STUDIES OF ELECTROPHORETIC SERUM PROTEIN PATTERNS IN SUBJECTS TREATED WITH PITUITARY-ADRENAL CORTICAL HORMONES, NITROGEN MUSTARD, OR X-RADIATION*

JULIAN FRIEDEN† AND ABRAHAM WHITE‡

Investigations during the past ten years have formed the basis for the working hypothesis that pituitary-adrenal cortical secretion exerts an influence on the structure and function of lymphoid tissue. The administration to rats of adrenal cortical extract, or of adrenal cortical steroids oxygenated in position 11 of the steroid nucleus, produced marked thymic atrophy.[26, 27, 35, 40, 44, 48] Experiments conducted by Dougherty and White[10] demonstrated that adrenal cortical secretion, regulated by the pituitary adrenotrophic hormone, is a normally existing mechanism which can affect lymphoid tissue mass. At a time when lymphoid tissue involution was maximal as a result of augmented pituitary-adrenal cortical secretion, a profound absolute lymphocytopenia was observed.[11] Histological studies revealed [10, 12] that the basis for these phenomena was the marked dissolution of lymphocytes in the lymphoid organs following augmented pituitary-adrenal cortical secretion. Thus, decreased lymphoid tissue weight was due to fewer lymphocytes in the lymphoid organs, and the lymphocytopenia appeared to be due to a failure of delivery of lymphocytes to the circulation because of their dissolution within the lymphoid organs.

It was further established by White and Dougherty,[50] and confirmed by Kass,[29] that lymphocytes contain a protein which is identical with serum globulin. Moreover, in the immunized animal, antibody globulin was demonstrated in the lymphocytes of mice[8] and of rabbits.[19] It is well known that the antibody of the blood is generally found in the β- and γ-globulin fractions.[16] This led to experiments which demonstrated that a single injection of adrenal cortical extract (aqueous or oil), or of adrenotrophic

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† Present address: Lincoln Hospital, New York, New York.

‡ Present address: School of Medicine, University of California, Los Angeles, California.

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hormone, in hyperimmunized rabbits resulted within six hours in a marked rise in antibodies in the blood.[5, 9, 14] The data established that higher titers of antibody appear in the immunized animal after adrenal cortical administration at a time when lymphocyte dissolution in the tissues is most marked, and when the most striking lymphocytopenia is present in the blood.[51]

The influence of pituitary adrenotrophic hormone on lymphoid tissue structure and function appears to offer an explanation for the similarities of changes in lymphoid tissue following many unrelated stimuli. Sayers *et al.*[42] have reviewed the literature suggesting that most of these stimuli augment the secretion of pituitary adrenotrophic hormone. This hypothesis would also explain why various nonspecific agents produce an anamnestic response[4] and would correlate this response with the concomitant lymphocytopenia.

With this background of experimental evidence, it was proposed to study the serum protein pattern in dogs and in human subjects receiving either adrenal cortical extract or pituitary adrenotrophic hormone. In addition, it has been possible to examine in the Tiselius apparatus the electrophoretic serum protein patterns of patients receiving x-ray irradiation or nitrogen mustard therapy. The radio-sensitivity of lymphoid tissue has long been known.[7] Several investigators have studied the possible effects of x-ray therapy on serum proteins.[2, 6, 13, 25] Inasmuch as the majority of the previous studies in this field have utilized chemical rather than electrophoretic measurements, it was considered desirable to examine the effect of x-radiation on the electrophoretic pattern of the serum proteins.

Nitrogen mustard therapy, in use currently in treatment of various lymphomata, is known to produce within 24 hours a marked lymphocytopenia, which becomes progressively more severe during the subsequent six to eight days.[28] Similar observations have been made in experimental animals[18] treated with nitrogen mustard. It was thought protein changes might be found at a time when lymphocyte destruction was striking.

Procedure and methods

All blood samples were drawn under oil, with a minimum of stasis. The blood was allowed to clot and the clear serum separated.

Total nitrogen analyses were conducted in duplicate by the macro-Kjeldahl technique, using mercuric oxide as a catalyst.

The method of Bock and Benedict[2] was employed for non-protein nitrogen determinations.

