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APPENDIX I. "

~~January 27, 1944~~

MEDICAL AND BIOLOGICAL IMPORTANCE OF RADIOACTIVE MATERIALS PRODUCED IN PILES, 39

by W. E. Cohn 13

~~To: S. K. Allison~~

~~From: W. E. Cohn~~

Last April (in MUC-HG-71), I proposed that neutrons escaping from the piles then planned be partially utilized in the production of useful radioactive isotopes instead of expending all of their energy in the biological shields. My interest was aroused by the demand and need in the fields of biochemistry and medicine for those atomic species which can be produced by n,γ and n,p reactions. This was a natural result of my experiences (in the five years prior to joining the project, and since) in biological tracer and radioactive therapeutic work, and the continual difficulties encountered, regardless of location, in procuring suitable radioactive material -- suitable from the twin viewpoints of total activity and specific activity (vide infra). Additional spurs to making the proposal were given by the facts that: a. certain war projects were in acute need of the type of material that could thus be produced, b. many of the materials, not needed (to my knowledge) in war work, were of such long half-life as to permit of their activation for the duration, thus involving during the war only the work of placing them in position, the product to be harvested at any time desired.

Biochemistry, in the broad sense, may be considered to have entered the era of the study of metabolism. Each living organism is, from the chemist's viewpoint, an extremely complex mixture of compounds, all intertwined and interdependent to a degree unknown in any artificial system, whose amounts, concentrations and structures may vary only within very narrow limits if life is to be maintained. Any adult living organism may serve as an example of the steady state; materials enter and materials leave, but the complex structure is maintained constant. The reactions and the sequences of events occurring during the stay in the organic whole constitutes metabolism. There is scarcely a department of biochemistry, physiology, anatomy, pharmacology, medicine, zoology, nutrition, plant physiology, or biological science, which does not (or did not prior to the war) devote a major part of its research program to problems which can be classed under the heading of metabolism.

This concentration of effort is no mere accident. Such studies are fundamental to any comprehension of the means by which life (including activity and reproduction) is maintained. The energy for all life on this planet is derived from the sun through photosynthesis, essentially a metabolic process. The utilization of this plant-stored energy by non-photosynthetic organisms is another important field of metabolic study (and one which has taken a new lease on life since the introduction of the tracer technique with carbon isotopes). Similar examples could be cited all the way from such relatively "pure" scientific inquiries to the more "practical" fields of medicine and industry.

The introduction and rapid expansion of the tracer technique and the surge in the study of metabolism are not easily dissociated. Tracers, stable and radioactive, offer many great advantages not available before to research men in this field, such as:

1. Aids to analysis. The extreme sensitivity, in terms of numbers of atoms, of radiochemical methods has been found useful in chemistry as well as

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in biochemistry (e.g., von Hevesy's use of radio-lead in determining the dissociation of $PbCrO_4$, also the metabolism of "trace" elements as studied by Greenberg, Whipple, etc.). Adaptions of note are

a. The radioautograph, making it potentially possible to determine the actual cells involved in an accumulation or secretion process (see Hamilton's review for photographic examples);

b. The "outside analysis", which takes advantage of γ-radiation to analyze for a tracer species without disturbance of the status quo in the animal or tissue analyzed (Lark-Horowitz, Hamilton, Moore and Tobin, Cohn, and others).

c. The "isotope dilution" technique, which has made it possible to analyze quantitatively for chemical or cellular constituents which cannot be isolated or separated in a quantitative manner (Rittenberg, Chargaff, Whipple, Ruben and Kamen, and others).

2. Equilibria in the steady state, including,

a. Permeability of cell membranes to essential ions, be these simple, such as Na or K, or complex, such as phospholipids or amino acids, where disturbances in the intra- and extra-cellular concentrations of these ions are not compatible with normal life processes (Cohn, Chaikoff, and others).

b. Establishment of mechanisms of cellular reactions under conditions compatible with life, such as the mechanism of CO_2 fixation and of carbohydrate metabolism. (Ruben and Kamen, Hastings et al, Evans and Slotin).

c. Rates of reaction in the steady state, such as are involved in enzyme studies (Meyerhof).

