um salts in guinea pigs and conclude that the carbohydrate disturbance is not directly related to the mechanism of the pulmonary edema.

Summary 1. The acute pulmonary edema which follows the administration of ammonium salts to guinea pigs may be entirely prevented by prior administration of the adrenergic blocking agents, N-(9-fluorenyl)-N-ethyl-B-chloroethylamine-HCl ("SKF-501") and N-(2-chloroethyl)-N-ethyl-benzhydrylamine-HCl ("SY-2"). Less marked lung edema is produced in rabbits by ammonium chloride and it is also prevented by the blocking agents.

2. A potent antihistaminic agent, N-B-dimethylamino-propylthiodiphenylamine-HCl ("Phenergan-3277 R.P."), was without effect on the incidence of ammonium pulmonary edema, which in this respect as well as the manner it is influenced by adrenergic blocking agents, resembles epinephrine-induced pulmonary edema.

3. A central nervous system depressant in the form of continuous ether anesthesia prevents the occurrence of ammonium pulmonary edema. Along with the effect of adrenergic blocking agents this is construed as evidence for the mechanism of this edema being due to adrenergic stimuli of reflex or direct central stimulation origin.

4. Ammonium salts cause a striking hyperglycemia. Since adrenergic blocking agents of the type used as well as tetraethylammonium which blocks epinephrine release by the adrenal medulla fail to prevent the hyperglycemia response it is believed that ammonium hyperglycemia is not directly related to the edema formation.

5. Although pulmonary edema sometimes may be the cause of death from toxic doses of ammonium salts, this is not true in the Slonaker strain of rats or our strain of albino mice both of which fail to develop edema. Nor is this the cause of death in guinea pigs or rabbits when an adrenergic blocking agent is used to prevent the edema, as the animals succumb as a result of other effects of the ammonium ion.

Addendum: When this manuscript was ready for submission to the Editors, an article by the Koenigs (Koenig, H., and Koenig, R., *Am. J. Physiol.*, 1949, **158**, 1) on the pathogenesis of ammonium pulmonary edema appeared. Insofar as our observations are similar the conclusions are parallel.

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Effect of Adrenal Cortical Extract and of X-Radiation on Serum Proteins of Mice. (17456)

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It has been reported previously from this laboratory that chronic injections of pituitary adrenotrophic hormone in mice produced an increase in the total serum proteins.[1] Moreover, a single injection of adrenotrophin or of adrenal cortical steroids in oil in rabbits was observed to cause an elevation of circulating β- and γ-globulins within a few hours after hormone administration.[2] Electrophoretic analysis also revealed an increase in these

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serum globulins in normal mice exposed to a single dose of X-rays.[3] It was suggested that the influence of radiation on blood globulin levels in intact mice was chiefly a result of the direct involution of lymphoid tissue by large radiation doses. An augmentation of pituitary-adrenal cortical controlled lymphocytolysis, with accompanying blood globulin elevation, was also demonstrated to occur as a result of the response of the endocrine mechanism to the action of radiation as a non-specific stimulus.

A review of the literature[4] indicates that conflicting data have been obtained in efforts to establish definitely a relationship between pituitary-adrenal cortical secretion and serum protein levels. Simonnet[5] injected adrenotrophic hormone or adrenal cortical extracts into rats of both sexes and of varying ages, and found a rise in total blood globulin occurring most consistently in the 2-month-old male rat. On the other hand, Li and Reinhardt[6] were unable to alter the electrophoretic serum protein pattern of normal or of hypophysectomized rats by giving single or chronic injections of adrenotrophic hormone. It may be noted that the data of these investigators show an approximately 100% difference in values for γ-globulin concentrations in 2 groups of control rats.

In rats and dogs exposed to physical and chemical stimuli which augment pituitary-adrenal cortical secretion, Chanutin and his associates[7,8] found rather uniformly an increase in the serum α-globulin fraction and the appearance of boundary anomalies in the β-globulin component. Arocha and De Venanzi[9] reported briefly that serum globulin increased in normal dogs following intravenous injections of epinephrine, and Mondolfo and De-Lerner[10] confirmed the observations of Chase, White, and Dougherty[11] that adrenal cortical extract injections, accompanying antigen administration, resulted in a greater rate and degree of elevation of blood immune globulin. However, Eisen and co-workers[12] found identical concentrations of serum antibodies and γ-globulin in adrenalectomized rats injected repeatedly during immunization with adrenal cortical extract, and in operated animals not receiving hormone.

