been tagged, but no reports on their metabolism have appeared.

II. Amino Acids

Cystine. Gutman and Wood have administered *l*-cystine-S^{35} and brombenzene simultaneously and recovered labeled bromphenylmercapturic acid to the extent of 4% during the first 24 hours (*281*). The administration of 3,4-benzpyrene produced no effect on the sulfur metabolism.

The ability of cysteine to aid in protecting against X-radiation seems well established (*149*).

Iso-cysteine. Iso-cysteine is largely excreted in "organic" form in the urine, and only a small amount is found as sulfate (*282*).

Glycine. As the simplest of the amino acids, and one quickly prepared, glycine was used in C^{14} studies.

The most recent report on this acid, C^{14}-labeled first in the carboxyl and second in the alpha carbon, is that of Barnett and Wick (*283*). They found that in the intact rat the carboxyl carbon of the administered glycine supplied approximately 1 carbon for each 28 carbon atoms incorporated into glycogen. One carbon atom out of 28.5 carbons in the glycogen molecule was derived from alpha-carbon-labeled glycine. As would be expected, more $C^{14}O_2$ is derived from the carboxyl than from the carbon in the alpha position. (See paragraph on "Globin" in section VIII on *Biological Materials*.)

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Borsook *et al.* (*284*) studied the incorporation of C^{14}-labeled amino acids into bone marrow cells in vitro. Glycine, leucine, and lysine were thus incorporated. However, only about one fourth of the counts in the protein after incubation with labeled glycine was due to glycine incorporated as such into the proteins. This is not true for leucine and lysine; a number of inhibitors of this reaction were found.

Methionine Ethionine, and Other Methyl Donors. The body distribution of fed methionine was studied by Maass, Larson and Gordon (*285*). It was rapidly absorbed when given with food, and was present throughout the body within 4 hours. There was no selective localization. Strain differences have been observed with respect to its uptake and utilization (*286*).

Klotz (*287*) used S^{35}-labeled methionine uptake as a measure of protein synthesis by *E. coli*. The rate was nearly constant for several hours and then fell off sharply, though 95% of the substrate remained.

Reference has been made in Section 7 to the vital role played by methionine as a methyl donor. Recent studies with C^{14}-methyl-labeled methionine (and also C^{14}-deuterium doubly labeled methionine) prove transfer to choline, creatine, taurine, and anserine (*288, 289*). Very recently, after methionine feeding to rats, the epinephrine was isolated by means of carrier and shown by constant isotopic composition as such, and after conversion to iodoadrenochrome, to have taken up the methyl from the amino acid (*290*). At the same time, an appreciable amount is oxidized to $C^{14}O_2$ and respired (*291*).

Stekol and Weiss showed that on a diet containing ample methionine as the sole sulfur amino acid but free of vitamin B-12 poor growth was obtained; supplementing the methionine-containing diet with B-12 resulted in good growth. None of the experimental animals receiving B-12 died of kidney lesions even when there was a complete absence of "labile methyl group" donors in the diet (*292*).

As would be expected, methionine is rapidly incorporated into proteins; the rate of such uptake varies greatly with the metabolic state of the animal and the size of the dose administered (*293, 294*).* Incorporation into liver, kidney, and plasma proteins is inhibited by the S-ethyl analog, though the mechanism does not seem to be a simple competitive inhibition.

Ethionine. Ethionine, C^{14}-labeled in the methylene carbon of the ethyl group was given to rats by Stekol and Weiss, and radioactive creatinine and choline isolated from the tissues. S^{35}-labeled ethionine was administered with brombenzene and *p*-bromophenyl, and mercapturic acid was isolated from the urine (*295*). This seems to establish that ethionine sulfur is available to the rat for cysteine syntheses, and that the ethyl group of ethionine is available for the ethyl analogs of substances known to participate in transmethylation reactions.

A somewhat surprising and certainly interesting result is the effect of testosterone on protein synthesis. In the body generally there is no effect, but in the comb of the capon, which is the "target organ" of that hormone, a striking increase in the specific activity (S^{35}) of the protein is found (*296*).

