tissue thromboplastin or viper venom, it is apparent that this factor is necessary for the action of both of these thromboplastic materials. It has been suggested that viper venom contains proconvertin or acts in place of proconvertin(4). It is also possible that there are two distinct systems for activation of the prothrombin conversion enzyme, one through the tissue thromboplastin route where proconvertin is necessary, and the other through the plasma thromboplastin route where proconvertin is not necessary. In this sense, proconvertin may be thought of as a true "cothromboplastin" but only for the tissue thromboplastin. Viper venom in this scheme appears to act in the same manner as plasma thromboplastin (platelets, antihemophilic globulin, PTC, PTA, and possibly other factors) for it does not require the presence of proconvertin for its normal action(8).

It seems probable from the findings that Hjort, Rapaport, and Owren's(9) method of assaying prothrombin by viper venom is also sensitive to Stuart factor.

Summary. The modification of Owren and Aas' test for proconvertin by substitution of viper venom-cephalin mixture for tissue thromboplastin is described as a method of assay for Stuart factor.

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Effect of Adrenocortical Hormones on Serum Proteins of Adrenalectomized Mice Infected with *M. tuberculosis*.* (23571)

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The electrophoretic distribution of serum proteins following experimental infections with *M. tuberculosis* has been studied in rabbits(1), guinea pigs(2-4), and rats(3). Also, the effects of adrenocortical hormone administration on serum protein patterns have been reported on rabbits(5,6), guinea pigs(7), rats(8), and dogs(6). It has been difficult to evaluate these results due to species variation, variation in severity and length of the infection, and differences in amounts and types of hormones administered.

This study was designed to investigate the effects of adrenocortical hormones on serum proteins of infected, adrenalectomized mice where there is no buffering effect on the intact adrenal gland, and to compare the effects of similar amounts of a glucocorticoid and a mineralocorticoid in this animal.

Materials and methods. Bilateral adrenalectomies were performed on 260 CFl albino mice. One hundred thirty of these mice and

* Hydrocortisone, desoxycorticosterone, and 2-methyl-9-fluorohydrocortisone were generously donated by Dr. Robert O. Stafford, Dept. of Endocrinology, Upjohn Co., Kalamazoo, Mich. Total protein determinations were performed by Jack A. Moorman and Jeanne LaCerte.

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TABLE I. Effects of Hydrocortisone (F), Desoxycorticosterone (DC), and 2-Methyl-9-fluorohydrocortisone (MFF) on Serum Proteins of Adrenalectomized Infected and Non-infected Mice after 5 Weeks of Infection and Hormone Administration.

Treatment	Total protein (g %)	Albumin*	Globulins*				
			Alpha$_1$	Alpha$_2$	Alpha$_3$	Beta	Gamma
Normal mean (45)†	6.07	54.87	4.06	9.73	5.16	20.21	5.97
" range (2σ)	5.25-6.89	45.05-64.69	3.28-4.84	8.29-11.17	2.20-8.12	10.27-30.15	1.33-10.61
Infected animals:							
Intact controls (8)	5.35	44.42	8.62	14.32	7.41	19.69	5.54
Adrenalectomized controls (3)	5.45	47.36	4.13	13.80	11.24	18.97	4.50
" + 500 γ F (8)	6.50	50.86	9.70	14.06	7.42	13.56	4.40
" + 500 γ DC (8)	5.50	47.44	5.18	13.34	10.54	18.62	4.89
" + 10 γ MFF (5)	5.50	52.30	4.30	8.61	9.26	19.92	5.61
Non-infected animals:							
Intact controls (9)	5.98	48.75	5.28	11.42	10.81	16.68	7.05
Adrenalectomized controls (10)	6.78	47.57	2.98	9.56	21.96	12.99	4.94
" + 500 γ F (6)	7.50	37.80	4.38	6.98	27.28	10.69	12.88
" + 500 γ DC (7)	6.72	42.36	3.80	11.65	15.11	19.71	7.37
" + 10 γ MFF (9)	7.08	28.49	3.18	10.03	38.45	11.97	7.88

* Protein fractions expressed as % area. † Total No. of animals/group.

