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Number 13 of 14 copies, Series A.

CLASSIFICATION CHANGED TO NOT CLASSIFIED
TID-1116 5-15-52
Authority of: USAEC

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To: Robert S. Stone

From: C.J. Watson

December 20, 1944

The following represents a summary of the information which I gained from the discussions with Drs. Dougherty and White in New Haven on Monday, December 18th. Their main thesis is that the adrenotropic hormone is one of the main regulators of the lymphatic system by mediation through the adrenal cortical hormone. They have obtained considerable evidence that pure adrenotropic hormone injected into animals causes at least transitory lymphopenia with a simultaneous reduction in the amount of sudanophilic material (lipid) in the adrenal cortex and a gravimetric decrease in the amount of cholesterol in the adrenals. They speak of the effect of adrenotropic hormone on the adrenal cortex as "adrenal firing". They state that the best method of testing for "adrenal firing" in acute experiments (3 to 6 hours) is the amount of sudanophilic material in the adrenals. If adrenotropic hormone is given in what they speak of as an intermittent continuous fashion, that is to say, once or twice daily for longer periods, a lymphopenia has been noted to persist in mild degree even up to a year. In some instances the lymphopenia has been noted to give place to a mild lymphocytosis. This is regarded by them simply as an evidence that regeneration in the lymph nodes has gained the upper hand over destruction. At this point it may be mentioned that they correlate the effect on the peripheral blood directly with histologic changes as noted in the lymph nodes. These consist mainly of a deterioration of many of the lymphocytes indicated by pyknotic nuclei and fragments of nuclei together with obviously degenerating cells. In acute experiments this change is quickly followed by an increase in young forms, the so-called reticular lymphocytes, as well as reticular cells, an increase which is regarded as an immediate compensatory regeneration. They believe that this regeneration can at times result in an actual peripheral lymphocytosis. The initial lymphopenia, however, is characteristic and in most instances persists with continued administration of the hormone. In addition to the lymphopenia, moderate early decreases in hemoglobin and red blood cells have also been noted. Dougherty and White believe that these decreases are not due to hemodilution but rather to increased erythrocyte destruction, as an evidence of which they have noted increased erythrophagocytosis.

The same sequence of events is produced by adrena[illegible] hormone, particularly a preparation of adrenal steroids in oil which is [illegible] concentrated. The adrenotropic hormone is effective only in animals with intact adrenals. In adrenalectomized mice or rats the effect on the lymphocytes and lymph nodes is observed only when adrenal cortical hormone is employed.

With increased ad[illegible]tivity, whether from adrenotropic hormone or adrenal cortical hormone, th[illegible] uniform and characteristic diminution in size of the lymph [illegible] as well as [illegible]chymus, whereas, after adrenalectomy the lymph nodes and thymus increase. [illegible] noted that fasting adrenalectomoized rats do not lose body weight or organ weights (spleen, liver, heart, lymph nodes) nearly as rapidly as do fasting normal rats. The differences here were quite striking. Dougherty and White visulaize this difference as being due to a lack of adrenal cortical hormone

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to "push" protein out of the lymph nodes and the lymphatic areas. In other words these animals cannot get at the stores of protein in the lymphocytes. They think it possible that protein may be yielded by normal cells not undergoing actual necrosis but the only evidence they have obtained relates to lymphocytes in which actual deterioration has been observed.

Histologic studies of the lymph nodes following adrenotropic hormone or adrenal cortical hormone (steroid in oil) reveals first the deterioration of the lymphocytes as already noted. This is followed by the appearance of phagocytes which clean up the pyknotic nuclei. It is believed that the protein has been metabolized before this stage is reached (vide infra). The lymphocytes in mi[illegible] of injury show a more deeply basophilic staining of the cytoplasm and areas of the same basophilic materials are seen in the phagocytes. The nature of this substance is not known, but the question arises as to whether it is protein which has become loosened or relatively more available and has been in part phagocyted. Significant increases of protein were noted in the blood serum after injection of adrenotropic hormone. In experiments lasting six hours the increase was from 6.0 to 6.30 gms. % In 15 day experiments an increase of 6.0 to 6.52 gms% was noted. These changes were ascertained to be statistically significant. Tiselius studies showed that the increase was due to beta and gamma globulin, particularly the latter. Lymphoid cell extracts were then made and Tiselius patterns studied. These revealed the presence of 4 proteins, 2 of which were entirely different from any proteins encountered in the serum; one was identical with gamma globulin and the other may be beta globulin although this was not proven.

