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Some suggested future developments.

SECTION I: ORGANIZATION AND DIRECTION OF THE HEALTH PROGRAM.

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In this discussion the following assumptions are made:

1. The immediate war purposes of the Metallurgical Project will be successfully realized.

2. Atomic power will, in our lifetime, radically change the nature of our industrial organization and will affect at least to some extent our social and political organization.

3. The biological affects of radiation will become an increasingly important medical problem.

Because of these assumptions a program for the fundamental study of radiation effects on tissues is proposed here and in Section II. The following observations, I feel, are pertinent to the carrying through of this program.

A national health committee working in close liaison with a larger "nucleonics" committee (see Section III) should be organized. It should have broad powers to initiate and carry through the following program.

1. Encouragement of fundamental research:

It should encourage fundamental radio-biology research through the following agencies:

a. Properly qualified individuals in universities, hospitals, etc.

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b. It should organize and maintain one or more institutes devoted to radiology study.

This, I feel, is of primary importance. In going through the radiobiology literature (like most other medical literature) one is struck by certain phenomena.

(1.) There is a deplorable lack of continuity in studies; preliminary observations are not adequately followed up. Because few investigators are able (either by inclination or by necessity) to stick at a program of research long enough, studies tend to be superficial and made up of partial truths or of wrongly interpreted whole truths.

(2.) Long range and difficult studies are ignored in favor of studies which promise early success.

(3.) Even relatively simple problems remain unsolved because the proper authorities have not had the time or inclination to work on them.

While the organization of a large full-time institute staff does assure the solution of all radiation problems, it should, nevertheless, offer at least a partial solution to the above difficulties.

Close liaison should, of course, be maintained between these institutions and groups such as the National Cancer Institute which are working on similar problems.

Special emphasis must be placed on the study of human subjects not only because human biochemistry is not absolutely reproduced in animals but also because humans have specific problems of exposure and chronicity.

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Thus, for example, chronic radium toxicity studies should be done on humans with chronic radium poisoning, and full advantage should be taken of opportunities to study humans receiving various forms of radiation therapy.

The directing body, as I see it, should not only encourage the development of original ideas by investigators all over the country, but should take the initiative in directing work into these channels where worthwhile results may be expected. Some of these channels are indicated in Section II. The list, is by no means exhaustive, and is obviously colored by my own experience. It does, however, indicate at least a few such channels where fruitful work may be expected.

2. Distribution of radioactive materials:

It should be responsible for the distribution of at least those radioactive materials whose control is deemed advisable.

3. Education:

(a.) It should make provision for the proper training of bio-physicists and others essential to the carrying out of this program.

(b.) It should make provisions for the adequate education of professionals as well as of the lay public regarding the health problems of radiation.

(c.) It should undertake to organize the pertinent literature so that it will be readily accessible to the workers in the field.

4. Monitoring:

It should make provisions to adequately monitor areas in which radioactive hazards exist.

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Discussion:

There is a widespread feeling that the really fundamental problems of radio-biology are so difficult and so hampered by our ignorance of general biology and biochemistry that the type of approach suggested in Section II is inadvisable. It is felt that the specific radiology problems are of minor importance as compared to the non-radiology problems in each of the fields discussed. It is suggested, for example, that radiation cancer is insignificant compared to the general cancer problem and radiation anemia is insignificant compared to the general anemia problem. A study of these problems would, therefore, mean the subsidization of the entire field of biochemistry.

Obviously, if radio-biology will always be of secondary importance, if it is felt that we either already know all that it is necessary to know or that the natural course of events will elucidate things that we should know but do not, then a directed program of research as herein suggested may not be advisable.

On the other hand, if it is expected that problems of radio-biology will become sufficiently important to warrant further research, then it is suggested (See Section II) that there be a reorientation of approach toward an emphasis on certain crucial unknowns. It is further emphasized that considerable advances are possible with the knowledge and tools scientists already have, and that lastly, if it is necessary to advance other specific fields in order to apply them to our problem, this should be done if the specific problem is important enough to warrant such "tangential" study.

The slow rate of accretion of radio-biologic information may have been suitable during the past three decades. With the increased tempo of discovery and application in the field today, a directed broadened program is essential if we are not to be embarrassed by future accidents and unforeseen hazards.