For electrophoresis, the Tiselius apparatus[33, 47] with the Philpot scanning device[39]

was employed. Four ml. of serum were diluted to 12 ml. with 0.1 molar veronal buffer (pH 8.6). The diluted serum was then dialyzed for 48 to 72 hours at 5° C. against two liters of the buffer.

Electrophoresis was conducted at approximately 2° C., using a potential gradient of 5 to 6 volts per cm. Each electrophoretic run was continued until complete separation of components occurred. Photographs were taken and the magnified projections of the plate diagram then traced. The areas under the peaks were measured with the planimeter, thus giving the relative percentages of the individual serum components. The descending boundary was used for measurements of all fractions except the β-globulin. Because of the lipid anomaly in this fraction of the pattern in the descending

TABLE 1

ELECTROPHORETIC SERUM PROTEIN PATTERNS IN DOGS INJECTED WITH ADRENAL CORTICAL EXTRACTS*

Dog #1	*Pre-injection*		*3 hrs. post-injection*	
	gm./%	*%*	*gm./%*	*%*
S.T.P.†	6.17		6.46	
Albumin	2.59	42.0	2.90	44.7
α_1-Globulin	0.76	12.3	0.85	13.1
α_2-Globulin	0.77	12.5	0.93	14.4
β-Globulin	1.30	21.2	1.10	17.5
γ-Globulin	0.74	11.9	0.66	10.3
Dog #2	*Pre-injection*		*3 hrs. post-injection*	
	gm./%	*%*	*gm./%*	*%*
S.T.P.†	6.53		6.58	
Albumin	3.90	61.1	3.74	57.0
α_1-Globulin	0.54	8.3	0.74	11.2
α_2-Globulin	1.03	15.7	0.79	12.0
β-Globulin	0.48	7.4	0.65	9.8
γ-Globulin	0.48	7.4	0.65	9.8

* Each animal received 12 ml. of aqueous adrenal cortical extract (Upjohn) subcutaneously.

† S.T.P.—Serum total proteins.

limb, the ascending limb pattern was utilized for β-globulin measurements in most of the experiments. In instances where the total protein concentration of the serum had been determined, the absolute number of grams of each fraction could be calculated from the percentage distribution of the various fractions.

Results

Tables 1 and 2 contain data obtained from electrophoretic examinations of the sera of dogs and human subjects injected with either adrenal cortical extract or pituitary adrenotrophic hormone. It will be seen from the tables

that no consistently striking alterations in the serum protein pattern occurred in any of the subjects studied.* This was surprising in that a concomitant lymphocytopenia was present in many instances. The observed

TABLE 2

ELECTROPHORETIC SERUM PROTEIN PATTERNS FOLLOWING PITUITARY ADRENOTROPHIC HORMONE OR ADRENAL CORTICAL EXTRACT INJECTIONS IN MAN*

Patient Diagnosis†		*Serum Protein (%)* *Albumin*	*Globulins* α_1	α_2	β	γ	*Total blood lymphocytes*
Patient #1	Before	63.2	2.4	12.0	11.0	11.3	3010
	After 4 hrs.	61.7	2.8	9.8	13.7	11.9	3390
Patient #2	Before	65.7	3.3	9.8	9.8	11.3	1750
	After 4 hrs.	62.9	4.0	10.2	9.5	13.3	2110
Patient #3	Before	68.8	2.8	6.7	11.0	10.6	4140
	After 4 hrs.	67.8	3.6	7.7	7.7	13.1	2500
Patient #4	Before	61.0	5.8	10.1	10.5	12.6	2070
	After 4 hrs.	59.3	6.2	11.9	10.4	12.1	1780
	After 8 hrs.	65.5	5.6	10.3	9.1	9.4	2540
Patient #5	Before	56.3	3.5	8.2	9.8	22.2	2160
	After 4 hrs.	56.5	3.7	8.1	9.6	22.0	1430
Patient #6	Before	44.8	6.7	17.6	9.1	21.7	37,765
	After 4 hrs.	43.0	7.3	16.3	10.5	22.7	18,644

* Patients #1 to #5 were injected with 25 mgm. adrenotrophic hormone intramuscularly. Patient #6 received 15 cc. adrenal cortical extract (Wilson) intramuscularly.