3. Mechanisms and pathways -- "tracer" work in the purest sense -- involved in the study of bone and tissue metabolism, growth and healing, secretion and filtration processes, identification of intermediates; the establishment of metabolic sequences, of growth versus exchange, of enzyme action, of rate-determining steps, of the reversibility of reactions; the specific point of action of drugs, hormones, vitamins and chemotherapeutic agents. (Examples are too numerous to state; see reviews of Lawrence, Hevesy, Schonheimer, Haven and Hodge, Chaikoff, Hamilton).

To the biochemist (using the term in the broad sense), the element of greatest interest is carbon, which is concerned with both the fueling and construction of living matter. Second in his list would be nitrogen, involved in both structure and maintenance of the life processes, without which no protein -- and no life -- can exist. Coupled with these are oxygen and hydrogen, which, together with C and N, are the organic framework of biological matter. Following these four, at a respectful distance, are sulfur, involved practically exclusively in organic form, and calcium, phosphorus and iron, concerned both with organic compounds essential to life and with the inorganic framework, chemical and skeletal, of organisms. A third group would comprise Na, K, Mg and Cl, which exist in the plant or animal body in, and exert their peculiar effects through, their common ionic forms. A fourth group, comprising those elements about which so much is known as to limit the scope of their importance or about which so little is known as to render classification difficult, would include I, Mn, Co, Cu, F and Zn. The fifth and last group would include the elements known to have biologi-

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cal effects but whose presence, in the organism, as of this date, seems to be non-essential or detrimental, such as Pb, As, Rb, Cs, Ba, Be, Se, Te and others (Ra is omitted since its biological effect is not exerted through its chemical properties).

There are three things about this sequence which merit attention. The first is that the order as given is not only the order of metabolic importance but also of metabolic complexity. Secondly, the list includes only a relative few of the lighter elements, particularly if we exclude group five. Third, the ease of production, if not the existence, of radioactive isotopes of the above elements suitable for biochemical investigation (at least prior to the advent of the chain-reacting pile) is almost inversely proportional to the position of the element in this list.

This last factor has had its effect on the work in the field as the biological tracer literature demonstrates. The lack of suitable radioactive isotopes of C and N (a complete lack until H^3 and C^{14} were discovered and, in view of the small amounts extant, still grossly deficient), forced some into the restricted (from the standpoints of amount of material and experimental dilution permissible) field of heavy isotope tracer work with C^{13}, N^{15}, H^2 and O^{18}. Many others, whose interest in metabolic problems was sufficient to keep them in the field even though the use of the heavy isotopes was denied to them, turned to the use of 88-day S^{35} and 34-hour Br^{82} as substitutes for N and C, to the 20-minute C^{11} for C, to the 55-day Sr^{89} for Ca, or confined themselves to those parts of the field which could be approached with the radioactive isotopes then available (P^{32}, Na^{24}, K^{42}, Cl^{38}, F^{18}, Fe^{55}, Fe^{59}, I^{131}, etc.). A third group, large in number, not desiring to deviate from the attack on the principal elements, C and N, and not being able to work with the heavy isotopes, have preferred to continue work along the classical lines until suitable isotopes, in sufficient quantity, became available.

The foregoing is an attempt to explain my conviction that the market for the isotopes which can be produced in the pile is in no way limited to those individuals nor to those problems which constituted the market either before the war or now. From the extent of collaboration already shown between physicists and biochemists all over the country, I regard the relatively small pre-war market for radioactive isotopes, and the relatively small number of investigators using them, as being due solely to the shortage of suitable material for the more important problems in the field, and not at all due to lack of electronic and other apparatus or of trained personnel, such as is the case in the heavy-isotope work. Certainly there will be no dearth of trained personnel in the radioactive tracer field from now on.

From this we may turn to a consideration of those isotopes which are known and needed, and to a more exact definition of the adjective "suitable" as used above. These will be considered in reverse order.

