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SUMMARY OF RESEARCH ACTIVITY

Donner Laboratory

John H. Lawrence, Director

PROJECTS

Donner Laboratory of Medical Physics

RESEARCH ACTIVITY

I. Study of lipoproteins and their relationship to the metabolism of fatty materials.

A. Serum lipoprotein classes native to the blood have been described for the first time, with methods for analyzing each class and separating and identifying the chemical properties of each one. At least 18 different lipoprotein classes have been identified. Considerable information has been reported on the chemical composition, structure, methods of isolation and identification of lipoprotein types. In all of these studies this laboratory has consistently done the pioneer development of this field.

B. Evidence has been established that the normal pathway of fat in fat metabolism is the entry into the blood stream of neutral fat contained in lipoprotein molecules of high molecular weight, high neutral fat content, and high S_f values. Subsequently, the high molecular weight lipoprotein is reorganized by a complex blood enzyme system to progressively lower S_f values with reduction in neutral fat and release of free fatty acids. In this reorganization the cholesterol, cholesterol ester, and phospholipid composition of lipoprotein passes through successive transformations. Thus, several hundred grams of fatty materials are transported by the blood each day, and in the course of lipid transport a "digestion" of parts of the lipid takes place releasing and distributing small soluble lipid fragments to the tissues.

C. The normal state of serum lipoproteins is marked by low concentrations of lipoproteins between the size of chylomicrons (lipoproteins of S_f 40,000) and lipoproteins of the major abundant class (S_f 6). This is true of all healthy laboratory animals and children, most young men and women, and a lesser percentage of older adults.

D. Many disturbances of this lipid transport system have been found. Each has its own characteristic features of elevation of lipoprotein of particular classes, changes which are readily classified by ultracentrifugal analysis. Every disturbance of lipoprotein appears to be the failure of some part of the lipoprotein conversion system to convert swiftly the lipoprotein load available in the blood for lower S_f conversion as described in "B". Some of these disturbing mechanisms have been described:

(a) High cholesterol and/or high fat intake may be a factor
(b) Thyroid hypofunction may be a factor
(c) Obesity may be a factor
(d) Diabetes may be a factor
(e) Hypertension may be a factor
(f) Steroid hormone balance may be a factor

E. The hormone heparin has been found to be the only generally effective agent capable of restoring serum lipoproteins to a normal balance when they are disturbed. Heparin does not alter lipoproteins in itself but, in small quantities, when it is introduced into the blood stream it causes the release of the enzyme system that affects rapid lipoprotein conversion. The properties of this material and its method of release have been described by reports from this laboratory, and the major aspects of this work have been confirmed by other investigators. The identification of these enzyme substances is of prime medical importance.

F. Altered lipoprotein metabolism resulting in an elevation of the S_f 10-100 classes has been associated with the disease atherosclerosis. This association is great enough to explain the major etiological factors of this disease, and the association is only with the S_f 10-20 and the S_f 20-100 classes of lipoproteins. Hence, the lipoprotein test is of clinical importance as a laboratory aid in diagnosis and therapy. A consistent finding has been made in every animal studies (man, rabbit, dog, and chicken) that marked elevation of these lipoproteins prelude the development of experimentally induced atherosclerosis. And further, it has been found that other classes of lipoproteins which can be elevated excessively (with associated disturbances of serum cholesterol, phospholipid, and neutral fat) under experimental circumstances (especially alloxanized-cholesterol fed rabbits or excessive ACTH administration in the rabbit both associated by a great elevation of serum cholesterol and S_f 100+ lipoproteins) are not associated with atherosclerosis regardless of the general elevation of blood lipids. Elevation of the S_f 10-100 classes of lipoproteins are highly associated with atherosclerosis in the diseases that are marked by severe atherosclerotic tendency even though other classes of lipoproteins may be higher or lower than the normal level. Xanthoma tendinosum, xanthoma tuberosum, nephrosis, hypothyroidism, and uncontrolled diabetes are diseases that are especially characterized by marked elevations of the S_f 10-20 and S_f 20-100 lipoproteins. The average concentrations of these molecules may exceed 1000 milligrams per cent of either S_f 10-20 or S_f 20-100; clinically these diseases are marked by severe atherosclerosis regardless of the age of onset of the disease. The average "normal" male 41-50 years of age has 45 mg.% of S_f 12-20 lipoproteins and 64 mg.% of S_f 35-100 lipoproteins; whereas the average coronary disease patient of this age has 64 mg.% of S_f 12-20 lipoprotein and 120 mg.% of S_f 35-100 lipoprotein. Serum cholesterol has a much lesser tendency to be elevated in the patient with coronary artery disease. Due to occasional decreases of other cholesterol containing lipoprotein classes, the average older patient with coronary artery disease may indeed have much less cholesterol than the average normal of his age.