† Patients #1 to #4 were cases of dementia praecox.
#5 was a normal man.
#6 was a patient with infectious mononucleosis.

* The data for dog #2 in Table 1 suggest that distinct increases in circulating α_1-, β-, and γ-globulins may have occurred following hormone administration. A similar trend is seen in the γ-globulin fractions of patients #2 and #3 following injection of hormone (Table 2). However, such trends are seen in too few instances to be considered significant.

lymphocytopenia is in good agreement with that originally demonstrated by Dougherty and White[11] to occur within a few hours following subcutaneous injection of either adrenal cortical extract or pituitary adrenotrophic hormone in mice, rats, rabbits, dogs, and humans. These authors postulated that pituitary-adrenal cortical secretion is one of the normal mechanisms influencing the level of circulating lymphocytes. The lymphopenic effect of pituitary-adrenal cortical secretion has been confirmed in a number of laboratories.[40]

The negative findings with respect to the serum protein changes in the human following adrenal cortical hormone administration are in agreement with certain other available data.[40] Milne and White[34] were unable to obtain consistent alterations in the serum protein patterns of mice injected with adrenal cortical extract. Li and Reinhardt[32] presented data showing an increase in albumin to globulin ratios in the sera of hypophysectomized rats treated with growth or adrenotrophic hormones. There was no increase in the globulin fractions of either hypophysectomized or normal rats injected with adrenotrophic hormone. These authors also found no change in the protein pattern of cervical duct lymph after adrenotrophic hormone administration.

The further experiments in the present study were designed to examine possible alterations in serum proteins under circumstances, other than extraneous hormone administration, which are associated with marked lymphoid tissue involution or lymphocyte dissolution. One of the most effective methods for the production of lymphoid tissue involution is x-ray irradiation. The decrease in lymphoid tissue size seen following exposure to x-rays is one of the most clearly established clinical and experimental observations. Of considerable interest in this connection is the equally well-documented observation that the dose of radiation required to cause lymphocyte dissolution *in vitro* is many fold that required *in vivo*. (See reviews of Dunlap,[15] Selling and Osgood,[43] and Murphy.[36]) This has led numerous investigators to suggest that a humoral mechanism may be concerned with the lymphocytolytic action of x-rays. This working hypothesis would aid in explaining the clinical observation that following irradiation of a lymphoid structure in the body, involution of distant nodes occurs frequently. Experimentally, Barnes and Furth[1] showed that, in a pair of parabiotic mice, the shielded animal has extensive lymphoid tissue destruction if his mate is irradiated, suggesting the involvement of a humoral mechanism. Of particular significance are the experiments of Leblond and Segal,[21] who shielded all but the thymic area of normal and adrenalectomized

rats and exposed this region to 3000 Roentgen. In the unoperated rats, there was generalized lymphoid, as well as thymic involution, whereas only thymic degeneration occurred in the adrenalectomized animals.

Dougherty and White[13] used x-radiation as a means of producing lymphocyte dissolution in mice in the absence of the adrenal glands. These authors found that in addition to the well-known acute lymphocytopenic action of x-rays, the total proteins and the γ-globulin fraction of the blood increased at a time when tissue lymphocytolysis and blood lymphocytopenia were most marked. Davy[6] had found that there was an immediate drop in globulin following irradiation of dogs, but that in 24 hours it was either at the pretreatment level or higher. This work agreed with that of Herzfield and Schinz,[25] although Breitlander and Lasch[3] had found no change in serum proteins under similar conditions.

From the foregoing evidence, one might anticipate alterations in the serum globulin levels of patients as a result of extensive lymphoid tissue dissolution due to radiation. It will be seen, however, from the data presented in Table 3, that no consistent alterations in serum protein patterns were found in the four patients examined at various intervals during and following x-radiation therapy. In only one case (patient A, Table 3) was a rise in the globulin fraction of the blood proteins seen following a course of x-ray therapy.