Practically all biological tracer work involves a large dilution of the administered material, and often the important measurements are made on samples containing from 1% to 0.01% of the total activity needed to start the experiment at all (this is one advantage that C^{14} has over C^{13}). Furthermore, biological material is so inconsistent and inconstant that usually a large number of experiments must be made to rule out the statistical fluctuations. Thus <u>total</u> activity can be a major problem to one planning experiments.

<u>Specific</u> activity is not so obvious a hurdle but has proved in the past to be the most formidable of all. Any substance, whether it be a "poison", such as arsenic, or the essence of life itself, such as water, is toxic if a sufficient quantity is given to the organism to upset the status quo. This has been shown in some of the investigations with K^{42} and P^{32} and is reflected in the efforts of the Biochemical Group in the Health Division to prepare "carrier-free" fission products. Translated into actualities, this

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means that n,p reactions are preferable to n,γ since specific activity (excepting those elements which are easily subjected to isotope-separation by fractionation or by Szilard-Chalmers methods) can easily be lowered but is difficult to raise. The n,γ reaction is comparable to the d,p reaction produced by cyclotrons and one cannot state a priori which -- d,p on the cyclotron or n,γ in a pile -- may result in the higher specific activity. The cyclotron offers a high degree of focussing and higher energies than are attained in a pile, but is completely tied up to the bombardment in hand if a d,p reaction is being produced. Higher energies give it an advantage in the realm of n,p and n,2n reactions. It is unique in the field of d,2n reactions. One cannot choose between these two instruments or between reactions without consideration of the particular result desired, but in general n,p in the pile would seem to have most in its favor.

My knowledge of war projects being somewhat limited, I will lump my discussion of the uses of isotopes in these with that of their uses in general biochemical fields.

C^{14}, produced either by C^{13} (n,γ)C^{14} or, preferably, by N^{14}(n,p)C^{14}, is of the greatest importance in biochemical work in peace or war. Ever since the advent of C^{14} (discovered by Kamen and Ruben, and to a slight extent used by them in their investigation of photosynthesis), there has existed a rosy glow of anticipation in the field of biochemistry. Among those already familiar with techniques of radiochemistry this has been dimmed to some extent by the realization of the many problems involved in production, handling, utilization, measuring and recovery. Past experience has taught these individuals that between discovery of a usable isotope and its utilization in experiment there are many problems to be solved, chief of which are the attainment of the total activity and specific activity necessary to the experiment in hand. It is likely that only the n,p reaction will produce high enough specific activities, unless isotope-fractionation processes are coupled with the n,γ reaction. Long-lived carbon can be used in such war projects as those involving gases and chemotherapeutic agents. At present those war projects that are using the tracer technique in this type of work are confined to S^{35} and other isotopes that are only poor -- and insufficient in quantity -- substitutes for the carbon isotope needed and are perforce limited to those compounds which contain the type of atom used as a tracer. The importance of long-lived radio-carbon to the field of biochemistry (embracing nutrition, pharmacology, etc.) is enormous and the demand for it, once its production is assured, will be such that a plant of this or W size may not be sufficient to supply it. In one field alone, that of photosynthesis and the fixation of carbon dioxide, the results to be obtained with a sufficient supply of C^{14} are potentially so far-reaching and significant to science, industry, and humanity at large that one could justify any means, no matter how expensive, that would produce suitable material. The preliminary results obtained by Ruben, Kamen and others, including Van Niel (one of the world's authorities on microbiology) in California before the war intervened on -- literally -- a shoe-string* and 20-minute C^{11}, are of such significance that an extrapolation of these, however made on predictable bases, can easily lead to just as far-reaching discoveries as those which initiated this project. Turning to the opposite side of the biochemical picture, the results obtained by Hastings and his collaborators at Harvard, using C^{11} in the study of carbon utilization in mammals - preliminary though they were up to the start of the war -- may well prove to be the start of a revolutionary upset in this field, which touches closely upon those of nutrition and medicine. One may include in this brief summary the work of Schonheimer and his colleagues in New York on fat metabolism, using H^2 as a tracer for carbon. This work upset many of the classical theories concerning the metabolism of the fatty acids and led to a radical revision of many concepts in this field. From what has been done here the predictions of what will result in the overlapping fields of photosynthesis and carbon fixation, carbohydrate and energy metabolism, enzymology, nutrition, pharmacology, and medicine when an adequate supply of carbon tracer is available, are difficult to hold within the bounds of reason. One can include in this discussion of what has been done

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* The Rockefeller Foundation came in to back this series of investigations just before the war put a stop to it.