20 intact mice were infected with *M. tuberculosis* var. *hominis* H37Rv by means of an airborne infection apparatus(9). The adrenalectomized mice were divided into 7 groups of 15-20 each the day after infection and treated as follows: oil-treated controls, 50 γ and 500 γ hydrocortisone/animal/day, 50 γ and 500 γ desoxycorticosterone/animal/day, a combination of 50 γ hydrocortisone and 50 γ desoxycorticosterone/animal/day, and 10 γ 2-methyl-9-fluoro-hydrocortisone/animal/day. The daily dose in each case was administered in 0.1 ml corn oil subcutaneously. The intact infected animals were treated only with oil. The 130 non-infected adrenalectomized mice were divided into the same groups, including a group of 20 intact, non-infected controls. The animals were bled to death by cardiac puncture. Approximately half were sacrificed at 2½ weeks and the remainder 5 weeks after injection or after the beginning of hormone administration. The blood drawn from each group was combined into one or 2 pooled samples due to the small amount obtained from each animal, and serum was obtained from the whole blood by centrifugation at 2,500 rpm for 10 minutes. Total protein was determined by a modification of the biuret method(10) and relative concentration of the serum protein fractions with the Spinco Model R Electrophoresis equipment consisting of a Durrum "hanging strip" type cell and a recording and automatically integrating densitometer(11). A normal range ($2\ \sigma$) was established from 10 pooled samples of blood from 45 untreated, intact, non-infected mice of the same age and weight (approximately 25 g). Degree of infection was determined by gross and histological examination of the lungs after autopsy.

Results. Over 90% of the normal determinations of each fraction fell within the normal ($2\ \sigma$) range (Table I). Since it was necessary to pool the blood from each experimental group, values which fell outside the normal range were considered to be significant changes.

After 2½ weeks of hormone administration all the non-infected mice, including both adrenalectomized and intact controls, showed a decrease in albumin and increase in the alpha$_2$ globulin. An increased alpha$_3$ globulin was observed in all of the groups after 2½ weeks of infection.

A summary of the results from 5 weeks of infection and/or hormone treatment is found in Table I. The results obtained with 50 γ hydrocortisone and desoxycorticosterone were similar to those demonstrated with the higher doses. In the non-infected animals, adrenal-

ectomy alone caused a slight decrease in the alpha$_1$ fraction and a marked increase in the alpha$_3$ globulin. With both doses of hydrocortisone, the total protein increased, the albumin decreased, and the alpha$_3$ globulin was slightly higher than in the adrenalectomized controls. The greatest decrease in albumin, an increase in the alpha$_3$ globulin, and a slight decrease in the alpha$_1$ globulin were caused by the 2-methyl-9-fluorohydrocortisone and the combination of hydrocortisone and desoxycorticosterone.

The infection with *M. tuberculosis* appeared to mask or possibly inhibit the effects of the individual hormones on the serum proteins. In the intact controls, the disease caused an increase in the alpha$_1$ and alpha$_2$ globulins and a slight decrease in the albumin.

All of the infected animals showed a marked degree of lung involvement both grossly and histologically. There was no correlation between severity of the disease and serum proteins as the high doses of hydrocortisone and desoxycorticosterone and the 2-methyl-9-fluorohydrocortisone caused the greatest degree of infection.

Discussion. There has been considerable variation in the reported effects of the adrenocortical hormones on the serum proteins depending on the species of the animal treated and length of time and level of administration of the compounds. Hoch-Ligeti and Irvine(8) reported elevation of albumin and total protein and decrease in gamma globulin in rats with large doses of hydrocortisone and cortisone, and no change with desoxycorticosterone following 5 days of administration. Cortisone has been shown to cause an increase in gamma globulin and decrease in albumin in guinea pigs after 15 days(7), but Snell and Nicol reported a decrease in gamma globulin in guinea pigs after 2 weeks of cortisone treatment(12). No changes, however, were observed in rabbit serum proteins after 30 days administration of cortisone and hydrocortisone(5,6). In dogs, a marked elevation of alpha$_2$ globulin was observed after cortisone and hydrocortisone treatments for 3 weeks (6). It is not too surprising, therefore, that the changes in serum proteins observed with hormone treatments in mice do not agree with those reported in other animals.

