aureomycin to the media(10). The observations in guppies are somewhat in agreement with the more recent work on the catabolic effects of aureomycin in man and dogs(11). We cannot at this point explain why aureomycin feeding stimulates growth in mammals and birds and inhibits growth in fish. It is yet to be determined whether these results are due to specific differences or based upon the possible catabolic propensities of large doses of aureomycin as previously described (11). An overgrowth of a mold identified as *Monilia albicans* was noted in the water of the aquarium in which aureomycin had been added. The increased growth of Monilia noted in the tank after the addition of aureomycin is in accord with some clinical observations on the apparent increase in moniliasis after the use of broad spectrum antibiotics. One must consider the possibility that this fungal growth might be a factor in inhibiting somatic growth in the fish.

Summary. Supplemental feeding of aureomycin to guppies caused a marked inhibition of somatic development and growth. There was, however, no gonadal inhibition. The possible mechanism of this action of aureomycin was discussed.

1. Jukes, T. H., Stokstad, E. L. R., Taylor, L. R., Cunha, R. R., Edwards, H. M., and Meadows, G. B., *Arch. Biochem.*, 1950, v26, 324.

2. Lepley, K. C., Catron, D. V., and Culbertson, C. C., *J. Animal Science,* 1950, v9, 608.

3. Carpenter, L. E., *J. Animal Sci.,* 1951, v10, 657.

4. Catron, D. V., Maddock, D. V., Speer, V. C., and Vohs, R. L., *Antibiotics and Chemotherapy,* 1951, v1, 31.

5. Stokstad, E. L. R., Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 523.

6. Oleson, J. J., Whitehill, A. R., and Hutchings, B. L., *Arch. Biochem.,* 1950, v29, 334.

7. Berg, L. R., Bearse, G. E., McGinnis, J., and Miller, V. L., *Arch. Biochem.,* 1950, v29, 404

8. Perrini, F., *Boll. Soc. Ital. Biol.,* 1951, v27, 1151.

9. Cohen, A. M., and Rachmilewitz, M., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 50.

10. Legine, P., Barski, G., and Maurin, J., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 252.

11. Bateman, J. E., Klopp, C. T., and Sedwitz, J., *Clin. Res. Proc.,* 1953, v1, 22.

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Comparative Effects of Intramuscular Injections of ACTH, Cortisone, and Saline on Serum Glycoprotein Levels.* (20532)

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Increases in the protein-bound carbohydrates of serum have been observed in such diverse conditions as human(1) and experimental(2) tuberculosis, human(3) and experimental(4) carcinoma, human(5) and experimental(6) thermal injury, late pregnancy and parturition(7), rheumatic fever and osteomyelitis(8), X-irradiation(9), immunization(10), experimental scurvy(11), and from the administration of parathyroid extract(12). The reports suggest that the elevated serum glycoprotein levels may represent a response to non-specific stress(13).

Although the mechanism is still speculative, it is now generally accepted that the pituitary-adrenal axis is concerned in some fundamental way with the ability of the organism to respond to stress. A wide variety of unrelated stimuli such as heat, cold, tissue injury, hemorrhage, infections, the injection of bacterial toxins, toxic chemicals, foreign proteins, epinephrine, thyroxine, and insulin have been found to augment the rate of secretion of

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TABLE I. Summary of Chemical Analyses.

Group	A	B	C	D
No. of animals	27	22	20	24
Treatment	None	10.0 mg ACTH/day	10.0 mg cortisone/day	0.5 ml saline/day
Avg wt before treatment		653	595	573
Avg wt after treatment		620	622	597
Total serum* polysaccharide (mg %)	111 ±2.5	127 ±4.3	119 ±3.8	131 ±6.0
Mucoprotein* polysaccharide (mg %)	29 ±1.1	31 ±1.1	27 ±1.7	38 ±2.5
Total serum* protein (g %)	5.4± .08	5.2± .09	5.4± .10	4.9± .09
Total serum poly.*/Total serum protein × 100 (%)	2.1± .05	2.4± .07	2.2± .08	2.7± .11

* Including stand. error of the mean calculated from the relation $E_m = [\Sigma d^2/(N(N-1))]^{1/2}$ where "d" is the deviation from the mean and N the No. of observations.

TABLE II. Statistical Comparison of Results.