Another agent which is known to have a destructive effect on lymphoid elements is the nitrogen mustard, methyl-bis (beta-chloroethyl) amine hydrochloride. This agent causes a marked lymphocytopenia, progressive for 6 to 8 days, with an accompanying striking disappearance of lymphocytes from lymphoid organs.[18, 28, 30] It has been possible in the present study to examine the serum proteins of five patients before, and at varying intervals following, administration of nitrogen mustard. The data are presented in Table 4. In 3 of the 5 patients studied, the results suggest a trend toward a decrease in either the β- or the γ-globulin fractions, or both, following therapy with nitrogen mustard. However, there is no correlation between the decrease in peripheral lymphocytes and the degree of the protein changes, since marked lymphocytopenias were seen in patients both with and without serum globulin alterations. It may be pointed out here that a quantitative relationship need not exist between the level of circulating lymphocytes and the concentration of a blood constituent derived from these cells. This is due to the fact that lymphocytopenia may be a result of either failure to produce new lymphocytes, in which case there will also be a reduced production of lymphocytic components, or a failure to deliver

TABLE 3

ELECTROPHORETIC SERUM PROTEIN PATTERNS FOLLOWING X-RAY IRRADIATION IN MAN

		Time after treatment				
	Before treatment	6 *hours*	7½ *hours*	9 *days*	14 *days*	1 *month*
Patient A[a]						
S.T.P.* Gm./%	6.54	6.52		7.08		
Albumin %	68.2	64.2		60.1		
α_1-Globulin %	5.5	5.7		5.4		
α_2-Globulin %	10.9	11.1		11.8		
β-Globulin %	9.2	12.0		15.5		
γ-Globulin %	6.2	6.9		7.2		
Patient B[b]						
S.T.P.* Gm./%	6.7	7.38				
Albumin %	54.6	56.8				
α_1-Globulin %	6.75	4.76				
α_2-Globulin %	13.8	13.7				
β-Globulin %	18.08	19.05				
γ-Globulin %	6.75	7.14				
Patient C[c]						
S.T.P.* Gm./%	6.76		6.75			7.46
Albumin %	56.1		59.8			53.5
α_1-Globulin %	2.75		2.9			4.5
α_2-Globulin %	9.35		8.7			9.8
β-Globulin %	10.5		10.7			11.4
γ-Globulin %	21.4		17.9			20.4
Patient D[d]						
S.T.P.* Gm./%	6.2					
Albumin %	62.9				60.9	
α_1-Globulin %	5.9				6.1	
α_2-Globulin %	11.7				13.9	
β-Globulin %	13.2				11.8	
γ-Globulin %	6.2				7.2	

[a] Man with carcinoma of larynx, received approximately 38 R/min. for 6-7 minutes (7 cm. field) every day for 8 days.

[b] Woman received deep irradiation to pituitary fossa. (Followed by vomiting and probable dehydration.)

[c] Man with chronic lymphatic leukemia. 7 treatments of spray irradiation over thorax and abdomen (11 R/min. for 2-3 minutes.) Over this period white blood-cell count dropped from 144,000 to 14,000.

[d] Woman with carcinoma of breast with pulmonary metastases. Deep therapy to dorsal spine.

* Serum total proteins.

TABLE 4
ELECTROPHORETIC SERUM PROTEIN PATTERNS FOLLOWING NITROGEN MUSTARD THERAPY*

		Days following final injection					
	Before treatment	1 *day*	3 *days*	4 *days*	6 *days*	10 *days*	12 *days*
Patient A[a]							
S.T.P.† Gm./%	6.85	6.26				5.80	
Albumin %	45.2	50.6				43.9	
α_1-Globulin %	9.5	9.7				13.0	
α_2-Globulin %	16.6	17.5				16.2	
β-Globulin %	11.7	12.8				13.5	
γ-Globulin %	16.8	9.2				13.3	
T.B.L.‡	425					175	
Patient B[b]							
S.T.P.† Gm./%	7.05				7.50		
Albumin %	68.8				67.0		
α_1-Globulin %	4.1				5.3		
α_2-Globulin %	8.8				9.7		
β-Globulin %	8.8				8.9		
γ-Globulin %	9.5				9.1		
T.B.L.‡	3390				425		
Patient C[c]							
S.T.P.† Gm./%	6.93		7.10				
Albumin %	50.2		55.3			66.1	
α_1-Globulin %	7.6		5.2			5.6	
α_2-Globulin %	15.2		11.7			8.6	
β-Globulin %	11.8		11.9			7.2	
γ-Globulin %	15.2		15.8			12.5	
T.B.L.‡	3248		882			1125	
Patient D[d]							
S.T.P.† Gm./%	5.70			4.86			
Albumin %	46.2			45.3			
α_1-Globulin %	11.7			16.2			
α_2-Globulin %	15.9			16.2			
β-Globulin %	17.7			13.1			
γ-Globulin %	8.6			9.3			
T.B.L.‡	861			335			
Patient E[e]							
S.T.P.† Gm./%							
Albumin %	39.0						43.1
α_1-Globulin %	7.8						6.8