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SECTION II: FUTURE PROGRAM OF INVESTIGATION BY HEALTH GROUP

The following discussion includes in broad outline form suggestions for future investigations which, I feel, should receive as much emphasis as it is possible to give them. Factors of time and space, unfortunately, do not permit complete discussion of these programs. At times, too, it may seem that we are reaching for the stars, yet I am convinced that some such approach as herein described is essential if we are to adequately answer the questions confronting us.

There are certain things about radiation on which we already have at least semi-quantitative data. We know that large doses can kill tissue, and that smaller doses of a chronic nature can stimulate it to malignant growth. We know that peripheral white blood counts are affected in a variety of ways. We know that radioactive materials tend to accumulate in certain definite tissues. We are familiar with histological and pathological changes induced by radiation.

We do not know <u>why</u> radiation acts as it does. We do not know what chemical or physical changes occur in tissues to either kill them or stimulate them to abnormal growth. We do not know why chronic internal radiation produces such severe anemia. We do not know what fundamental biochemical systems are most sensitive to its primary effects and we are familiar with few reactions along the long chain of secondary, tertiary, etc., reactions which must follow. Since these things are unknown we are unable to either prevent toxic radiation effect or to reverse such effects once they have occurred. Nor are we yet in a position to clinically evaluate moderate overexposures.

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Future studies, I feel, should emphasize these unknowns rather than the statistical evaluation of the knowns or partial knowns. It is agreed that this is by far the more difficult and possible more thankless task. Yet, even partial successes in even a small proportion of these studies would make the entire program well worthwhile.

For sake of convenience, we might consider the suggestions in the following categories:

A. Biochemical Effects

B. Biological Effects

C. Clinical Considerations

It is understood, of course, that there is considerable overlapping of subject matter among these three groups.

A. Biochemical Effects

We have many clues but few proven facts concerning the nature of these effects. I would, therefore, suggest a comprehensive study of radiation effects on groups of substances which play crucial roles in cellular metabolism with special emphasis on those substances which we have reason to believe are affected by radiation. Studies with which our group has had some contact will be considered first.

1. Porphyrins

A large group of metallo-porphyrins occur in most, if not all, of the cells of the body. These include hemoglobin, cytochrome, catalase and peroxidase. These compounds are all iron complexes of either proto-porphyrin or similar porphyrins. (Chlorophyl is a somewhat similar magnesium complex of a porphyrin in plants). These compounds obviously play a crucial role in cellular metabolism.

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(a.) Because of its known effect on oxidation reduction-systems radiation might affect the state of metal in these porphyrins. Thus, for example, large doses of radiation are known to convert hemoglobin to methemoglobin (ferrous--ferric iron).

(b.) Evidence is accumulating that porphyrins are essential for cell growth. Whereas urinary coproporphyrin excretion is increased following stimulation of erythropoiesis, we have found a consistently marked decrease in the urinary excretion of this porphyrin following acute radiation exposure.

(c.) Anemia is the main cause of death in humans with chronic radium exposure. A study of hemoglobin metabolism is obviously necessary to an elucidation of the nature of this anemia. We have already shown that acute radiation anemia, contrary to generally accepted opinion, is chiefly hemolytic in nature. The study of hemoglobin derivatives in bile, feces and urine should be very worthwhile in humans with chronic radiation exposure.

(d.) Studies of more immediate derivatives of hemoglobin would also be worthwhile. The in-vitro conversion of hemoglobin to methemoglobin has already been noted. It has been shown that the porphyrin ring in hemoglobin can be broken by peroxide with the consequent formation of verdohemoglobin, a precursor of the bile pigments. Since the formation of peroxide is one of the few known primary effects of radiation it may be that this reaction (i.e., formation of verdo/hemoglobin) might have biological significance.

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(e.) It might be noted here, too, that porphyrin metabolism is markedly affected by metal poisoning. This property is discussed further in the section on metal toxicity studies.

(f.) The ease with which metal combines with porphyrins may possibly be of some importance as regards product effect. Dr. Watson has suggested that the localization and excretion of product may be influenced by this property.

(g.) Mention might also be made of certain studies which have appeared recently in the literature claiming a correlation between porphyrin and tumor incidence in mice. The significance (and certainty) of these studies is unknown.

(h.) There are suggestions in the literature that the various respiratory enzymes may be affected by radiation. Thus, Radsill and Hosh claim that death may be due to inactivation of cytochrome.