Pituitary adrenotrophic hormone or adrenal cortical extract injections have failed generally to influence the level of serum globulin in human subjects. This has been the experience of Kelley and Adams,[13] Forsham and co-workers,[14] Mason and co-workers[15] and Frieden and White,[16] following administration of adrenotrophic hormone or adrenal cortical extracts. In addition, exposure of patients to X-radiation, nitrogen mustard therapy, or electric shock treatment was rather uniformly without influence on the electrophoretic pattern of the serum proteins, although in two instances significant rises were seen in the serum γ-globulin fraction after x-radiation.[16]

In view of the nature of the conflicting evidence in a variety of species, experiments have been initiated to investigate systematically the possible variables which may be involved in influencing the nature and extent of the response of the serum protein pattern to circumstances of augmented levels of cir-

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16 Frieden, J., and White, A., unpublished results.

culating adrenal cortical steroid hormones. The present investigation deals with the response in mice. The previously reported[3] electrophoretic experiments with serum obtained from mice exposed to X-radiation were conducted in phosphate buffer at pH 7.8. Under these circumstances, the separation of the γ-globulin component from the salt boundary is unsatisfactory, and errors may arise in evaluating correctly the contribution of the γ-globulin component to the total area in this region of the electrophoretic pattern. In addition, when this phosphate buffer is employed, a satisfactory separation of the α-globulin fraction into its two commonly recognized individual components is not obtained. Another possible source of error in electrophoretic studies of sera results from the fact that the precise concentration of the β-globulin component may be somewhat obscured by the presence of lipid in this protein fraction, resulting in an apparent, but not true, increase in β-globulin concentration. This factor would seem to be of particular significance in circumstances of augmented pituitary-adrenal cortical secretion, particularly since the adrenal cortical steroids produce a mobilization of lipid to the liver.

The present study has involved the electrophoretic examination of the serum proteins of normal mice, and of mice bearing a transplantable lymphosarcoma, both prior to and following either the injection of adrenal cortical extract, or exposure to a single, relatively large dose of X-radiation. Under the experimental conditions employed, no significant alterations were seen in the serum protein patterns as a consequence of exposure of the animals to either the hormonal or radiation treatment.

Methods. Mice of both sexes of the CBA (Strong) strain, 8 to 13 weeks of age, were used in all experiments. The animals were maintained on a diet of Purina Fox Chow supplemented with calf meal; food and water were available at all times. The tumor employed was obtained from Professor W. U. Gardner of the Department of Anatomy at Yale University. This tumor arose in an estrogen-treated mouse of the C_3H strain and has been transplanted for many successive generations. A transplant of the tumor takes in 100% of CBA mice. The transplanted tumor grows rapidly and kills the host in approximately 3 weeks. The tumor has been described in detail[17] and is composed of large cells which resemble reticular cells. Tumor transplants were made by implanting small pieces of the tumor subcutaneously. Since the mice with this tumor transplant live generally a maximum of three weeks, the tumor-bearing animals were used 2 weeks after the transplant had been made, at a time when a clearly palpable tumor was present.

Two preparations of adrenal cortical extract were used, one an aqueous adrenal cortical extract (Wilson) and the other a preparation of adrenal cortical steroids in oil (Upjohn). The animals subjected to X-rays received total body radiation. This was accomplished by placing 4 to 6 mice in a light cardboard box, which because of its shallowness prevented the mice from shielding one another. Radiation of all animals was delivered at the rate of 43 r per minute by using the following conditions: 125 Kv., 5 ma.; 30 cm skin target distance; 3 mm aluminum filter.‡

In order to obtain blood samples, each mouse was pithed, and the heart exposed and blood obtained by direct heart puncture, using a finely drawn glass pipette. Blood was pooled from groups of animals varying in number from 15 to 32, in order to obtain adequate serum for each electrophoretic experiment. The total serum protein and non-protein nitrogen contents of the pooled serum were determined by the micro-Kjeldahl technic. The serum was then diluted with 3 parts of 0.1 M sodium barbital buffer, pH 8.6, and dialyzed against the buffer for 2 or 3 days in the ice box. Electrophoretic runs were conducted in the Tiselius apparatus at 2°C for a period of approximately 3 to 4 hours, at which time satisfactory separation of the individual serum protein components had occurred. The concentrations of the various protein fractions were estimated from the descending diagrams

[17] Gardner, W. U., Dougherty, T. F., and Williams, W. L., *Cancer Research*, 1944, **4**, 73.

‡ Grateful acknowledgement is made to Dr. H. S. Kaplan for assistance in radiation of the animals.

TABLE I.
Electrophoretic Analyses of Mouse Sera.

Electrophoresis exp. No.	Animal group type	No. of mice for pooled sera	Total protein, g/%	Albumin,		Globulins									
						α_1		α_2		β		γ		$\beta + \gamma$	
				%	g	%	g	%	g	%	g	%	g	%	g
1	Normal, ♂	32	4.53	59.70	2.70	15.70	.71	9.45	.43	9.45	.43	3.48	.16	12.95	.59
2	,,	23	4.82	63.43	3.05	16.50	.79	6.74	.32	9.75	.47	3.61	.17	13.76	.64
3	Normal, ♀	15	4.75	67.60	3.21	11.63	.55	6.71	.32	10.62	.51	3.35	.16	13.97	.67
4	Tumor, ♂	15	3.57	61.36	2.19	18.80	.67	5.45	.19	10.90	.39	2.48	.09	13.38	.48
5	,, ♀	15	3.41	63.44	2.16	14.75	.50	7.05	.24	11.53	.39	3.21	.11	14.74	.50
6	Normal, ♂ and ♀[1]	16	6.08	63.05	3.84	18.22	1.11	5.61	.34	10.75	.65	2.39	.15	13.14	.80
7	Tumor, ♂ + ♀[2]	16	5.18	61.98	3.22	16.81	.87	5.43	.28	13.05	.68	2.73	.14	15.78	.82
8	,, ♂ + ♀[3]	18	5.22	65.80	3.43	15.42	.81	3.72	.19	11.54	.60	3.72	.19	15.26	.79
9	Normal, ♂ + ♀[4]	18	4.96	66.20	3.28	15.10	.75	4.04	.20	11.60	.57	3.03	.15	14.63	.72
10	,, ♂ + ♀[4]	18	5.86	68.40	4.01	12.55	.73	5.20	.31	10.82	.63	3.03	.18	13.85	.81