Determination	Groups compared	Difference between means	D.F.	t	P
Total serum polysaccharide	Normal (A) vs. ACTH-treated (B)	16 mg %	47	3.312	<.01
	" " " cortisone-treated (C)	8	45	1.824	.1
	" " " saline-treated (D)	20	49	3.180	<.01
	Saline-treated (D) vs. ACTH-treated (B)	4	44	0.528	.6
	" " " " cortisone-treated (C)	12	42	1.608	.2
Mucoprotein polysaccharide	Normal (A) vs. ACTH-treated (B)	2 mg %	47	1.284	.3
	" " " cortisone-treated (C)	2	45	1.044	.4
	" " " saline-treated (D)	9	49	3.672	.001
	Saline-treated (D) vs. ACTH-treated (B)	7	44	2.653	<.02
	" " " " cortisone-treated (C)	11	42	3.696	.001
Total serum protein	Normal (A) vs. ACTH-treated (B)	.2 g %	47	1.685	.1
	" " " cortisone-treated (C)	.0	45	0.000	—
	" " " saline-treated (D)	.5	49	4.123	.001
	Saline-treated (D) vs. ACTH-treated (B)	.3	44	2.385	<.05
	" " " " cortisone-treated (C)	.5	42	3.616	.001
Total serum polysaccharide ÷ total serum protein × 100	Normal (A) vs. ACTH-treated (B)	.3 %	47	3.561	.001
	" " " cortisone-treated (C)	.1	45	1.144	.3
	" " " saline-treated (D)	.6	49	5.136	.001
	Saline-treated (D) vs. ACTH-treated (B)	.3	44	2.175	<.05
	" " " " cortisone-treated (C)	.5	42	3.470	<.01

pituitary adrenotrophic hormone(14).

If the increased secretions of ACTH and adrenal cortical hormones following stress are responsible for the elevation of serum glycoprotein levels, then the administration of the hormones to normal animals might be expected to cause an increase in the concentrations of the circulating conjugated proteins. The present investigation was undertaken to study the effects of exogenous ACTH and cortisone on the total serum glycoprotein, serum mucoprotein, and total serum protein levels of normal guinea pigs. Sterile 0.85% sodium chloride was administered to control animals in order to evaluate the stress effects of the injection procedure.

Experimental. Male and female guinea pigs were maintained *ad libitum* on a diet of rabbit pellets plus greens, and tap water. Injections of 5 mg ACTH,† 5 mg cortisone,‡ and 0.25 ml of 0.85% saline were made intramuscularly, at 12-hour intervals, for 11 days. After the final injection, the animals were bled by cardiac puncture, the sera separated and stored in the frozen state until the chemical analyses could be conducted.

† The ACTH (Actrope) employed in the investigation was obtained through the courtesy of Mr. S. S. Kingman and Mr. Richard C. Bruner, United Laboratories, Ltd., Pasadena, Calif. One mg of Actrope was equivalent to one U.S.P. unit.

‡ Cortone acetate, Merck and Co., Rahway, N. J.

Total serum polysaccharide, mucoprotein polysaccharide and total serum protein were determined as previously described(2). The total serum polysaccharide and mucoprotein polysaccharide values were employed as indices of the levels of the total serum glycoprotein and serum mucoprotein, respectively. For comparative purposes, the data obtained from the serum analyses of normal, untreated animals and which have been previously reported(2) are included. The group means for all determinations were compared by the "t" test of Fisher(15).

Results. The summary of the results of the chemical analyses and weight determinations is presented in Table I. A statistical comparison is shown in Table II. *Total serum polysaccharide.* Statistically significant increases were observed when the means of the groups which received ACTH (B) and saline (D) were compared with the mean of the untreated animals of Group A. The elevation that occurred in the cortisone-treated group (C) was not significant. No significant differences were found when the means of the groups administered hormones (B, C) were compared with the average of the saline-treated guinea pigs of Group D. The individual values within the group which received saline (D) exhibited a greater degree of variability as indicated by the magnitude of the standard error of the mean. *Mucoprotein polysaccharide.* The serum mucoprotein values remained within the normal range for the groups administered ACTH (B) and cortisone (C). The guinea pigs of Group D, which received saline, exhibited a significant increase in mucoprotein polysaccharide when compared with the untreated animals of Group A. The serum mucoprotein content of the saline-treated group (D) also differed significantly from the means of the groups (B, C) which received injections of hormones. *Total serum protein.* A statistically significant decrease occurred in the total serum protein content of Group D, which received saline (Table II). The mean of the cortisone-treated group (C) was identical with the average value of the untreated guinea pigs of Group A. Group B, which was injected with ACTH, exhibited a small, but non-significant decrease in total serum protein. *Total polysaccharide as % of protein.* Highly significant increases were observed in the total serum polysaccharide levels with respect to the total serum protein in the ACTH (B) and saline-treated (D) groups. In the case of Group D, the increased percentage was due in part to the marked decrease in the amount of circulating protein. *Weight.* The weight responses of the hormone-treated groups (B, C) were similar to those previously observed in uninfected guinea pigs which received the dosages of ACTH and cortisone employed in the present study(16). The effect of saline administration approximated that of cortisone, causing a small retardation in the normal rate of growth.