Ferger and du Vigneaud have synthesized C^{14}-labeled choline, betaine, dimethyl thetin and dimethyl propiothetin, and studied the rate and extent of the oxidation of methyl groups with methionine (*297*). The last three were found to be particularily labile in this regard. Creatine and creatinine do not similarily serve as a source of oxidizable carbon, and S-methyl thio-

* An excellent review by H. Tarver on "Radioactive Sulfur and Applications in Biology" is in press.

glycolic acid and several simpler thetin compounds were quite inactive (*298*).

Phenyl Alanine. Phenyl alanine was C^{14}-labeled in the alpha and/or COOH position (*299*). It was also tritium labeled in the ring and possibly the side chain by heating at 50° with 85% H_2SO_4 made from SO_3 and TOH. Both samples were injected into rats intraperitoneally. The epinephrine was isolated by means of carrier, and in each case, there was evidence of the incorporation of activity in it.

Lerner has prepared C^{13}-C^{14} doubly labeled phenylalanine and studied its metabolism in liver slices (*300*). Two carbon atoms of the benzene ring and two of the side chain were converted to ketone bodies, and the remaining four of the ring to malic acid. Tyrosine apparently behaves similarily.

Tryptophane. Heidelberger fed C^{14}-labeled tryptophane to dogs, rabbits, and cats (*301*). Kynurenine was isolated and labeled on the methylene carbon; kynurenic acid was labeled in the 3 position. The N-methyl nicotinamid was not active, indicating that tryptophane was not a precursor. Tryptophane (beta labeled) has been given to phloroglucinized rats (*302*). Acetate seemed to be a metabolite, and tryptophane itself was incorporated into proteins.

Tyrosine. When beta-labeled tyrosine was incubated with rat liver slices, it was converted to acetoacetate labeled in the alpha position (*303*).

III. Vitamins

Ascorbic Acid. Burns and King have prepared COOH-labeled *l*-ascorbic acid (*304*). Of the injected dose, 20–30% appeared as $C^{14}O_2$ during 24 hours, and the urine contained 3–6%. The principal urine constituent was oxalate. There appeared to be no marked differences in tissue incorporation in the normal and scorbutic guinea pig on a weight basis.

The biosynthesis of glucuronic acid has been studied by Mosbach and King using borneol-treated guinea pigs (*305*). Following administration of C^{14}-labeled glucose, 1.8–4.0% was recovered as urinary bornyl glucuronide within 24 hours. Ascorbic acid did not act as such a precursor.

Nicotinic Acid. Carboxyl C^{14}-labeled nicotinic acid and nicotinamid were made by Leifer *et al.* (*309*). Half of the (small) dose given intraperitoneally was metabolized and excreted in 100 hours; 45% appeared in the urine, 4.2% as $C^{14}O_2$, and 1.5% in the feces. It was observed that red blood cells in vitro took up nicotinic acid, but not nicotinamid, presumably indicating that the former is the active agent. Carboxyl-labeled nicotinic acid was given to rats, who excreted N-methyl nicotinamid containing all of the C^{14} (*310*).

Thiamin. More than 10 years ago Borsook and associates prepared thiamin labeled with S^{35} (*307*). These early studies showed that thiamin rapidly enters the tissue from the blood, and that there is rapid interaction of the injected vitamin with that present in the body. A significant amount remains in the tissues even after 36 days on a B-1 free diet.

Chandler recently repeated the thiamin synthesis and used it to study the host-parasite relationship of *Hymenolepsis dimenuta* in white rats (*308*). The conclusion is that the worms obtained their vitamin supplies from the host rather than by synthesizing it for themselves.

Vitamin B-12. Vitamin B-12 has been tagged with Co^{60} by the Merck group (*306*): 100 mg of crystalline material was obtained having an activity of 0.2 μc/mg and the physical characteristics of the "cold" material. Studies with it, or material of higher activity to be prepared, should aid in shedding light on the nature of this interesting vitamin.

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IV. Dyes

Trypan Blue. Tobin, Moore, and Aub apparently were the first to report on the synthesis of radioactive dyes (*311, 312*). Dibrom trypan blue was given to tumor mice, but twice as much of the activity appeared in the liver as in the tumor. Since bromine, of course, has the disadvantage of a half-life of only 34 hours, Stevens *et al.* used iodo^{131}-trypan blue (*313*). The material was quite impure. Tumor 151091a in mice showed a higher activity than skeletal muscle or skin but less than liver, spleen, or kidneys.