			Days following final injection					
		Before treatment	1 *day*	3 *days*	4 *days*	6 *days*	10 *days*	12 *days*
α_2-Globulin	%	14.5						12.6
β-Globulin	%	16.0						14.6
γ-Globulin	%	22.7						22.8
T.B.L.‡		4550						700

[a] 33-year-old female. Known Hodgkins disease for 8 months. Several x-ray treatments previously. After nitrogen mustard, nodes and breast mass decreased in size. Less dyspnea and pain.

[b] 37-year-old man. Pulmonary metastases of myxosarcoma of thigh. Under therapy there was marked pain relief.

[c] 30-year-old man. Hodgkins disease, 1 year. Had received x-ray therapy previously. Back pain improved following treatment.

[d] 47-year-old man. Bronchogenic carcinoma. 25-pound weight loss.

[e] Young adult man with Hodgkins disease. No previous therapy.

* All patients received 0.1 mgm. of nitrogen mustard per kilogram every day for 4 days.

† Serum total proteins.

‡ Total blood lymphocytes.

normal numbers of lymphocytes to the circulation, despite normal lymphocyte production. The former case is seen in chronic nitrogen mustard or x-ray therapy where damage to lymphocyte production is a primary factor. On the other hand, adrenal cortical steroids produce lymphocyte dissolution in the nodes, and, if this continues, fewer cells are delivered to the blood. In this instance, the components of destroyed lymphocytes are still available for addition to the circulation, even though the number of lymphocytes is reduced.

A single dose of x-rays has been reported to produce globulin rises in well-nourished animals.[13] However, repetition of these experiments, while revealing increases in total serum proteins, showed no changes in the relative amounts of the individual protein components of the serum.[84] Moreover, chronic radiation is known to depress antibody globulin production,[20, 22, 23, 37, 45] as do such leukotoxic agents as benzene,[21, 46] sulfur mustard,[24] and nitrogen mustard.[38] Apparently under these circumstances there is a destruction of the chief tissue contributing to the synthesis of new globulin, and this results in an ultimate depression of the serum globulin levels. Philips, Hopkins, and Freeman[38] demonstrated a delayed increase in the circulating antibodies in goats treated with nitrogen mustard following antigen injections, as compared with animals receiving antigen alone. These authors pointed out that nitrogen mustard may act directly on the lymphoid elements without endocrine mediation,[30] thus causing persistent lymphoid

involution which may not necessarily be associated with the release of stored antibody. It is of some interest that the animals which had been given nitrogen mustard were capable of showing an anamnestic reaction, despite lymphocyte depletion of lymph nodes. This observation suggested that under conditions of lymphoid depletion, antigenic stimulation may be effected in other immunologically active tissues containing reticulo-endothelial elements. With this viewpoint, it is perhaps possible to understand why no striking diminution in β- and γ-globulins occurred in patients given chronic doses of nitrogen mustard, since other cell types may participate in globulin production.

The present study has sought to investigate further the possible contribution of the lymphocyte to the production of serum β- and γ-globulins. The data presented, however, suggest that there were no consistent changes in serum globulins in conditions which might be classified broadly as characterized by either pituitary-adrenal cortical induced lymphocyte dissolution (hormones, x-ray, or nitrogen mustard), or by an apparent lack of lymphocyte formation (nitrogen mustard). These essentially negative results suggest that a number of as yet unevaluated factors may be of significance in establishing lymphocytes as a significant source of serum globulins. The techniques of measurement employed may not be adequately sensitive to detect changes which perhaps did occur. In a human subject with a blood volume of 5 liters, the addition of at least 15 grams of protein to the circulation is necessary to produce a 5 per cent rise in total blood proteins. Moreover, this addition must be continuous to compensate for the removal of protein in the peripheral circulation. It may also be noted that a significant degree of hemodilution may occur as a result of increased secretion of adrenal cortical steroids, thus obscuring significantly elevated concentrations of blood components.