There are also considerable variations in the serum protein changes with *M. tuberculosis* infections in different species and with different strains of bacilli. An elevation in beta globulin and decrease in albumin has been reported in rabbits(1), while an increase in gamma globulin and decrease in albumin has been observed in guinea pigs(2,4).

It is felt that these results present some interesting possibilities for future study. To our knowledge, there has been no previous report of a disease inhibiting the effect of hormones on serum proteins and this observation alone warrants further investigation.

Summary. Following administration of varying amounts of hydrocortisone, desoxycorticosterone, and 2-methyl-9-fluorohydrocortisone to adrenalectomized non-infected mice and adrenalectomized mice experimentally infected with *M. tuberculosis,* changes in serum proteins were determined by a paper electrophoretic technique. An increase in total protein was observed with hydrocortisone; a decrease in albumin and an increase in alpha$_3$ globulin occurred with hydrocortisone, high doses of desoxycorticosterone, a combination of hydrocortisone and desoxycorticosterone, and 2-methyl-9-fluorohydrocortisone; and an increase in gamma globulin resulted with a high dose of hydrocortisone. The individual effects of the hormones on serum proteins were not apparent in those animals infected with *M. tuberculosis.* All of the animals, except those treated with 2-methyl-9-fluorohydrocortisone, showed an increase in alpha$_2$ globulin while only the high dose of hydrocortisone produced a decrease in albumin.

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Modification of Antibody Response in X-irradiated Rats by Injection of Spleen Homogenates. (23572)

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Sublethal doses of x-radiation are capable of inhibiting antibody response in experimental animals if antigen is given before or after irradiation(1,2). This inhibition can be reversed by lead shielding of the spleen or appendix during irradiation even if the spleen is removed soon afterward(3,4). Injections of homogenates of spleen and bone marrow have been shown to accelerate recovery from the hematopoietic damage caused by total-body x-irradiation(5-7). It was demonstrated recently that this protection by cell transfer is characterized by a "recolonization" process in which the injected cells proliferate and substitute for some of the animal's own tissues that were destroyed by x-rays (8,9). There has been relatively little study of the effects of heterologous and homologous post irradiation cell transfer upon the immunological process. The present work was undertaken to study antibody response of rats receiving homologous spleen cell injections up to 24 hours after 500 r total-body x-irradiation and stimulated from 3 to 48 hours later with an intravenous dose of sheep erythrocytes. Other investigators have attempted to restore or protect the immune mechanism of experimental animals by injection of various materials following irradiation. Of these, Jaroslow and Taliaferro have been able to produce considerable protection in rabbits by means of injection of whole spleen cells and of HeLa and yeast extracts when the material is given *mixed with the antigen* 24 hours after irradiation(10). The present experiments, on the other hand, have always involved separate administration of spleen cells and antigen from a *few hours to 24 hours* apart at different intervals after irradiation. A previous study, with a somewhat similar pattern, is that of Smith and his associates(11) in which spleen or bone marrow cells were given immediately after x-irradiation, immunization was carried out from 1 to 7 weeks later, and the circulating antibody titer was studied on the day of peak response. Under these conditions no difference in the rate of recovery of the antibody-forming capacity was noted between "protected" mice and mice receiving x-irradiation alone. *Spleen cells* were transferred into the irradiated animals in this study because of the demonstrated importance of the spleen in antibody formation in the rat. Under these circumstances, splenectomized animals form only a very small amount of circulating antibody(12). Rats injected intravenously with a particulate antigen show characteristic cellular changes in the spleen which appear to be closely correlated with the appearance of circulating antibody(13). These changes can be inhibited by a variety of treatments known to depress antibody formation(2,14,15).

Materials and methods. Young adult Sprague-Dawley albino rats were used throughout. Irradiation was administered by means of a General Electric Maxitron thera-

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