2. Nucleic Acids.

Nucleic acids are known to play an important role in cell division. Mitchell and others have postulated that these compounds are affected by therapeutic doses of radiation.

Heretofore, studies of these compounds in living substances have been limited primarily to skin and tumor tissues. Our group has been engaged in the study of these compounds in white blood cells. These compounds are being studied following suitable fractionation by means of the following three techniques:

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1. Beckman analysis of the ultraviolet absorption.

2. Phosphorus estimation.

3. Ribose and desoxy-ribose estimation.

By means of these procedures a fairly accurate picture is obtained regarding the concentration of both ribo-and desoxyribonucleic acids as well as of various other fractions present in the cells.

Dr. Bruns and others have been especially interested in the rate of turnover of these compounds. There is reason to believe that this might be a much more sensitive index of radiation exposure than is the total content of nucleic acids at any given time. Clinically practical procedures for this type of study, however, have not been developed. (See appended suggestions by L. Wattenberg.)

3. So-called "shock producing" substances.

(a1) Adenosine-like compounds.

Several investigators have postulated that adenosine-like compounds liberated from traumatized tissues are responsible for much of the ensuing clinical picture. These compounds have important physiological effects on the cardio-vascular systems. They are also important in energy transfer mechanisms. In one patient and in two dogs in whom careful post radiation studies were made, significant rises in the adenosine-triphosphate fraction of the white blood cells occurred before any significant change in the total white blood count. Dr. Fressar tells me that he has found significant increases in blood adenosine of dogs given sub-lethal doses of total body x-ray.

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(b.) Histamine.

Several investigators have suggested that histamine is liberated from radiated tissues and constitues the toxic factor produced by radiation. S. Painter and L. Prosser of the Biology Section have found frequently elevated histamine values in dogs given sub-lethal doses of radiation.

4. Phosphorus Metabolism.

Phosphorus containing compounds (including carbyhydrates, fats, and proteins) and phosphorylating enzymes play a crucial role in many phases of cellular metabolism. Mention has already been made of the possible significance of adenosine triphosphate in radiation effect. Preliminary studies of various phosphorus constituents of white blood cells indicate too that there may be a significant fall in the so-called glycero phosphate fraction. It is well established that sugar absorption from the intestine is interfered with by radiation. Since phosphorylation is necessary to absorption it may be that the inhibition is due to a change in intestinal phosphotase activity.

A thorough investigation of tissue phosphatases is strongly recommended for an elucidation of possible radiation effects on these compounds. Gomorri has described a technique whereby liberated phosphorus in the cell may be precipitated and stained in situ, thus demonstrating the local presence of phosphatase. Preliminary studies of bone marrow in our laboratory indicate that alkaline phosphatase activity (pH 9) is localized in the cytoplasm of metamyelocytes. Acid phosphatase activity (pH 4) is localized in the nucleus of more immature cells.

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In these studies glycerophosphate has been used as the substrate.

These studies might be elaborated somewhat as follows:

A. Substrates

(1.) Adenosine triphosphate

(2.) Hexose phosphate

(3.) Glycerophosphate

(4.) Nucleic acid (ribonucleic acid or, desoxyribonucleic acid)

(5.) others such as phosphocreatin, adenosine diphosphate, etc.

B. Tissue Sections to be Analyzed.

(1.) Bone marrow

(2.) Spleen

(3.) Peripheral blood (i.e., buffy coat)

(4.) Testicle or ovary

(5.) Intestine

(6.) Liver

(7.) Adrenal

(8.) Pituitary

C. pH

(1.) 4.0

(2.) 7.4

(3.) 9.0

These may bemodified as indicated by the progress of the study.

In these studies note the following:

(1.) The optimum pH at which phosphatase activity, if any, occurs in each cell type.

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(2.) The correlation, if any, between the type of phosphatase and radio-sensitivity.

(3.) Changes produced by irradiation.

Wherever histochemical changes following radiation are suggestive, chemical analyses should be made of the phosphatase activity to get quantitative data.

5. Protein Effects

A. Tissue Proteins

Numerous investigators have shown protein changes with large doses of radiation. While it is true that these doses are much greater than those given therapeutically, it is not unlikely that in vivo changes might occur with much smaller doses. It is suggested that protein studies be carried out not only on blood but on other tissues as well. White blood cell proteins, for example, might be especially worthy of study. Various chemical and physical methods available for these studies should be utilized to the fullest possible extent.