G. Thirty-nine recurrences of myocardial infarction in a population of 359 patients with known coronary artery disease clearly show that recurrence tendency is related to the level of the S_f 12-20 lipoproteins,

the average recurrence rate being 15% per year at 100 mg.% of S_f 12-20 concentration and 5% per year at 40 mg.% of S_f 12-20.

H. Dietary restriction of fat and cholesterol as a means of lowering S_f 12-20 levels is effective in some cases and statistically lower lipoprotein levels lower the recurrence rate of myocardial infarction.

I. Obesity as a factor in lipoprotein metabolism has been evaluated. It is highly associated with the S_f 35-100 lipoproteins but much less related to the S_f 12-20 lipoproteins. The S_f 35-100 lipoproteins have their fat in the form of glycerol esters (neutral fat), while the S_f 12-20 lipoproteins contain fat largely in the form of cholesterol ester molecules. Weight reduction on the average lowers the concentration of S_f 35-100 lipoproteins by 0.7 mg. of S_f 35-100 per pound of weight lost.

J. Other agents may be of value in control of lipoprotein metabolism. Of the so-called lipotropic substances--choline, inositol, methionine, phospholipid, mixed vitamine--no agent has been found that influences lipoprotein metabolism favorably, using as a criterion the serum lipoproteins. Heparin is the only substance so far discovered that can restore lipoprotein metabolism toward the normal state. It appears likely that heparin released in small quantities is the mechanism by which lipoprotein metabolism is normally controlled. Therapeutic administration of heparin (100 mg. subcutaneously per day) will markedly shift high S_f lipoprotein metabolism toward lower S_f levels, lowering the serum lipoprotein level, as well as reduce the lipid of xanthoma lesions in xanthoma tuberosum. Since the lesion of xanthoma tuberosum is remarkably similar to atheroma, and the serum lipoproteins disturbance is characteristic of atherosclerotic tendency, it is a very leading conclusion whether indeed heparin might similarly reduce atheroma of atherosclerotic vessels. This question should be answered objectively during the next year. The relationship established--between heparin and fat metabolism, especially that "hypoheparinism" may be a primary underlying cause of lipoprotein disturbances and that under these circumstances arteries become sclerosed and the vessels have a marked tendency to become thrombosed--has caused a reevaluation of the anticoagulant therapy, favoring a shift from dicumerol to heparin in the management of coronary artery disease.

K. The full job of evaluation of heparin therapy requires a longer time, facilities for followup, and the knowledge of lipoprotein metabolism.

L. The evaluation of lipoprotein metabolism with respect to normal and diseased states is now maturing. While all indirect evidence has pointed to the causative association between elevation of serum lipoproteins of the S_f 10-100 classes and development of atherosclerosis, it is the followup study of 20,000 normal men that will provide the most useful standards for the evaluation of this test of atherosclerotic tendency.

