The Influence of the Adrenal Cortex on Serum Protein Metabolism in Normal and Malignant Lymphoid Tissue*

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The hormonal secretions of the adrenal cortex are known to be capable of causing a striking regression of normal lymphoid tissue (cf. 22) and of exerting at least a transient growth-arresting effect on various types of lymphoid tumors (1, 2, 5, 6, 8, 12, 13, 20, 21, 24). In contrast, a selective action of corticosteroids on the metabolism of normal and malignant lymphoid tissue has not been clearly demonstrated (cf., however, 7, 19).

The observation has recently been made that pituitary-adrenal cortical activation may result in the enhanced mobilization of specific proteins from normal hepatic and splenic tissue (15, 18). These proteins appeared to require serum albumin for their formation (16), and were translocated about the body as plasma proteins (15). In view of these findings, it seemed of interest to investigate the effects of the adrenal cortical hormones on serum protein metabolism in the presence of normal and malignant lymphoid tissues. Analyses were made of the alterations in total, nonprotein, and amino acid nitrogen and in the electrophoretic pattern of rat serum media in incubation. Serum samples were prepared for electrophoretic analyses were performed on the serum before and after incubation. Serum samples were prepared for electrophoresis by the dilution of 1-ml aliquots with 2.5 ml. sodium diethylbarbiturate buffer at pH 8.6 (ionic strength, 0.10) and were dialyzed against this buffer for 2 days in the refrigerator. All electrophoretic studies were conducted at a temperature of 0° C. in the Perkin-Elmer Tiselius apparatus, with a constant current of approximately 10 ma. passing through a 2-ml. cell. The term a-globulin is employed below to designate the combined areas of the a-globulin and a-globulin boundaries.

In vivo investigations were conducted on rats bearing the transplantable lymphosarcoma. At frequent intervals after subcutaneous transfer, tail blood samples were obtained and total and nonprotein nitrogen analyses made on the sera. Groups of animals were sacrificed at 3-day intervals after inoculation by exsanguination from the abdominal aorta. Electrophoretic analyses, as well as nitrogen determinations, were performed on these sera. The transplantable lymphosarcoma employed in these studies was found to “take” in 100 per cent of the Sprague-Dawley rats inoculated. The implant in each instance was introduced by trocar into the subcutaneous tissues of the left flank. The rate of growth of this tumor was found to be quite consistent in otherwise untreated animals, reaching a weight of 54 ± 3 gm. (28 rats) 80 days after transplantation to Sprague-Dawley rats weighing 250-300 gm. In adrenalectomized animals, the corresponding figure was 29 ± 5 gm. (28 rats). The tumor weight was only 18 ± 2 gm.

Materials and Methods

The animals used in these investigations were adult, male, Sprague-Dawley rats, weighing between 250 and 300 gm. and maintained ad libitum on Purina Laboratory Chow, with a weekly supplement of fresh lettuce. Adrenalectomized animals were maintained on 0.9 per cent sodium chloride. Endocrine preparations were injected intraperitoneally; these included Wilson's aqueous adrenal cortical extract (ACE), Armour's adrenocorticotropic hormone (ACTH), and Merck's Cortone (cortisone acetate).

Studies in vitro were conducted in Warburg flasks, containing 100 mg. of tissue mince and 2 ml. of fresh serum, obtained from rats under Nembutal anesthesia by exsanguination from the abdominal aorta. The tissue mince consisted mainly of intact cells (17). The tissues studied included normal thymus and mesenteric lymph nodes1 and a transplantable lymphosarcoma2 maintained by subcutaneous passage in Sprague-Dawley rats (18). Incubations were carried out at 38° C. with a constant stream of 95 per cent oxygen-5 per cent carbon dioxide passing through the flasks. Total (17), nonprotein (17), and amino acid (8) nitrogen determinations and electrophoretic analyses were performed on the serum before and after incubation. Serum samples were prepared for electrophoresis by the dilution of 1-ml aliquots with 2.5 ml. sodium diethylbarbiturate buffer at pH 8.6 (ionic strength, 0.10) and were dialyzed against this buffer for 2 days in the refrigerator. All electrophoretic studies were conducted at a temperature of 0° C. in the Perkin-Elmer Tiselius apparatus, with a constant current of approximately 10 ma. passing through a 2-ml. cell. The term a-globulin is employed below to designate the combined areas of the a-globulin and a-globulin boundaries.

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1 Mesenteric lymph nodes refers to the mesenteric chain of nodes dissected free of fat and connective tissue prior to mincing.

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(21 rats) in animals periodically injected with cortisone acetate (2.5 mg intraperitoneally/100 gm body weight every 12 hours after implantation). A similar inhibition of lymphosarcoma development after the administration of cortisone acetate has been reported by Ingle and Nesamis (6).