Nile Blue. The I^{131} derivative of nile blue BB has been prepared by Sloviter (*314*). In the dog, activity was high in the bile and the thyroid. In tumor-bearing mice, the concentration in the tumor was of the same order of magnitude as in the liver, kidney and spleen. He reports that such treated mice show "marked" prolongation of life. It would seem desirable that such experiments be repeated on a much larger scale with material of higher activity.

Di-iodofluorescein. The preparation and use of this dye for use in brain tumor localization has been described in detail in Section 3 of Part I.

Tetraiodophenolphthalein. We have tagged this material in 95% yields and better than 98% purity. No reports of its use are yet available.

Iodo-tetrazolium Salt. Seligman *et al.* studied the body distribution of an iodotetrazolium salt (*315*). It disappeared rapidly from the circulating blood, and appeared in greatest amount in kidney, liver, and lung. Sarcoma 37 contained less radioactivity than most other tissues.

V. Alkaloids and Glycosides

Digitoxin. Some work has appeared regarding the metabolism of C^{14}-labeled alkaloids and glycosides (*316, 317*). According to Geiling, Kelsey, *et al.*, radio-digitoxin (purity not accurately known) has been injected into rats, guinea pigs and dogs. The amounts found in tissues, including liver, kidney, heart, and skeletal muscle, were small and less than would be expected if the dose were evenly distributed. Most activity was found in the excreta, as a water-soluble fraction.

Nicotine. Labeled nicotine, obtained by growing tobacco in $C^{14}O_2$ was given in 5–10 mg doses to rats. The urinary excretion represented only 8–12% of the total amount given (*318*).

Morphine. Morphine, having an activity of 152,000 cpm/mg, was also secured by this group and purified by chromatography. No in vitro results seem to have been reported.

Codeine. Prepared synthetically by Adler and Latham, codeine, tagged by C^{14} in OCH_3 group, is demethylated in vivo, the methoxyl being oxidized to $C^{14}O_2$ which is expired by lungs. The reaction takes place primarily in the liver, 40% being demethylated in 4 hours. Transmethylation was not observed in vitro (*319*).

VI. Carcinogens

Dibenzanthracene. A biological problem that remains unanswered is the mechanism of the production of tumors by carcinogens. Heidelberger, first at California and now at Wisconsin, has made very careful studies in this field using C^{14}-tagged cancer producing agents. According to papers he and others published during 1948–1950, 9,10-labeled dibenzanthracene was given by a number of routes; in every case, degradation of the absorbed portion was extensive, with elimination through the bile of at least four substances (*320–323*). Tumors induced by the drug showed varying amounts of degradation, but there always remained some of the agent.

20-Methyl-cholanthrene-11-C^{14}. 20-Methyl-cholanthrene-11-C^{14} was

given intramuscularly to mice. At the end of 7 weeks, 50% had disappeared from the injection site. Both the rate of disappearance from tumor tissue and the distribution of degradation products were similar to the observations with dibenzanthracene.

Dimethyl-aminoazobenzene. Boissonnas, Turner, and du Vigneaud synthesized dimethyl-aminoazobenzene, and labeled one of the methyl groups with C^{14} (*324*). When fed to rats, 57% of the ingested C^{14} was removed in the expired air over 10 days, and 1.3% appeared in the urinary urea. The detoxification is obviously an oxidative demethylation, but one cannot help wondering about the fate of the remainder. The analogous azo dye, 3′-methyl-C^{14}-4-dimethyl amino azobenzene, has just been prepared, but no tests have been reported (*325*).

Salzberg and Griffin studied the distribution of this dye in normal rats and in rats previously fed inactive dye (*326*). No difference in the two classes of animals was found. A large percentage of the administered activity was excreted rather rapidly in the urine. No C^{14} activity was found in expired CO_2.

8-Azaguanine. Studies with 8-azaguanine (guanazolo) in normal and adenocarcinoma-bearing mice have shown that a little of the 2-carbon atom is oxidized to $C^{14}O_2$ and exhaled but that most of that atom is excreted in the urine. No evidence was obtained that the compound in question, or that the 2-carbon therefrom, was preferentially accumulated, fixed, or incorporated in Eo771 tumors which are known to be inhibited by 8-azaguanine. The nucleic acid fraction from the tumor was no higher in radioactivity than that from normal viscera tissue (*122*).