1 6 hr after 0.2 cc adrenal cortical steroids (oil), subcut.
2 3 hr after 0.5 cc ,, ,, ,,
3 3 hr after 1.0 cc aqueous adrenal cortical extr., subcut.
4 6 hr after X-radiation (200 r).

of the electrophoretic patterns.

Results and discussion. Table I presents the results of the various analyses under the experimental conditions indicated in the table. It will be seen from the data that a single injection of adrenal cortical extract, or the exposure of mice to 200 r produced an increase in the total serum protein concentration, as compared to appropriate controls, in all experiments except that designated as No. 9. This increase is in agreement with previously published data.[1] However, it will also be seen from the table that no significant alteration occurred in the percentage distribution of the various serum protein components as determined electrophoretically. The slight alterations which were evident in several of the experiments, *e.g.*, No. 7, occurred in the β-globulin fraction. In view of the influence of altered lipid concentrations on the value for this protein component, it is unlikely that this increase is indicative of an elevated concentration of β-globulin.

It is unknown at the present time whether the relatively low initial level of total serum proteins in the control animals was a factor in determining possible responsiveness to the experimental conditions employed. In all experimental groups, there was a striking elevation in total serum proteins. It is possible that in a relatively protein-depleted animal, there is a generalized stimulus to protein production following hormone administration or exposure to X-radiation. However, in the experimental animals, there was no significant alteration in the relative distribution of the individual serum components.

The precise relation of pituitary-adrenal cortical secretion to the normal pattern of serum proteins would appear to wait further investigation. The conflicting data in the literature, taken together with the present experiments, suggest that variables exist which remain for further careful evaluation. These comments relate not only to the pattern of normal serum globulins, but also to immune globulins, about which there also have appeared conflicting data.

It may be pointed out that serum protein levels alone do not provide a complete evaluation of the possible relation of endocrine secre-

tions to blood protein production and utilization. Thus, it has been demonstrated recently[18] that although the antibody level in the *blood* of immunized, normal and adrenalectomized rats may be at similar levels, the *tissues* of the adrenalectomized rats are producing antibody at a rate which is distinctly less than that of the unoperated animals. This suggests that the adrenalectomized animals are also removing antibody from the circulation at a rate which is less than normal. Thus, although the blood titer is similar in the two groups of animals, the presence of the adrenal does appear to influence the rate at which the antibody globulin enters and leaves the circulation. It may also be of significance that Mondolfo and DeLerner[10] reported recently that whereas relatively small doses of adrenal cortical extract may augment the rate of antibody production, significantly larger amounts of the same extract produced a suppression of antibody production as compared to the rate seen in animals receiving antigen alone.

Summary. Normal mice, or mice bearing a transplantable lymphosarcoma, were given single injections of adrenal cortical extract or exposed to a single radiation dose of 200 r (total body radiation). Examination of the serum protein patterns of these mice at 3 and at 6 hours after hormone injection, or at 6 hours after exposure to X-rays, revealed a striking increase in total serum protein levels. However, electrophoretic examination did not reveal significant alterations in the relative distribution of the serum proteins.

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18 Roberts, S., and White, A., unpublished results.

Cinchophen Choleresis and Production of Peptic Ulcer in Various Species. (17457)

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Brugsch and Horsters[1] were the first to show that cinchophen, in both dogs and man caused an increase in the bile flow. Stransky[2] carried this study to rabbits and was unable to detect any choleretic effect of cinchophen in this species. Berman and Ivy[3] confirmed this finding and found in addition that the excretion of cinchophen in the bile of rabbits was very small.

Churchill and Van Wagoner[4] demonstrated that dogs receiving 20 to 30 times the human dose of cinchophen developed gastric or duodenal ulcers. This finding has since been confirmed very many times.[5-7] Shoji[8] found that rabbits were very resistant to the ulcerogenic action of this drug. Okada[9] likewise failed to produce cinchophen ulcers in either rabbits or guinea pigs. Schwarz and Simmonds[10] were unable to induce cinchophen gastric, or duodenal ulceration in rabbits or guinea pigs, but found cats very susceptible. The ability of cinchophen to cause liver damage in rats[11] has been noticed but there has been no report of its effect upon the gastrointestinal tract.

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