B. Protein Derivatives

1. Polypeptides in blood and in urine

(a.) Polarographic study.

Several workers have described changes in the polarographic pattern of serum in patients with increased tissue breakdown. These changes, it is suggested, are due to polypeptide changes.

(b.) Chemical study

2. Other non-protein nitrogen compounds

Break down the NPN into as many fractions as possible. Study in tissue, blood and urine.

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3. Beckman analysis of blood and urine. Numerous amino acids have appreciable ultraviolet absorption. We have recently shown marked changes in the ultraviolet spectrophotometric pattern of urine in dogs given lethal doses of x-ray. Substances responsible are most likely amino acids or similar compounds. These studies deserve considerable future amplification. Thus, preliminary studies have indicated that ion exchange resins may be valuable aids in the purification of these compounds. Such purification combined with spectrophotometric analysis would be a powerful tool indeed.

4. A study of antibody formation due to protein changes. Protein alterations, it might be expected, would make them behave as foreign proteins. The literature concerning the formation of specific antibodies associated with radiation and tissue breakdown is indefinite. Some positive studies have been reported.

5. Enzymes.

Detailed consideration of these compounds is left to those better qualified. The possible importance of the respitory enzymes and phosphatases has already been mentioned. As a layman in this field, I might suggest that wherever possible enzymes be studied in tissues of exposed animals as well as in the purified state, since the latter study alone may yield negative results, if necessary co-enzymes are lacking.

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In addition, because of the natural occurrence of protective compounds, enzyme analysis might be done whenever possible during the period of irradiation, i.e., with radioactive materials in solution.

6. Ketosteriods

Radiation effects on the adrenals and gonadal systems might be expected to involve changes in the pattern of 17-Ketosteriods excretion.

The big bottle-neck to date in this type of study is the non-specificity of the Zimmerman reaction used to quantitate these compounds. This necessitates extremely laborious preliminary purification of these compounds. I would suggest, therefore, that the Zimmerman reaction be applied to the crude urine extract and an attempt made to separate the various fractions by suitable chromatographic analysis of the various pigment fractions.

7. Oxidation-Reduction Systems.

Oxidation-reduction effects of various types of radiation have been repeatedly demonstrated in vitro. These effects apparently may or may not be mediated through the action of peroxides.

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Mention has already been made of possible oxidation-reduction effects on porphyrin compounds. Similar studies of numerous other redox systems in the body might prove very valuable. Thus, ascorbic acid, glutathione, sulfhydryl compounds, etc., might be affected.

Numerous therapeutic possibilities suggest themselves, i.e., administer substances such as methyline blue in both oxidized and reduced states. Note the effect on state of compounds and on such things as survival.

8. The Detoxifying Mechanisms

The body normally detoxifies large amounts of potentially dangerous compounds by means of such mechanisms as conjugation with sulphate or glucuronic acid. Increases in either sulphate or glucuronic acid excretion have been reported in association with various industrial exposures such as benzene.

Radiation might be expected to increase the total amount of conjugated material. It may be, too, that failure of these mechanisms may be associated with the rapid deteration of the clinical conditions of these animals before death. It is suggested, therefore, that urine analysis for these compounds be carried out to detect evidences of over exposure. In addition, therapeutic studies such as those recently reported by Martin, Rosenbaum, and Thompson (Ann. of Int. Med. 18, 57, 1943) might be carried out. These authors showed that ~~the~~ detoxifying agents such as glucuronic acid, sulphur containing amino acids and other substances protect against lethal doses of histamine, guanidine and indole.

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10. Electrolight Balance

Several authors have called attention to the similarity between radiation effect and adrenal insufficiency. The data on electrolight balance are somewhat confusing. As pointed out by Plasencia this varies as a function not only of dose but of time. The accurate nature of these changes are still uncertain.