VII. Estrogens

Dibrom-estrone. Dibrom-estrone was prepared by the bromination of equiline and given to rabbits, monkeys, and dogs (*327*). It was not selectively localized in the adrenals, spleen, uterus, ovaries, or testes. There was high concentration in the gall bladder within 6 hours, and later appeared along the bowel. It was presumably excreted as a phenolic steroid. The product was only weakly, if at all, estrogenic.

Sodium Estrone Sulfate. Sodium estrone sulfate (S^{35}) has been made by the Ayerst, McKenna, & Harrison, Ltd., and submitted to several laboratories for study. It was found that the rat liver contained an enzyme that rapidly and completely hydrolized this material, with the elimination of inorganic sulfate (*328, 329*).

Iodo-alpha-estradiol. An iodo-alpha-estradiol has been prepared by Albert, Heard, LeBlond, and Saffran (*330*). The most noteworthy aspect of the metabolism in male and female mice is accumulation in the gastrointestinal tract via the liver and the bile. There was a marked increase in the mammary glands of mice during pregnancy. Only a small part apparently exists in conjugated form.

Methyl-estradiol. Much more logical is the labeling of methyl-estradiol by C^{14} in the 17 position (*331*). At 1 mg levels in female rats, most of the activity was recovered from the feces over a 72-hour period, with a small amount in the urine. Excretion was primarily through the bile.

Brom-dimethyl-ethyl-allenolic Acid. Brom-dimethyl-ethyl-allenolic acid (an artificial estrogen) was studied briefly by a French group (*332*). Unlike thyroxine, there was no measurable penetration into the hypophysis. The accuracy of their experimental methods may be open to some question.

Progesterone. The Doisy group prepared progesterone-21-C^{14} in 38% yield (*333*). When given intramuscularly, most of the activity was excreted in the bile over a 4-day period, less than 10% appearing in either the urine or expired air. The bile appeared to be a preferred route of elimination, with the urine an easy alternate.

Testosterone. Tagged testosterone has been prepared by Turner in a very clever way, but we have not noted any reference to its use in experiments (*334*).

VIII. Biological Materials

Cytochrome C. Cytochrome C tagged with Fe^{55} was synthesized biologically and its metabolism studied (*335*). It seems to be very rapidly disposed of by the body, and little if any is found in the brain and heart. This leaves the mechanism of its beneficial clinical administration quite unknown (*336*).

Creatine and Creatinine. Mentioned previously under "Methyl Donors" in section II on *Amino Acids*.

Cholesterol. The California group incorporated doubly (C^{14}) labeled acetic acid with adrenal cortex slices and after incubation isolated labeled cholesterol (*337*). In intact rats, cholesterol production was the same in normal as in hepatoma-bearing animals. It has also been observed that the rat artery can convert acetate to fatty acids and incorporate P^{32} into phospholipids, a significant observation in light of the current interest in arteriosclerosis (*338*).

4,5-Cholestenone. 4,5-Cholestenone was found to be well absorbed and apparently converted to dihydrocholesterol, with a trace to cholesterol (*339*).

Tagged Bacterial Spores. Pearson, Hammer, and Corrigan have published a set of papers which do not seem to have received the attention they deserve (*340–342*). Bacterial spores, tagged by growing them in the presence of I^{131}, were injected into animals. Non-pathogens gave an increase in counts in the reticulo-endothelial system and the thyroid, while the pathogens studied (such as *Blastomyces* and *Tubercle bacilli*) resulted in the retention of activity in lesions.

Globin. Glycine (COOH labeled) feeding to dogs and rats led to incorporation of the C^{14} in the globin portion of hemoglobin, but not in the heme. The globin of the erythrocytes apparently does not take part in the dynamic protein interchange and is not re-utilized (*343–344*).

Phosphoryl Choline. This "type compound" was prepared by Riley in a study of phosphorylation mechanisms (*345*). Following intraperitoneal injection, it disappeared rapidly, the P^{32} appearing in the blood as inorganic phosphate. The same has been found true for injected P^{32}-labeled proteins (vitellin, liporitellin, etc.) (*346*).