Discussion. Qualitative and quantitative differences have been observed with respect to total serum glycoprotein, serum mucoprotein, and total serum protein following the administration of ACTH, cortisone, and saline to normal guinea pigs. The total serum glycoproteins were elevated in the 3 groups which received injections, the increases being of statistical significance in the ACTH- and saline-treated groups. Serum mucoprotein values were within the normal range for the groups which received hormones, but were significantly elevated for the group administered saline. A significant hypoproteinemia was observed in the guinea pigs injected with saline. The decrease in the total serum protein did not occur in the groups administered hormones.

Since the concentrations of total serum glycoprotein and serum mucoprotein of the hormone-treated groups were less than the corresponding serum levels in the animals which received saline, the conclusion may be drawn that increased secretions of ACTH and cortisone are not directly responsible for the elevation of serum glycoprotein levels observed in many types of stress.

The pattern of alterations in the serum components of the saline-treated guinea pigs, presumably due to the trauma and emotional stimuli of the injection procedure, was remarkably similar to the serum changes reported for patients with neoplasms(3) and tumor-bearing rats(4). The deviations from

the normal serum values observed in the saline-treated group were partially prevented by the administration of ACTH or cortisone. A homeostatic effect of the hormones was suggested since the concentrations of serum mucoprotein and total serum protein in the groups which received ACTH or cortisone, did not differ significantly from the corresponding values of the untreated control group.

The studies reported above support in part the concept, emphasized by Roberts(17), that the primary role of the adrenal cortical secretions in protein metabolism is stimulation of the mobilization of tissue protein. Roberts (17) has demonstrated that the depression of total serum protein following partial hepatectomy in the rat may be prevented by treatment with ACTH or ACE. In the present investigation, the hypoproteinemia which followed the intramuscular injection of saline did not occur in the guinea pigs which received ACTH or cortisone. The data obtained from the total serum glycoprotein and serum mucoprotein determinations are not in complete agreement with this interpretation. If the labile protein-bound carbohydrates of tissues were released under hormonal influence as postulated above, greater concentrations of the conjugated proteins should be present in the sera of the hormone-treated animals than in the circulation of the guinea pigs which received saline. The observation that the serum glycoprotein and serum mucoprotein levels of the saline-treated group were higher, suggests that more than one physiologic mechanism may be involved in the metabolism of the glycoproteins and the carbohydrate-free proteins of serum.

Summary. The effects of intramuscular injections of ACTH, cortisone, and 0.85% sodium chloride on total serum glycoprotein, serum mucoprotein, and total serum protein levels of normal guinea pigs have been investigated. Statistically significant increases in total serum glycoprotein were observed in the ACTH- and saline-treated groups. Serum mucoprotein values were significantly elevated and the total serum protein values significantly decreased in the group which received saline. The conclusion was drawn that increased secretions of ACTH and cortisone are not directly responsible for the elevated total serum glycoprotein and serum mucoprotein levels following stress.

1. Seibert, F. B., Seibert, M. V., Atno, A. J., and Campbell, H. W., *J. Clin. Invest.*, 1947, v26, 90.

2. Weimer, H. E., and Moshin, J. R., *Am. Rev. Tuberc.*, 1953, v68, 594.

3. Greenspan, E. M., Lehman, I., Graff, M. M., and Schoenbach, E. B., *Cancer*, 1951, v4, 972.

4. Shetlar, M. R., Erwin, C. P., and Everett, M. R., *Cancer Research*, 1950, v10, 445.

5. Keyser, J. W., *J. Clin. Path.*, 1952, v5, 194.

6. Knobloch, W. H., Jr., Nagle, P., Shetlar, C. L., and Shetlar, M. R., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 417.

7. Shetlar, M. R., Kelly, K. H., Foster, J. V., Shetlar, C. L., and Everett, M. R., *Am. J. Obstet. and Gynecol.*, 1950, v59, 1140.

8. Kelly, V. C., Good, R. A., and McQuarrie, I., *Pediatrics*, 1950, v5, 824.

9. Shacter, B., Supplee, H., and Entenman, C., *Am. J. Physiol.*, 1952, v169, 508.

10. Hammerstrom, R. N., Adams, F. H., Bussman, J., and Lillehei, C. W., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 257.

11. Pirani, C. L., and Catchpole, H. R., *Arch. Path.*, 1951, v51, 597.

12. Engel, M. B., *Arch. Path.*, 1952, v53, 339.

13. Boas, N. F., and Peterman, A. F., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 19.

14. Sayers, G., Sayers, M. A., Fry, E. G., White, A., and Long, C. N. H., *Yale J. Biol. and Med.*, 1944, v16, 361.

15. Snedecor, G. W., *Statistical Methods*, 4th Edition, Iowa State College Press, Ames, Iowa, 1946.

16. Weimer, H. E., Boak, R. A., Bogen, E., Drusch, H. E., Miller, J. N., Moshin, J. R., and Carpenter, C. M., *Am. Rev. Tuberc.*, 1953, v68, 31.

17. Roberts, S., *J. Biol. Chem.*, 1953, v200, 77.

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