M. Ability to segregate normals based upon atherosclerotic tendency has received the attention of the armed services as well as commercial airlines and life insurance companies. Evaluation of the lipoprotein test is currently being made by the U. S. Air Forces, by several commercial airlines and by several large industries. The gain of reduction of vascular accidents in critical commands would be a very obvious military gain. Both the aspect of classification of persons as to atherosclerotic tendency and the reduction of atherosclerotic tendency through lipoprotein management are considered potential contributions to our military security.

N. The large problem of radiation damage has gained some solutions from the lipoprotein research study. Several important biochemical changes are known which are induced by radiation and which have helped in an evaluation of the mechanism of radiation damage. An alteration of lipoprotein metabolism is one of these but it differs in a special way from other known changes in that the latter represent graded degrees of change in function proportional to irradiation damage, whereas the characteristic disturbance of lipoprotein metabolism is only observed if a lethal level of irradiation has been given. Even though two animals may have been given the same quantity of irradiation dosage, one may survive and he most certainly will not show the lipoprotein alteration at any time, but the animal that is intrinsically more susceptible to irradiation and dies will most likely show the lipoprotein change. The irradiation induced lipoprotein change consists of three phases: (1) An immediate increase in lipoprotein converting ability with a release of detectable quantities of "heparin lipoprotein active factor". This lasts for 10-30 minutes after irradiation. (2) Immediately followed by a period of two days in which the ability to convert lipoproteins is largely lost; during this period there is a rapid accumulation of unconverted lipoprotein of S_f 30. (3) A period of super recovery of lipoprotein converting ability marked by reduction in the lipemia of stage "2" and by the presence of readily detectable amounts of heparin-lipoprotein active factor. These observations reestablish some earlier suggestions that heparinemia and bleeding may accompany late irradiation damage. Because of great difficulties in detecting heparin by conventional methods these observations have been refuted, but they are real and reestablished and belong in stage "3" above of severe irradiation damage. The early onset of the heparin-lipoprotein disturbance may be extremely useful in the diagnosis of degree of irradiation and recommended treatment. Only rarely in unintentional irradiation can the dose be estimated, and the best treatment of irradiation depends upon some evaluation of the degree of exposure. In the study of the mature radiation damage this alteration of lipoprotein metabolism and its relation to disturbed heparin release is of promise. Research findings in this area depend upon the extension of general knowledge of lipoprotein metabolism. The results are certainly a clue to an understanding of the lethality of the 200-700 r range of whole body radiation. The study of heparin biochemistry requires the special skills of this laboratory. It is possible that heparin can be involved in radiation damage effects with other protein systems in addition to lipoproteins. It is also known from the work of Heilbrun that heparin imbalance may interfer with the process of cell division, which is another marked consequence of radiation damage.

II. The study of the factors controlling the formation of the cellular elements of the blood.

A. The thought and technical development of this laboratory has led to measurement of the life span of the red cell and the white cell in the circulating blood and the rate of formation of red blood cells (hematopoiesis).

B. The rate of marrow hematopoiesis especially can be measured in man on a routine basis and has become a guide to the investigation and evaluation of red cell production as a factor in the blood dyscrasias, leukemia, polycythemia, and anemia.

C. The rate of formation of red blood cells has been shown to depend upon an anoxic stimulus. Increased production of red cells begins promptly upon exposure to conditions of low oxygen availability. The findings are relevant to understanding the mechanism of acclimatization to either anoxia or high altitude with its tendency to produce a polycythemia. These problems have particular bearing upon the problems of high altitude flight and anoxia.

D. The study has helped to evaluate the anoxic factors that may normally be involved in stimulating red cell production especially in the polycythemias.

E. Anemias can be classified on a basis of red cell production and destruction using this quantitative clinical test.

F. This convenient and precise method of red cell production has permitted a search for humoral factors that may control red cell formation. Several pituitary fractions have been shown to possess bone marrow stimulating--red cell producing ability. This is especially true of whole pituitary and some of the commercial ACTH preparations. Pituitary preparations relatively free from other humoral properties have been achieved which could be designated erythropoietic hormone.