Tagged Serum Albumin and Globulin. This has been discussed in detail in Sections 4 and 10.

Tumor Necrotizing Polysaccharide. Tumor necrotizing polysaccharide has been iodinated directly (*347*). It has also been coupled with iodobenzene-diazonium chloride (*315*). The highest concentrations were found in liver and lung, while the lowest occurred in tumor tissue. Disappearance from the blood stream was rapid. There was no evidence that the clinical course of the disease was favorably affected by the administration of iodopolysaccharide.

IX. Metals and Organic Derivatives

Bone Seekers. Since protection against the hazards of handling the highly dangerous fission products was an essential part of the Manhattan Project, a rather large literature has developed of direct medical significance (*348*). Yttrium, cerium, and plutonium, while not taken up from the gastro-intestinal tract, on inhalation remain for a long time in lung tissue. The predilection of such other elements as strontium for the bony structures where it remains localized is also well known (*349*).

Schubert and White have shown that certain citrate salts can assist in the

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excretion of beryllium and possibly other elements (*350*). Studies involving the fate of some 33 "nonessential" elements on a tracer scale are summarized (*120*). Since these subjects have been reviewed recently, further reference will not be made to them here.

Arsenic Compounds. A good method of preparing $AsCl_3$ has been developed and from this has been made labeled atoxyl, arsacetin and salvarsan (*351*). No biological experiments have yet been reported.

Reference has already been made to the use of As for irradiation purposes. Lawton *et al.* gave sodium arsenite to cotton rats infected with *Litomosoides corinii* and found a higher activity in the tissues of the parasite than in the host (*352–354*).

Lewisite. In addition to labeling mustard gas with S^{35}, Axelrod and Hamilton have tagged lewisite with As^{74} (*355*). By means of radioautography, they demonstrated that, following application to the skin, most of the irritant was concentrated in the epidermis and little in the dermis or the corium.

Barium Sulfate. Crandall has shown that traces of barium are absorbed (by rats) from $BaSO_4$, but probably not enough to mitigate against its use in X-ray studies (*356*).

Gold. For the therapeutic use of gold colloids, see Section 5.

Gold Sodium Thiosulfate. Bertrand and Tobias find that shortly after the introduction of gold sodium thiosulfate into the blood stream of animal and of man, the compound is so altered as to be nondialyzable and is closely associated with the plasma (*357*). Such injected gold sodium thiosulfate has been found to cross the placental barrier. In an earlier paper, they observed that in chemically produced "arthritis" there was a concentration of gold over normal, but that this was not specific for joint tissue (*358*). In a human arthritic, the synovialis contained a somewhat higher concentration than muscle, skin, etc. Certainly the activities achieved are such as to preclude radiation therapy.

Iron and Iron Preparations. Iron is, of course, one of the oldest of drugs, but its use has usually been empirical and on none too sound a scientific basis. The minute amount of iron absorbed from any given dose in comparison with the total body stores, and the complexity of its life cycle, have made particularly valuable the extremely sensitive isotopic methods. Two isotopes are available, Fe^{55} with a 2.91-year half-life, and Fe^{59} with a 46.3-day half-life. It is only recently that the latter, and preferable, isotope has been available from Oak Ridge.

The initial biological experiments were those of Hahn, Bale, Lawrence and Whipple (*359*). Their first contribution was the observation that the body has the unique ability to accept or reject iron following ingestion, depending on the need for the element. This observation was made possible by the ability to recognize the presence of the newly administered tagged iron in the plasma, and later in the red-cell hemoglobin, and to show that in iron-deficiency anemia, the absorption was increased many times over normal. This finding led this group to postulate an acceptor mechanism in the gastrointestinal mucosa which normally is saturated with iron, and therefore allows a minimal passage from the lumen to the plasma.

They further showed isotopically that upon the destruction of red cells, the hemoglobin iron was converted to ferritin in the liver and spleen, thus being biologically conserved and in part stored until needed.

Human experiments have been carried out by several groups (*360–362*). It was found that in human beings, tagged ferrous iron was absorbed many times more efficiently than ferric. Feeding a standard test dose to growing 7 to 10-year-old children showed that the uptake was closely related to estimated yearly increases in body iron during growth (*363*). In 1,000 pregnant women, it was found that there was from 50–100% greater absorption of iron in the last quarter of pregnancy than in the first quarter (*364*). It was further shown that controlled inflammatory processes depressed iron absorption considerably (*365*).