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with the pre-war supply of isotopes in the field of carbon metabolism, insufficient and unsuitable though this supply was, such significant work as that of Nier and associates (with C^{13}), of Meyerhof (with P^{32}) and of Evans and Slotin at Chicago (with C^{11}) on intermediate carbohydrate metabolism (I omit those, like Cori, Hogness, Krebs and many others whose work is ripe for the most fruitful application of carbon tracers, but who have not yet been able to use them).

One may consider nitrogen and sulfur together. Both are chiefly concerned with protein and amino acid metabolism, although the importance of the fixation of atmospheric nitrogen by organisms and the significance of the metabolism of sulfur-containing thiamin and the sulfa-drugs should not be forgotten. N^{15} has been the only nitrogen isotope suitable for biological work (some use has been made of 10-minute N^{13}). The principal work in this field is that of Schonheimer and his co-workers on amino acid metabolism, with results no less spectacular than those mentioned above. The power of the method in establishing the facts concerning the interconversions of organic molecules in the animal body is shown to best advantage in Schonheimer's work on creatine (with H^2 and N^{15} as tracers for C and N) and in that of du Vigneaud in the case of choline. The difficulties in using S^{35} in the amounts and specific activities hitherto available is shown in the work of Borsook et al on thiamin. S^{35} has also been used as a tracer for whole proteins in animals by Fine and Seligman in war work on the shock problem. I have personal knowledge to the effect that more than one war project concerned with sulfur-containing gases is in great need of adequate supplies of S^{35}, more being needed than even Berkeley can supply. Carbon would supplant sulfur as a tracer in most of this work, as well as making possible an organic tracer for non-sulfurous compounds, if it were available.

Phosphorus, in addition to its combination with calcium to form the skeletal structure in vertebrates, exists in many organic forms in the animal body and plays key roles in the metabolism of fats, carbohydrates and proteins. P^{32} has found wide application in each of these fields as a tracer for phospholipids (Chaikoff and Perlman, Hevesy), for the phosphorylated intermediates and coenzymes in the glycogen-CO_2 cycles (Meyerhof and others), and for nucleoproteins (Marshak, Cohn). Because of its occurrence in such organic molecules and its desirable radioactive properties it has to a slight extent filled the need for a carbon tracer. Along with Sr^{89} (as a tracer for Ca) it has been used in the study of skeletal metabolism, including growth and the healing of fractures; a direct outgrowth of these tracer studies has been the use of P^{32} and Sr^{89} in the treatment of certain types of blood and bone cancer by Lawrence, Stone et al in Berkeley, Warren in Boston, and others. While P^{32} has always been available in much larger quantities than any of the isotopes mentioned above, there has not been enough of it nor has the necessary specific activity always been attained ($P^{31}(d,p)P^{32}$ has been the usual readtion). The small amounts of 180-day Ca^{45} have not been nearly adequate, in quantity or quality, to the need.

Radioactive iron, chiefly Fe^{59} (although Fe^{55} has recently been produced in usable quantities at M.I.T.), has been used by several independent groups concerned with blood formation, red cell regeneration, and the utilization of dietary iron (Whipple et al, Greenberg, Ross). It has found use in war projects concerned with the shock problem as a method of labelling and tracing red blood cells in this condition. The importance of Fe in respiration indicates a wide field for tracer work.