G. Erythropoietic hormone, when it can be produced in sufficient quantities may be a factor of great importance in the control of anemias.

H. One of the great problems of radiation damage at any level of radiation is the associated anemia. It is quite probable that erythropoietic hormone may be of value in correcting the anemia of irradiation injury.

III. The study of nucleic acid metabolism and the use of nucleic acid turnover measurements in the understanding of multiplication of cells in normal metabolism, in cancer, in the irradiated state.

A. The formation of desoxypentose nucleic acid (DPNA) in theory (Casperson, Hammerstein and Hevesy) and in our studies the measurement of turnover of DPNA is a good index of the number of dividing cells in a given tissue. This measurement is possible under any circumstance where a labeling agent can be applied, that is incorporated into nucleic acids (P^{32}, formate C^{14}, glycine C^{14}), and when tissue samples are available at an optimum time after administration of the labeling agent.

B. Normally there is a rate of new synthesis of desoxypentose nucleic acid and cell division which is characteristic for such tissues as have been measured--liver, intestine, kidney, spleen, neoplastic tissue and embryonic tissue.

C. This process is depressed by irradiation regardless of whether the tissue concerned is directly irradiated or whether it is merely in an animal that is receiving irradiation at another tissue site. The depressive effect is proportional to the severity of radiation, being approximately 0.2% depression of cell division per r of whole body irradiation.

D. A humoral basis for the mediation of part of the depressive effects of irradiation on the normal process of cell division has been established. The depressive effect of irradiation induced indirectly is of very much shorter duration than in a tissue which is directly irradiated.

E. Further evidence for humoral control of the normal process of cell division, and additionally relating it to the biochemistry of tissue proliferation itself, is the observation that tumor tissue even of very small size or very early stages of embryonic tissue will markedly stimulate tissue proliferation of liver, spleen, and kidney. Eighty milligrams of tumor tissue is sufficient to double the rate of nucleic acid synthesis or cell division of the liver of a host animal with a subcutaneous tumor transplant. This effect is uniformly observed in all varieties of tumor transplants thus far studied on several strains of mice and rats.

F. One of the effects of irradiation of critical consequence is the depressive effect upon the general process of tissue proliferation. This is well known with regard to depression of blood cell formation. It is estimated that at the lethal level of irradiation the entire production of all new cells in all tissues in the body is probably reduced to 20 to 25% of its normal rate. In the mouse new cells of the order of 1 gram of tissue may be formed per day; in man the daily production of new cells may amount to several pounds of equivalent new tissue. This recycling of protein and other materials through the process of newly forming cells and with nearly equal tissue destruction

in the normal state constitutes an area of metabolic function that is only slightly explored, but in obvious scope must represent a rather large segment of metabolic activity. Interference with this process can be inferred to by a major part of radiation injury.

G. It has been a reasonable working hypothesis that restoration of tissue proliferation may be an aid in irradiation sickness. This problem has been pursued using formation of nucleic acids as a measure of the process of cell division. Also it has been found that cell free extracts of embryo, tumor and liver (and perhaps other tissues) have the ability to stimulate nucleic acid metabolism in the normal or the irradiated animal; and in addition, the extract will prolong life and increase survival of irradiated rats and mice.

H. Preliminary work toward the identification of the substances that stimulate cell division and give radiation protection in this manner shows that it is associated with a crude nucleoprotein fraction which can be obtained by very gentle methods of chemical purification. Thus it may be related to other growth factors (as defined by tissue culture requirements) which may also be nucleoprotein in character and it is possible that the restoration after irradiation damage may restore a similar system that is critically depleted by severe irradiation.

I. The study of this system is adding to information on the understanding of tissue proliferation and cancer.

J. The study of this system may lead to a method of achieving some lessening of the severity of irradiation sickness by an agent that may be applied after exposure to irradiation.