Extensive studies have been made on iron needs in various types of anemia, particularly those resulting from hemorrhage (*366*); ~0.5 mg of iron needs to be absorbed for each cm^3 of blood lost.

The Parke Davis & Co. research group has attempted to modify the course of the malaria infection in ducks and canaries through the use of radioactive colloidal iron. Even blood levels of 1 $\mu c/cm^3$ at the time of infestation seemed quite without effect (*367*).

Selenium. The incorporation of Se^{75} in body tissues was recently reported (*368*). At 24 hours after the injection of sodium selenite, Se^{75} was present in albumin, and globulin in roughly similar amounts. Dialysis indicated a fairly stable protein-like combination. Since incorporation of selenium, unlike sulfur, does not seem to take place in bone marrow, the suggestion is made that it enters the molecule through some sort of an "exchange" reaction (*369*).

Silver. West, Johnson, and Johnson have attempted to use Ag^{111} of 7.5-day half-life as well as $Ag^{108-110}$ of 225-day half-life for concentration in infected areas. After an injection of the nitrate into one leg, relatively high counts were found in the other leg in which an infection had been developed (*370*). The isotope is subsequently excreted by the liver into the intestine, presumably by way of the bile. The 8.5-day Ag^{106} is being studied, but formidable difficulties are presented by the difficulty of securing activities in the therapeutic range.

Antimony—Tartar Emetic. Tartar emetic has been synthesized containing mixed Sb^{122} and Sb^{124} from the cyclotron. It was found that the blood level drops rapidly and reaches a very low level after 6–8 hours (*371–373*). The highest concentration was found in the liver; the thyroid and parathyroid had the next highest concentration. Dogs naturally infected with *Dirofilaria immitis* were given this drug, as well as sodium antimonyl xylitol and As_2O_3. It was found that the adult filaria ranked high in antimony content, and considerably higher than would have been characteristic of random distribution. It was found that if hamsters injected with *S. mansoni* were used the adult flukes contained a relatively high concentration of antimony.

As much as one month was required for a man to eliminate 50% of a single dose of potassium antimony tartrate, during which time the liver had been subjected to 35 rep of radiation (*374*).

X. Miscellaneous Compounds

2-6 Diaminopurine. This material, C^{14}-labeled in the 2 position, did not undergo extensive oxidation, but was eliminated (*122*) through the urine largely as allantoin. No localization was noted in the neoplastic tissues of mice. It is interesting that at the end of 24 hours the 2 carbon atom was laid down heavily in the bone marrow, with little in epiphyseal cartilage or diaphysis.

Anthranilic Acid. The Los Alamos group have studied the metabolism of anthranilic acid and its p-isomer (*375*). In the first 24 hours, 79–83% appeared in the urine and 2.5–3% in the feces. The o-isomer gave 3.8% in exhaled $C^{14}O_2$, and the p-isomer less than 1%. In neither instance was there any tissue activity after 24 hours.

Acetone. Acetone, C^{14}-labeled in the methyl positions, was studied in

rats. Less than 10% of the activity was expired as carboxyl compounds. At least half of the activity was oxidized to $C^{14}O_2$ and exhaled within 24 hours. Some was converted to an acetylating agent, and some showed up in such diverse organic compounds as urea, liver cholesterol, glutamic acid, heme, liver glycogen, aspartic acid, arginine, and fatty acids (*376*).

Carbon Tetrachloride. Beamer has reported upon C^{14}-labeled CCl_4. When applied to the skin of monkeys for 270 minutes, C^{14} activity was not measurable in the blood after 48 hours, or in the expired air after 5 days. When inhaled, 30% was absorbed and metabolized; 10–20% in the blood and expired air was present as $C^{14}O_2$; 20% of that absorbed was exhaled as $C^{14}O_2$ during 18 hours. The remainder was in the urine and feces (*377*, *378*).