Because of the known relation of antibodies to the globulin fraction, rabbits were immunized against sheep red blood cells. After six weeks, when hyperimmunization had been achieved, adrenotropic hormone was given and a marked further rise was observed. In similar animals in which the titre of antibody was allowed to fall to zero over a period of time, administration of hormone was followed by a prompt and marked rise often to the previous hyperimmune levels. This in other words constitutes an artificial "anamnestic" response. In much the same way mice were immunized with sheep red blood cells after which they developed high hemolysin titres in the serum. The lymph nodes were then taken from these mice and extracts were made of the lymphoid tissue. These were found to have a very high hemolysin titre. On the basis of protein nitrogen this was about 8 times that of the serum, that is to say that the titre of the extract was actually twice that of the serum but it only had one fourth as much nitrogen so that a dilution factor of four could be presumed to have entered in. For purposes of control extracts of salivary gland and muscle from the same animals had a zero titre. In my opinion this would have been an excellent opportunity to find out whether reticuloendothelial cells as compared with lymphocytes were high in antibody (globulin) titre. This might be achieved by making similar extracts of the liver after thorough washing.

Administration of 200r in mice in the form of total body radiation was found to fire the adrenal but it also effected the lymphatic tissue [illegible]ectly since in adrenalectomized mice entirely similar changes were noted in the [illegible] nodes. 10r however, failed to produce any histologic changes or peripheral lymp[illegible] in the adrenalectomized animal. but this amount of radiation did produce [illegible]ite histological changes and ly[illegible]openia in normal mice over a four hour period.

It was of inter[illegible] that 200r in an adrenalectomized mouse, although causing lymphopenia and an [illegible] serum proteins, is nevertheless associated with a "spry[illegible] and hap[illegible]on" than is noted in a simply adrenalectomized mouse not receiving radia[illegible] This striking difference was believed to be due to a failure on the pa[illegible] the latter to get a supply of globulin from the lymph nodes

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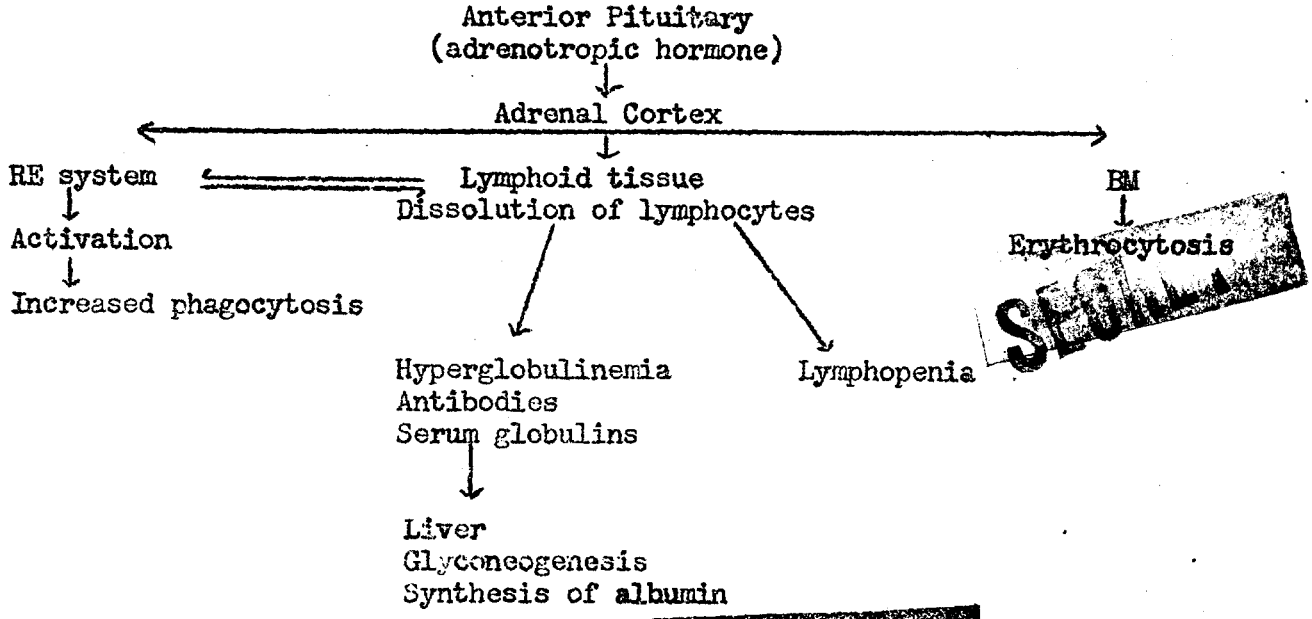
while in the former mice the direct effect of radiation on the node was sufficient to liberate this protein.

"Adrenal firing" was believed to be brought about in all instances [redacted] less of the initial stimulus (whether radiation, cold, fever, [redacted] by the adrenotropic hormone from the anterior lobe of the pituitary [redacted] implies, of course, that something must stimulate the latter to [redacted] adrenotropic hormone. The nature of this stimulus is unknown, but there is fairly clear evidence that epinephrine under certain circumstances, as for example, fever, insulin, cold, fear is the responsible agent. It is also known, however, that histamine stimulates the pituitary, and since radiation may temporarily increase the blood histamine the possibility exists that the chain of events may be primary elaboration of histamine with secondary stimulus to the anterior lobe, release of adrenotropic hormone, and firing of the adrenal. All of this Dougherty and White admit to be wholely theoretical insofar as radiation effect is concerned. The fact remains, according to them, that there is not radiation effect (with 10r) in hypophysectomized animals in contrast to that seen in normal animals. They are inclined to believe, speaking broadly, that a nervous or chemical stimulus derived from injured irradiated cells first fires the hypophysis thus liberating adrenotropic hormone which in turn stimulated the adrenal cortex to the liberation of cortical hormone.