IV. Study of regional blood flow.

Based upon a concept that tissue perfusion by blood is the primary factor in the exchange of many substances of low molecular weight, methods have been devised that permit evaluation in the undisturbed subject of the circulatory patterns of various regions of the body including muscle, liver, bone marrow, spleen, thyroid, and so forth. These studies have contributed to basic understanding of circulatory physiology, and they provide a basis for the investigation of disease processes to the extent that tissue function related to circulatory sufficiency or deficiency.

A. A decline in circulation that may reflect the pattern and associated causes of the aging problem has been found in these studies. Circulatory diminution of blood flow to the muscles is declining rapidly from a peak in early adolescence. Apparently this flow of blood decreases progressively throughout adult life and parallels the ebb of athletic prowess and the advancing infirmities of age. This problem is being pursued vigorously and involves a number of investigators.

B. Liver blood flow can now be simply and safely measured in man by the chromic phosphate colloid method without the use of venous catheters. These studies have a perspective importance in the study of human metabolism, as the liver and tissues of the hepatic portal drainage may easily account for 30 to 50 per cent of metabolic effort of the body. Liver blood flow is particularly enhanced in leukemia, and this may be related to the long observed increase in basal metabolism of this disease. It is likely that in the active leukemic state the metabolism of the tissues involved in liver blood flow have an increased metabolism relatively greater than the other tissues of the body, which may be a clue to metabolic burden of the disease and perhaps a lead toward a basic understanding of the metabolic basis of the disease itself.

In normal man a systematic study of liver blood flow is under way with regard to possible interdependency of subtle shifts in the blood flow to these major viscera, as compared to the peripheral connective tissues of the body carcass. The division of the blood flow between the major tissue regions is not only related to the functional activities and regulation of any area of the body, but it is suspected that there can be a metabolic interplay between the tissue areas that can be reflected in concentrations of materials within the blood stream. Thus blood levels may reflect a balance between blood flow to sites of production of a material and blood flow to sites of utilization of the materials.

C. The study of the blood flow to the thyroid gland continues. Frequently in the normal balance of function of the thyroid gland the blood flow to the gland (which in man varies from 1.0 to 20.0 volumes of blood per volume of thyroid per minute; average normal, 3.0 volumes/volume/minute) is barely sufficient to carry to the thyroid gland the quantity of iodide (approximately 10 ugm of iodide/hour) that constitutes the amount that must be synthesized to "thyroxine" for the body's steady state requirements. In the hypothyroid state, the thyroid perfusion rate falls below 1.0 volume of blood/volume of tissue/minute, at which level

of perfusion the thyroid could never succeed in capturing enough iodide to meet normal metabolic demands, and it would not, therefore, pour forth organic "thyroxine" into the blood stream at normal quantity. Thus, thyroid circulation must be considered as a possible channel of the control of thyroid function. A part of the measurement of thyroid function is the measurement of thyroxine formation and utilization rate which is being measured at this time.

V. The study of body composition as it is related to metabolism and health.

A. Several aspects of body composition lend themselves to measurement. These are: the quantity of body fat which can be estimated from a measurement of body specific gravity when simultaneously evaluated with a measurement of the water content of the body. Highly refined methods for obtaining both of these measurements have been achieved in this laboratory. Accurate estimation of body fat is a critical consideration in the study of lipoprotein metabolism. Indeed, in some men, faulty lipoprotein metabolism has been associated with obesity, and metabolic improvement has resulted from weight reduction.

The estimation of changes in body fat and body water is important in evaluating the adjustment of the body to weight reduction. It appears that reduction of the nonfat mass of the body may readily take place during weight loss by calorie restriction. Such measurements are in process of evaluation with their relationship to lipoprotein metabolism and other metabolic studies. Also, a part of this study is an accumulation of data which may comprise normal standards of body composition for reference purposes.