RESULTS

Influence of adrenal cortex on serum protein metabolism in normal and malignant lymphoid tissue in vitro.—The rate of release of total protein from splenic and hepatic tissue in vitro has previously been shown to be proportional to the level of circulating adrenal cortical hormones in the animal (15). In the present investigation a similar relationship was found to exist in surviving normal and malignant lymphoid tissue.

Mesenteric lymph nodes, thymus, and lymphosarcoma tissue obtained from untreated rats were observed to release proteins to a serum medium in vitro at a rate intermediate between that observed for animals adrenalectomized 2–4 days earlier and those injected intraperitoneally 2 hours before autopsy with either ACE (2 ml.) or cortisone (2.5 mg/100 gm of body weight) or ACTH (equivalent to 4 mg of Armour standard LA-1-A/100 gm of body weight). These results are depicted in Chart 1. Electrophoretic examination of the serum media before and after incubation with these surviving tissues yielded the results shown in Chart 2. It is apparent that the major protein released by both normal and malignant lymphoid tissues in vitro possessed an electrophoretic mobility characteristic of serum β-globulin (cf., also, Chart 5). Lymphosarcoma tissue appeared to release larger than normal amounts of "α-globulin," as well as "β-globulin." In all instances, albumin disappeared from the serum medium concurrently. This disappearance was most rapid in the presence of malignant lymphoid tissue. It was, in fact, so rapid during the 1st hour of incubation, in the presence of tumor tissue obtained from adrenalectomized rats, that the total protein content of the serum medium dropped significantly (Chart 1).

In other experiments, the rate of release of amino acids to a serum medium by normal and malignant lymphoid tissue was followed under conditions identical to those described above. Chart 3 reveals that the release of amino acids was appreciably greater in tumor tissue than in normal lymphoid tissue, when these tissues were obtained from otherwise untreated rats (open circles). Adrenalectomy or hormone treatment was without influence on this process in normal lymphoid tissue. In contrast, the release of amino acids by lymphosarcoma in vitro was slightly de-
pressed by previous adrenalectomy of the donor rat, and markedly depressed after the administration of pituitary ACTH or adrenal cortical hormones (ACE or cortisone).

Influence of adrenal cortex on serum protein metabolism in rats carrying a transplantable lymphosarcoma.—The effects of the development of the lymphosarcoma and of variations in adrenal cortical activity on total serum proteins in tumor-bearing rats are illustrated in Chart 4. It will be observed that the serum proteins exhibited a biphasic response to the presence of the tumor. The tumor became palpable about 5 or 6 days after implantation and then continued to grow at a moderate rate for the next 5 or 6 days. During the latter period, the first depression in serum protein levels occurred. About 12 days after implantation the lymphosarcoma appeared to begin growing with markedly increased rapidity. This continued for about 10–12 days, at the end of which time the tumor attained its maximum size. The host became moribund and died soon thereafter. During the period of most active tumor growth, 12–17 days after implantation, the level of total serum proteins exhibited a rapid rise which continued until a few days before the animal's demise. The average length of survival of adrenalectomized, untreated, and hormone-injected rats bearing the lymphosarcoma is indicated by the last point on each curve in Chart 4.

The nature of the changes in the individual serum proteins during development of the lymphosarcoma is revealed in Charts 5 and 6. Chart 5 depicts the electrophoretic pattern of serum obtained from otherwise untreated rats 20 days after subcutaneous implantation of the tumor. It will be noted that a marked reduction in serum albumin occurred, accompanied by a large increase in β-globulin and a somewhat smaller increase in the α-globulin area. These changes were remarkably similar to those produced in a serum medium in vitro after incubation with surviving lymphosarcoma tissue (Chart 5). Chart 6 reveals that the decline in serum albumin was most rapid during the first 10 days after tumor implantation and did not appear to be markedly influenced by adrenalectomy or cortisone treatment. The elevation in α- and β-globulins was progressively more

![Chart 4](https://example.com/chart4.png)

![Chart 5](https://example.com/chart5.png)
rapid until about 20 days after inoculation and then declined rapidly until the animal's death. Hormone treatment significantly accentuated the rise in β-globulin; adrenalectomy tended to produce the opposite effect.

Total serum nonprotein-nitrogen levels exhibited a consistent rise during the course of tumor development (Chart 7), doubling by the time of the host's death. Adrenalectomy markedly accentuated this rate. Cortisone injection also caused some elevation in serum N.P.N. levels, especially during the first 2 weeks of tumorigenesis.