Carbon Disulfide. S^{35}-labeled CS_2 was injected intraperitoneally, subcutaneously, intramuscularly, and intracardially into guinea pigs and mice, and also inhaled. Some 20–50% of the intracardial dose was retained, and it was generally distributed throughout the body tissues, with a larger amount in the liver. The nonmetabolized portion was excreted in 30–60 minutes; of that retained, some 30% appeared in the urine as inorganic sulfate (*379*).

Ethyl Alcohol. Rats orally oxidized 75% to $C^{14}O_2$ in 5 hours, and 90% in 10 hours. There was no acceleration of destruction in habituated rats (*380*).

Hexa (1-C^{14}-ethyl) Tetrapolyphosphate. This has been found to form a stable complex with plasma cholinesterase (*381*). About 83% of the fixation was related thus to enzyme inhibition; the rest to less specific reaction.

Histadine-2-C^{14}. This compound was prepared by Reid and Landefeld (*382*), and its distribution studied in rats bearing chemically induced hepatomas. About 8% was excreted as $C^{14}O_2$ during the first 72 hours, 7% appeared in the urine and 6% in the feces. The remainder was scattered in the tissues, the highest concentrations being found in plasma, tumor, spleen, and kidneys.

Mustard Gas. During the war, the Wormall group in England labeled mustard gas with S^{35} (*383*, *384*). They found that such labeled material combines readily and rapidly with various proteins (in vitro) to at least the extent of 22 moles per "unit" of protein. This combination involves the SH group but is apparently only a minor one of the actual reactions taking place. The corresponding sulfone reacts chiefly with amino groups, but the sulfoxide does not. Given intravenously it was rapidly removed from the blood stream, as much as 17–22% of the S^{35} being excreted in 4–7 hours, with 7% being found in the bile. There was widespread distribution in the body tissues, particularly in the kidneys, lung, and liver. The sulfoxide and sulfone have similar body distribution, and also reacted with tissue to give what they term "fixed" sulfur.

In an effort to gain a better understanding of the obviously complex reaction between the vesicant and biological materials, the Cornell group has S^{35}-tagged the "one-handed" mustards (R-S-C-C-Cl). (R equals benzyl and butyl). These were then reacted with the typical proteins, insulin, pepsin, and tobacco mosaic virus. In each case some reaction took place when the reactants were present in the magnitude responsible for vesicant effects. The combined sulfur residues were partly removed by alkaline hydrolysis, as R-S-C-C-OH, as shown by isotopic dilution. Acid hydrolysis resulted in the removal of R-S-C-C-substituted phenylalanine, as again demonstrated by isotopic dilution. This constituted evidence for at least this amino acid being involved in the reaction (*385–387*).

Serum proteins labeled with mustard gas have been used in immunological investigations (*388*).

Urea. In mice, the Los Alamos group found urea-C^{14} to be converted to the extent of 21% into $C^{14}O_2$ and exhaled; 79% was excreted unchanged (*389*). The biological half-life is five hours, and only 4% remains after 24 hours. Radioautographs showed urea to be the only labeled compound in the urine. Initial distribution in the body tissues is random.

Krypton and Inert Gases. While one may not, strictly speaking, refer to the "metabolism" of the inert gases, studies with them, particularly radiokrypton, have contributed considerable information to our knowledge of circulation in the extremities, particularly under conditions of abnormal pressure (*390*). A rather surprising observation is that gas given by stomach tube appeared rapidly in the circulation of the extremities and in the exhaled air.

Conclusions

In this report, an attempt has been made to summarize briefly progress made to date in certain specific fields in which isotopes have been applied to the solution of pharmaceutical and medical problems. We have likewise noted that progress has been made in the diagnostic and therapeutic use of isotopes. This progress, seen from day to day, seems discouragingly small. Taken altogether, it represents a very much worth-while contribution.

Four great obstacles to the full use of isotopes in chemistry, pharmacy and medicine, are being dissipated: **(1)** Research workers in general are rapidly losing their fear of isotopes and coming to look upon them as a very useful tool. **(2)** A well-trained group of "isotopologists" is gradually being built up who understand the limitations and hazards, as well as the advantages of isotopic studies. **(3)** Basic data, methods, and equipment are being developed to the point where one can proceed with a problem without spending months in preliminary work. **(4)** The availability of tagged compounds, reagents, and research medicinals of a purity and pharmaceutical quality equal or superior to ordinary drugs is well on the way to being an established fact.

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