As noted above, a slight decline in hemoglobin and red cells occurred in the acute experiments with adrenotropic hormone. In chronic experiments in rats a slight persistent polycythemia was observed when adrenotropic hormoze was given in an "intermittent continuous" fashion. This is, of course, of some interest in relation to the frequent occurrence of polycythemia in cases of adrenal cortical hyperactivity such as Cushing's syndrome.

The accompanying chart shows the relationships which Doughtery and White believe the anterior pituitary has to the lymphatic and reticuloendothelial systems.



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From the standpoint of the Metallurgical Laboratory I should think it would be desirable to confirm or deny Dougherty and White's results using 10r in mice and rats with respect to lymphopenia and changes in the lymph nodes. Since this is admittedly a mild or border-line dose it would seem to me that the histologic study should be carried out in a quantitative fashion. By this I mean that actual differential counts of damaged and undamaged lymphocytes ought to be made.

Assuming that these results are confirmed it would appear that there are several applications of possible importance from the standpoint of the Metallurgical Project. Some of these may be given as follows: a) the response of immunized subjects to small single doses of x-ray or other radiation to note whether significant increases in antibody titre might appear. More specifically one might immunize a human subject with typhoid vaccine, then, after stabilization of the antibody titre had been noted by serial study 10r of total body radiation might be given followed by frequent determination of titre of antibody in the circulating blood. b) The content of gamma globulin of normal lymphocytes might be compared with that of the lymphocytes of irradiated individuals and as controls of those receiving cortical hormone or adrenotropic hormone. If such a study were to be carried out it might be well first of all to study the lymphocytes from a patient with chronic lymphatic leukemia in which, because of the high count, a relatively large number would be available. This would permit an insight into the number of lymphocytes necessary to detect and characterize any globulin present. For this study, Dr. Schwartz' method of isolating lymphocytes from the blood ought to be of the utmost value. c) The assay of urine and/or blood serum for cortical hormone by means of a biological test with adrenalectomized mice. According to Dougherty and White this method has already been used and is believed to be quite sensitive. They state that Dr. Koch, director of biochemical research at the Armour laboratories, Chicago, has had more experience with this technique than anyone else and that he believes it to be very useful. Since it would not appear feasible to inject urine or blood serum directly into adrenalectomized mice because of the dilution factor, an extraction and concentration would probably be necessary. Dr. White suggested that a large amount of urine might be extracted with benzene which would remove all of the cortical hormone. After addition of a small amount of sesame oil the benzene could be distilled off. The cortical hormone would then be concentrated in the sesame oil which could be injected into the adrenalectomized mice. The probable difficulty here would be presence of concentrated impurities which would be toxic. If this method failed it is quite possible that some preliminary fractionation or chromatographic analysis could be carried out without too much difficulty. It is likely that Dr. Koch would be able to answer these questions o the baiss of the experience he has already had.

Some of Dougherty and White's work has already been published. The references are as follows:

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1) Dougherty, T.F. and A. White, Influence of Hormones of Lymphoid Tissue Structure and Function, the role of the Pituitary Adrenotropic Hormone in the Regulation of the Lymphocytes and Other Cellular Elements of the Blood, Endocrinology, 35 , I, 1944.

2) White, A. and T.F. Dougherty, Influence of Pituitary Adrenotropic Hormone on Lymphoid Tissue Structure in Relation to Serum Protein, Proceedings, Society for Experimental Biology and [redacted] 56, 26, 1944.

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3) Dougherty, T.F., A. White and J.H. Chase, The Relationship of the Effects of Adrenal Cortical Secretion on Lymphoid Tissue and on Antibody Titre, Proceedings, Society for Experimental Biology and Medicine, 56, 28, 1944.

Assuming that the early lymphopenia and histologic chang[redacted] following 10r, can be confirmed, it might be well to "farm out [redacted] experiments. For example a and b in the above could be assigned to Dougher[redacted] White who could carry them out, if need be, without further information other than that of our interest in radiation effects. C, in the above, might be assigned to Dr. Koch, or at least he might be invited to make certain pilot studies sufficient to determine whether more elaborate ones ought to be undertaken.

I should like to make the further comment that I am inclined to accept Dougherty and White's work in a general way, and that it probably constitutes a very important advance in our knowledge of the finer mechanisms of adjustment for defense between lymph nodes and blood.

Sincerely yours,

C J Watson

C.J. Watson MC

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