Evaluation of the blood level of cellular elements, or of the protein elements derived therefrom, requires consideration of a diversity of variables. The first of these is the structural and physiological integrity of the tissues concerned with the synthesis of the cells and their constituents. For example, destruction of the synthetic site by x-ray or nitrogen mustard would impair both synthesis and delivery to the circulation of the lymphocytes. Secondly, the supply and quality of materials from dietary sources for the construction of new cells is of prime importance. Thus, Wissler and his colleagues[52] have demonstrated that, under conditions of undernutrition, globulin synthesis is greatly impaired. In addition, there are several processes occurring at the site of synthesis whose rates may be affected by

endocrine secretions. These processes are (a) the rate of new cell (and its constituents) synthesis, (b) the rate of continuing cell turnover at the site of synthesis, (c) the rate of delivery of cells and/or their constituents to the circulation. Finally, the blood levels of a particular cell, or of constituents derived therefrom, may be affected by the rate at which the reticulo-endothelial system removes these materials from the circulation. The rate of this process may also be influenced by endocrine secretions. Indeed, Reiss and Gothe[41] reported that the capacity of reticulo-endothelial cells to store a circulating dye is increased one hour after injection of an extract rich in adrenotrophic hormone. Furthermore, Gordon and Katsh[17] showed a relation between the activity of the adrenal cortex and the capacity of reticulo-endothelial cells to take up injected thorium oxide. Other factors, such as renal loss and passage into tissues, may also be of significance in the removal of blood constituents from the circulation.

Thus, the maintenance of the normal state of lymphoid tissue function, and of its contributions to the circulation, is a result of a dynamic balance among a number of processes. One or more of these may be affected by experimental or clinical conditions, and until a clear evaluation of the contribution of each is available, unequivocal interpretation of data derived from studies of the type presented here is not possible.

Summary and conclusions

1. No significant alterations in serum protein levels were seen in man or in dogs following a single injection of adrenotrophic hormone or adrenal cortical extract.

2. X-ray irradiation of several patients with carcinomata produced no consistent changes in serum protein levels.

3. Nitrogen mustard administration had no definite effect on the serum proteins, despite a marked involution of the lymphoid tissue throughout the body.

References

1 Barnes, W. A. and Furth, O. B.: Am. J. Roentg., 1943, *49*, 662.
2 Bock, J. C. and Benedict, S. R.: J. Biol. Chem., 1915, *20*, 47.
3 Breitlander, K. and Lasch, G. M.: Klin. Wschr., 1927, *6*, 743.
4 Cannon, P. R.: J. Laborat. Clin. M., 1942, *28*, 127.
5 Chase, J. H., White, A., and Dougherty, T. F.: J. Immun., Balt., 1946, *52*, 101.
6 Davy, L.: Am. J. Roentg., 1931, *25*, 255.
7 Desjardins, A. U.: Science, 1932, *75*, 569.
8 Dougherty, T. F., Chase, J. H., and White, A.: Proc. Soc. Exp. Biol., N. Y., 1944, *57*, 295.
9 Dougherty, T. F., Chase, J. H., and White, A.: Proc. Soc. Exp. Biol., N. Y., 1945, *58*, 135.