11. Radioactive Materials

Brief mention should be made of thes use of radioactive elements in biochemical research. These materials will undoubtedly find application in the elucidation of the various reactions discussed above. For example, P^{32} has already been employed to measure the rate of metabolism of phospholipid and nucleic acids. Considerable more work remains to be done in these fields. C^{14} should be used similarly for studies of compounds for which phosphorus or other easily obtainable radioactive elements are not suitable or other easily obtainable

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B. Biological Effects

These will be considered only briefly. Again I would emphasize the advisability of fundamental studies such as the following:

1. Vascular responses to irradiation, i.e., employ microscopic study of living tissue....check with Keisley's technique of microphotography.....Investigate humoral vs. nervous mechanisms... Attempt to prevent these changes by use of suitable substances....

2. Effects on cell membranes.

Study permeability of various substances...Are changes reversible?....Can these changes account for radiology effects?... What is the nature of the diminished intestinal absorption of sugars?....

3. Nervous system effects.

Is the flaccid state of animals immediately after a lethal dose of x-ray a nervous phenomenon?....Study the relative therapeutic effects of cholinergic vs.adrenergic drugs....

4. Distribution of radioactive materials in tissues.

Radioautorgraphy to be developed to as high a degree of precision as possible.

5. Effects of shock producing substances such as adenosine-like compounds and histamine have been considered in the section on "Biochemical effects".

In addition to the type of experiments now in progress, studies like the following might be considered.

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Magnesium has been shown to potentiate the effect of adenosine-like compounds and of most types of shock. In one rabbit Simmons and Prosser showed that a sublethal dose of Mg quickly killed a rabbit given 800r 10 days previously. A sublethal dose of nembrutal, however, had a similar effect. Since both these substances are narcotic it might be worthwhile to further pursue this type of investigation. Further, investigation of substances with the opposite type of action might be considered.

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C. Clinical Considerations

These, of course, are largely dependent upon results of investigations in the biochemical and biological fields. In turn, they must influence to a considerable extent the pattern of research in these fields.

In general, we may consider the clinical aspects of radiation effect in the following categories:

1. Detection of radiation exposure.
2. Evaluation of harm from radiation.
3. Therapy

Problems of prophylaxis, including monitoring, advice regarding shielding, etc., will not be considered since I have no experience in this field. Obviously, this program must receive a very high priority.

1. Detection of radiation exposure.

a. White blood cell studies.

Routine white blood count and differential study are still of prime importance.

These studies, however, do not give a sufficiently dynamic picture of the metabolism, physiological stability, and life span of these cells.

Factors, such as the rate of liberation from bone marrow and distribution in tissues vs. peripheral blood are not adequately apparent from these studies alone.

There are indications that the following studies would yield valuable information as to the degree of abnormality of these cells.

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(1.) Phagocytic activity

(2.) Motility

(3.) Brownian movement

(4.) Supravital staining

The rate of turnover in these cells might be investigated by the following techniques.

(1.) Arneth count

(2.) Chemical studies of nucleic acid derivatives in urine.

(3.) Search for relatively specific substances in white cells (analagous to hemoglobin in red cells) whose excretion might be followed.

The condition of these cells might be further studies by chemical means.

(1.) Nucleic acids and other phosphorus containing compounds as already described. (p.8).

(2.) Metabolic activity and enzyme studies such as the following:

(a) Respiration

(b) Glycolysis

(c) Adenosinase (This enzyme is apparently very highly concentrated in white cells).

(d) Pyruvate utilization

(e) Oxidase

(f) Phosphatase

(g) Catalase

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B. Hemoglobin metabolism.

This problem is especially important in chronic radiation exposure. In cases of chronic radium poisoning it is apparently of primary importance. The following studies are suggested.

1. Routine hematological studies such as hemoglobin, red blood count, hematocrit, and reticulocyte count.
2. General pattern of cell size and type.
3. Fecal urobilinogen (as an index of the rate of red cell destruction.)
4. Hemoglobin derivatives (See p.7)
5. Urine coproporphyrin (as an index of erythropoietic activity.
6. In dogs, urinary bile pigment excretion is a valuable index of hemolytic activity.)

C. Liver function.

1. Urine urobilinogen
2. Serum bilirubin
3. Serum cephalin cholesterol
4. Serum thymol turbidity
5. Urine bilirubin

D. Miscellaneous studies.

1. Sedimentation rate
2. Urine pigments
   (a) Spectrophotometric pattern with special reference to absorption at 243,260-280, and 330 mu.
   (b) Urorosein and related pigments
3. Studies sperm count and motility may at times be indicated.

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