B. Improved methods for blood volume and plasma volume measurements have long been needed. The laboratory has an advanced program to extend the catalogue of information on the blood volume in various states of health and disease in comparative animals and a critical evaluation of the technical exactitude of the measurement as it may or may not permit fine evaluation of changes in the circulating blood volume, and as it relates to total body volume, body weight, body water, body fat and lean body mass. Blood volume studies, for instance, showed that dextran is as satisfactory as plasma protein as a blood volume extendor.

C. Similarly a catalogue of information has been accumulated on the normal blood content of various tissues and organs of the body.

VI. Study of the physical theory of the mechanism of radiation injury including the "hit" or "target" theory of radiation injury.

Variations in biological response to irradiation have long been noted to be associated with the mass, intensity and energy of ionizing radiations. Continuation of studies of this field must always be kept abreast of the physical production of high intensity particles in the Radiation Laboratory. Previously, the systematic study of biological effects of ionizing radiation have been limited to the radioactive materials and to rather low energies per particle compared with the great particle energies at the cosmic ray level. In this laboratory, it has previously been shown that neutron irradiation effects were greater in relative biological damage than x-rays or γ-rays, also that nuclear fission at tissue sites is extremely more deleterious on a relative scale than other types of ionization. Still to be evaluated are the effects of protons, electrons, and neutrons at energies of >100,000,000 electron volts. Since laboratory energies of the order of 10,000,000,000 electron volts are anticipated, estimation of hazard is of great importance.

Above stratospheric levels the concentration of high energy, highly ionized particles becomes very great. Laboratory estimation of the hazard to man of such exposure can be made at this time in the Radiation Laboratory using the large accelerative devices to reproduce the particles and energies that are in the energy range estimated for the "cosmic cloud".

Thus biological tolerance and hazard of almost any kind may be predicted accurately from current work. These studies are also valuable in extending the systematic study of intensity, energy, and duration of exposure. The study has a direct bearing upon interpretation of the site of primary disturbances of tissues by ionizing radiation. This work has already established an explanation for the inherent difference between radio-resistant and radio-susceptible strains of yeast based upon the number of sensitive genetic loci of radiation target within the cell.

VII. Irradiation with high energy beams.

High energy beams of the accelerator devices have found their place in experimental radiology. The beam of the 184-inch cyclotron has remarkable properties of focus and control, and since the entering ionization dose to the tissues is considerably less than the dense ionization at the end range (Bragg effect) of the penetration of the beam, small volume precision irradiation can be achieved without damage to overlying or surrounding tissues. The method is of good potential value in radiological treatment of tumors and it has been especially useful as a means of producing experimental injury in areas of the pituitary and hypothalmus without otherwise disturbing the experimental animal. Unlike 200 kv x-rays where at 10 cm. in tissue the depth dose falls off to 50%, in the case of 190 mev deuterons from the 184-inch cyclotron the depth dose is four or more times the surface dose. The therapeutic possibilities of such a surface depth dose relationship are obvious, and the University of Chicago group is utilizing this work in planning a program of therapeutic treatment.

VIII. Infrared spectroscopy.

Analysis by infrared spectroscopy has been developed especially for lipid studies. Lipid analytical methods by usual chemical means are difficult and deceptive. The study of the chemical composition of large molecular weight proteins and lipoproteins has been possible for the first time on a firm basis using as an analytic tool, the infrared spectroscopy. Bond structure in the infrared region of the spectrum is attuned to the energies of specific atomic bonds in molecular structure with precise dependence upon the wave length in the infrared. Thus from the pattern of absorptions of infrared wave lengths, a great deal can be guessed both as to the atomic composition of material and to the nature of the interatomic bonds. This has become our most powerful tool in the identification of substances involved in fat metabolism. The methods that have been devised can be applied to reflection type microscopy so that at least coarse resolution of tissue composition may be made at the microscopic level with regard to the distribution of recognizable fatty materials.