10 Dougherty, T. F. and White, A.: Proc. Soc. Exp. Biol., N. Y., 1943, *53,* 132.
11 Dougherty, T. F. and White, A.: Endocrinology, 1944, *35,* 1.
12 Dougherty, T. F. and White, A.: Am. J. Anat., 1945, *77,* 81.
13 Dougherty, T. F. and White, A.: Endocrinology, 1946, *39,* 370.
14 Dougherty, T. F., White, A., and Chase, J. H.: Proc. Soc. Exp. Biol., N. Y., 1944, *56,* 28.
15 Dunlap, C. E.: Arch. Path., Chic., 1942, *34,* 562.
16 Enders, J. F.: J. Clin. Invest., 1944, *23,* 510.
17 Gordon, A. S. and Katsh, G. F.: Ann. N. York Acad. Sc., 1949, *52,* 1.
18 Graef, I., Karnofsky, D. A., Jager, B. V., Krichesky, B., and Smith, H. W.: Am. J. Path., 1948, *24,* 1.
19 Harris, T. N., Grimm, E., Mertens, E., and Ehrich, W. E.: J. Exp. M., 1945, *81,* 73.
20 Hektoen, L.: J. Infect. Dis., 1915, *17,* 415.
21 Hektoen, L.: J. Infect. Dis., 1916, *18,* 69.
22 Hektoen, L.: J. Infect. Dis., 1918, *22,* 28.
23 Hektoen, L.: J. Infect. Dis., 1920, *27,* 23.
24 Hektoen, L. and Corper, H. J.: J. Infect. Dis., 1921, *28,* 279.
25 Herzfeld, E. and Schinz, H. R.: Strahlentherapie, 1923, *15,* 84.
26 Ingle, D. J.: Proc. Soc. Exp. Biol., N. Y., 1938, *38,* 443.
27 Ingle, D. J.: Proc. Soc. Exp. Biol., N. Y., 1940, *44,* 174.
28 Jacobson, L. O., Spurr, C. L., Barron, E. S. G., Smith, T., Lushbaugh, C., and Dick, G. F.: J. Am. M. Ass., 1946, *132,* 263.
29 Kass, E. H.: Science, 1945, *101,* 337.
30 Kindred, J. E.: Arch. Path., Chic., 1947, *43,* 253.
31 Leblond, C. P. and Segal, G.: Am. J. Roentg., 1942, *47,* 302.
32 Li, C. H. and Reinhardt, W. O.: J. Biol. Chem., 1947, *167,* 487.
33 Longsworth, L. G.: Chem. Rev., Balt., 1942, *30,* 323.
34 Milne, J. and White, A.: Proc. Soc. Exp. Biol., N. Y., 1949, *72,* 424.
35 Moon, H. D.: Proc. Soc. Exp. Biol., N. Y., 1937, *37,* 34.
36 Murphy, J. B.: *Monographs of the Rockefeller Institute,* 1926, No. 21.
37 Murphy, J. B. and Sturm, E.: J. Exp. M., 1925, *41,* 245.
38 Philips, F. S., Hopkins, F. H., and Freeman, M. L. H.: J. Immun., Balt., 1947, *55,* 289.
39 Philpot, J. S. L.: Nature, 1938, *141,* 283.
40 Reinhardt, W. O. and Holmes, R. O.: Proc. Soc. Exp. Biol., N. Y., 1940, *45,* 267.
41 Reiss, M. and Gothe, I.: Endokrinologie, 1937, *19,* 148.
42 Sayers, G., Sayers, M. A., Fry, E. G., White, A., and Long, C. N. H.: Yale J. Biol., 1944, *16,* 361.
43 Selling, L. and Osgood, E. E.: In: *Handbook of Hematology.* Edited by H. Downey. New York, Paul B. Hoeber, Inc., 1938, *4,* 2693.
44 Selye, H.: Endocrinology, 1937, *21,* 169.
45 Simonds, J. P. and Jones, H. M.: J. Med. Res., 1915, *33,* 183.
46 Simonds, J. P. and Jones, H. M.: J. Med. Res., 1915, *33,* 197.
47 Tiselius, A.: Tr. Faraday Soc., 1937, *33,* 524.
48 Wells, B. B. and Kendall, E. C.: Proc. Mayo Clin., 1940, *15,* 133.
49 White, A.: Annual Rev. Physiol., 1949, *11,* 355.
50 White, A. and Dougherty, T. F.: Endocrinology, 1945, *36,* 207.
51 White, A. and Dougherty, T. F.: Ann. N. York Acad. Sc., 1946, *46,* 859.
52 Wissler, R. W., Woolridge, R. L., Steffee, C. H., Jr., and Cannon, P. R.: J. Immun., Balt., 1946, *52,* 267.