DISCUSSION

The rapid growth of all tissues, including tumors, is, of course, associated with the enhancement in such tissues of the rate of protein deposition. A considerable body of evidence now suggests that much of this new tissue protein may be derived from protein formed elsewhere in the body (9-11, 15, 17) and perhaps translocated to the growing tissue in the form of specific plasma proteins (10, 15, 17). The mobilization of this protein appears to be under adrenal cortical control (15).

The present investigations suggest that both normal and malignant lymphoid tissues, like other tissues previously studied (15, 17), have the capacity of utilizing serum albumin for vital processes. Evidence suggests that this protein may serve as a precursor for other specific proteins elaborated by the tissue (16). During the course of serum albumin utilization by lymphoid structures, a protein possessing the electrophoretic mobility of serum β-globulin appeared to be formed in largest amounts, and was released to the circulation in the intact animal or to the incubation medium in vitro. Lymphosarcoma tissue, in addition, released appreciable quantities of an “α-globulin,” possibly identical with the mucoprotein characteristic of plasmas obtained from animals or patients afflicted with a wide variety of malignancies (cf. 23). The protein (non-lipid) nature of the substances causing an elevation in the α- and β-globulin peaks in the conventional electrophoretic patterns appeared to be established by the occurrence of similar changes in protein-staining material separated by filter paper electrophoresis of the various sera. The release of these proteins was accentuated in normal and malignant lymphoid tissues in animals with elevated levels of circulating adrenal corticosteroids.}

*S. Roberts, unpublished observations.
cal steroids and was depressed in adrenalecto-
mixed rats. Inhibition of tumor growth in rats
infected with cortisone was associated with an
increased rate of release of proteins to the circula-
tion by the lymphosarcoma and may have been
causally related to the latter phenomenon.

Studies of nonprotein-nitrogen metabolism
during tumorigenesis have not been especially
instructive. During the growth of the tumor,
N.P.N. levels in the blood exhibited a progressive
increase, presumably associated with the enhance-
ment of protein metabolism and, later in the de-
velopment of the tumor, with necrotic degenera-
tion (cf. 4). Previous adrenalectomy of the host
seemed to elevate these levels, but to a lesser ex-
tent. The latter changes were similar to those
which occur in normal animals subjected to ad-
renalectomy or hormone treatment. The extent
of their modification by the presence of the de-
veloping tumor is at present unknown. In vitro,
it will be recalled, surviving lymphosarcoma tis-
ue released appreciably larger amounts of amino
acids to a serum medium than normal thymus
and lymph nodes. The level of activity of the
adrenal cortex was without effect on this latter
process in normal lymphoid tissue, but signifi-
cantly influenced the rate of release of amino
acids by malignant lymphoid tissue. In particular,
hormone injection caused a marked depression
in the rate of release of amino acid nitrogen by the
lymphosarcoma. This depression of amino acid
release was, as noted earlier, associated with an
enhanced release of protein to the serum medium.
It may be suggested that the adrenal cortical
steroids facilitate the utilization of amino acids
for protein synthesis, particularly in rapidly grow-
ing tissues such as tumors.

The present studies, considered together with
earlier ones (19), strongly suggest a marked de-
pendence of lymphosarcoma tissue on plasma
albumin for growth and on carbohydrate for
energy (cf. 4). Adrenal cortical hormones appear
to magnify this dependence, which may be re-
sponsible for tumor regression noted after their
administration. The release of apparently specific
proteins to the circulation by different tissues,
and the accentuation of this process during tu-
morigenesis, suggest that a diagnostic tool for
the detection and localization of early malignancy
may eventually be forthcoming. The usefulness
of such a tool, however, must await the develop-
ment of more sensitive and reliable methods than
those already available for distinguishing be-
tween normal and "abnormal" proteins, includ-
ing those released to the circulation by malignant
tissues (cf. 14).

SUMMARY

Normal lymphoid tissue and a transplantable
lymphosarcoma in the rat were found to release to a serum medium in vitro a protein possessing
the electrophoretic mobility of serum β-globulin.
In addition, the tumor released appreciable
amounts of an "α-globulin." Coincidentally, albu-
min was observed to decline in the serum medium.

Similar changes occurred in the blood of rats dur-
ing the development of the lymphosarcoma follow-
ing subcutaneous implantation. The rate of re-
lease of the "β-globulin" by both normal and
malignant lymphoid tissues seemed to be directly
proportional to the level of circulating adrenal
cortical steroids. Attention is drawn to the pos-
sible relationship between these findings and the
growth and regression of the lymphoid tumor
under these circumstances.

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Announcements

ERRATUM

The word "tissue" should have appeared in place of the word "metastases" in the running head for the article "The Influence of the Adrenal Cortex on Serum Protein Metabolism in Normal and Malignant Lymphoid Tissue" which appeared on pages 582-87 of the September (No. 8, 1954) issue.
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