The equilibria between intra- and extra-cellular fluids in animal organisms has offered a "natural" problem for the tracer technique. Such equilibria usually involve widely varying concentrations of Na^+, K^+, Mg^{++}, and Cl^- on the two sides of a semi-permeable membrane. Classical theory, with only the steady-state concentrations for evidence, maintained that the membranes were impermeable to cations as well as to proteins. Work with Na^{24} and K^{42}, in particular, has shown that the membranes are quite permeable to

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univalent cations (Fenn, Hevesy, Cohn) and has thrown the field into a search for other means, probably involving energy, of maintaining the vital concentrations. As indicators of intra- and extra-cellular fluids Na^{24} and K^{42} have been used in war problems in which these were under scrutiny (e.g., shock). 37-minute Cl^{38} has also been used, but the 10-minute Mg^{27} is too short.

I^{131}, with an 8-day period, has been used extensively in uncovering and measuring the function of the thyroid gland, with which it is exclusively concerned, and the mechanims of the production of the thyroid hormone (Hertz et al, Chaikoff). Because of the unique structure of the thyroid gland, the most successful radioautography has been done here (Hamilton). I^{131} has been used therapeutically in the treatment of certain hyperthyroid disorders -- again a medical use based on tracer studies.

The functions of the so-called "trace elements" has been studied with the aid of Mn^{54}, Co^{57}, Cu^{64}, F^{18} and Zn^{65}. These elements appear to be essential in minute quantities, and therefore probably are concerned with certain enzyme systems. Further studies with these isotopes will probably lead to the discovery of new enzymes concerned in metabolism of foodstuffs.

Although not "biological" in a strict sense, the radioactive isotopes of Pb, As, etc. have been used as tracers in attempts to uncover the mode of action of these toxic elements or their compounds. Here it may be pointed out that many of the non-essential elements (which list includes most of the fission products) may prove to have certain properties that render them useful as biological tracers or as radioactive therapeutic agents.

No attempt will be made to list the potential uses of artificial radioactive isotopes in chemistry and in industry, but it should be born in mind that the list of their potential uses in these fields is large indeed, ranging from tracers in chemical reactions (see Seaborg review) and industrial processes to sources of gamma radiation as a substitute for industrial x-ray analysis and detection (Pecher). Rates, mechanisms, analyses, etc., are as common to chemistry and industry as they are to biochemistry, and are ideally suited to the tracer technique. At least one large chemical corporation indicated, before the war, its intention to build a cyclotron for tracer production.

Medical applications have been touched upon in the discussions of P^{32}, Sr^{89} and I^{131}, which are the only ones so far being used. Enough evidence has been accumulated to show that artificial radioactive isotopes can be used in certain medical cases to check or even cure certain specific diseases and to alleviate pain, and the lists of uses, and of usable isotopes, will grow in proportion to the tracer studies performed and the types and quantities of isotopes available. The actual use of these materials poses no new problem in control and the safeguarding of the public; all drugs are potentially dangerous and the mechanisms extant for the control of drugs in general can also be used to control radioactive therapeutic agents.

The study of radiation damage is another large biological field in which isotopes of all kinds will be used, as is clearly shown by the history of the project. Radiation is to become an increasingly large industrial and public health hazard, yet precious little is known regarding its mode of action. As sources of beta and gamma radiation with varying loci of internal deposition, all species, fission and others, will be widely used in uncovering the biophysics of radiation damage.

These last categories of radio-isotope uses bring in a word concerning quantity. One may estimate the range of activity needed for the usual biological tracer experiment as 1-100 μC per animal, or on the order of 1 mC per series of experiments. This

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estimate can be made no more accurate than an order of magnitude without considering specific isotopes and specific experiments. However, a radiological or therapeutic case may require between 1 and 100 mC -- roughly 1000 times the activity needed for tracer work. There is thus quite a case to be made for large-quantity production. For example, if 1000 leukemics were being treated with P^{32}, the total used would be roughly 5 curies per week.

From the arguments so hastily sketched above, which by no means exhaust the field, I believe that the utilization of the chain-reacting pile as a source of artificial radioactive isotopes of all kinds has a significance and a relationship to the advance of humanity which is of such magnitude that laying plans for this utilization during the war is no more out of place than is the laying of plans for the post-war peace, and the production of these materials in wartime as urgent as the training of men now going on for the administration of the peace.

Waldo E. Cohn

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