given subanesthetic doses of hexobarbital. 4) Following the injection of small doses of hexobarbital, potassium can abolish the righting reflex in a fraction of the dose required when injected alone.

1. Lamson, P. D., Greig, M. E., and Hobdy, C. J., *J. Pharm. and Exp. Therap.*, 1951, v103, 460.

2. Fenn, W. O., *Phys. Rev.*, 1940, v20, 377.

3. Amberg, S., and Helmholz, H. F., *J. Pharm. and Exp. Therap.*, 1918-1919, v12, 19.

4. Boyer, P. D., Lardy, H. A., and Phillips, P. H., *J. Biol. Chem.*, 1943, v149, 529.

5. Stewart, J. M., *Am. J. Physiol.*, 1950, v163, 622.

Received May 15, 1952. P.S.E.B.M., 1952, v80.

Effect of Cortisone on Lipids of Serum, Liver and Testes in Intact and Adrenalectomized Rats.*† (19694)

CLAUDE J. MIGEON.‡ (Introduced by Lytt I. Gardner)

From the Department of Pediatrics, Johns Hopkins University School of Medicine, and the Harriet Lane Home, Johns Hopkins Hospital, Baltimore, Md.

Cortisone therapy in man increased the serum concentration of cholesterol and phospholipid, and decreased serum neutral fat (1,2). The increase of serum cholesterol, total and esterified, has been reported in rats treated with cortisone(3). In rabbits, adrenal extract and cortisone administration also increased cholesterol, lipid phosphorus and total fatty acids of serum(4-6). ACTH was shown to cause fatty infiltration of the liver in the rabbit(6,7) and rat(8). Adrenalectomy prevented fat mobilization into the liver in mice given ACTH(9). Cortisone caused no fatty infiltration of the liver in intact or adrenalectomized mice(10). However, lipid granules were found in hepatic cells of intact rabbits treated with cortisone(6). Cortisone was not found to cause a severe involution of testis in rats(3,11). ACTH was found to cause a slight reduction in the weight of rat testes with atrophy of the Leydig cells(12).

This study reports the effect of cortisone administration on total cholesterol and lipid phosphorus concentration of serum, liver and testes in adrenalectomized rats maintained with desoxycorticosterone pellets, as compared with the effects of the hormone in intact rats.

Experimental. The animals used in this study were 56 adult male rats of the Sprague-Dawley strain, selected for uniformity and with a weight range of 270 to 320 g. They received Rockland Rat diet and tap water *ad libitum*. Vit. A (200 I.U.) and vit. D (100 I.U.) were given orally once a week.

The experimental groups are shown in Table I. Adrenalectomy was performed through a flank approach. Each rat was maintained with a 15 mg pellet of desoxycorticosterone acetate (DCA).§ For the first 2 days following operation, the animals received by mouth 5% glucose in 0.3% saline, after which they were kept on the same diet as the other rats. Cortisone acetate¶ was given subcutaneously, 4 mg every day (0.5 ml in 0.9% saline) for 28 days. The rats were weighed twice weekly. The cortisone-treated rats lost about 50 g. At different times, daily food intake was weighed in each group. Blood was drawn under ether

* Research of the endocrine division is supported in part by Grants-in-Aid from the American Cancer Society, and by a research grant from the National Institutes of Health.

† The author is indebted to Drs. Lawson Wilkins, Walter Fleischmann, Lytt Gardner, Gordon Kennedy and Samuel Silverman for helpful suggestions. Thanks are due to Mr. Henry Schulte, Mrs. Mary Ellen Crafton and Miss Betty Youngblood for technical assistance.

‡ Fellow of the André and Bella Meyer Foundation (France) on recommendation of the Institute of International Education, New York.

§ The author is indebted to Dr. F. E. Houghton, of Ciba Pharmaceutical Products, Summit, N. J., for Percorten pellets.

¶ The author is thankful to Dr. L. V. Curtin of Merck and Co., Rahway, N. J., for cortisone.

TABLE I. Liver Weight and Concentration in Total Cholesterol and Lipid Phosphorus. (Mean value ± stand. dev. of group.)

	Exp. groups	Liver wt, g — Total	Liver wt, g — Per 100 g B.W.	Total cholesterol, mg — Total	Total cholesterol, mg — Per g of liver	Lipid P, mg — Total	Lipid P, mg — Per g of liver
I	Normal (10)*	10.99 ± .30	3.45 ± .78	34.29 ± 1.4	3.14 ± .28	7.82 ± .55	.714 ± .037
	Normal + cortisone (12)	9.758 ± .67	4.30 ± .29	30.4 ± 2.7	3.10 ± .29	7.43 ± .72	.764 ± .074
II	Normal (6)	10.55 ± 1.04	2.96 ± .37	32.98 ± 5.3	3.12 ± .17	7.66 ± 1.20	.725 ± .06
	Normal + cortisone (6)	10.60 ± .97	4.15 ± .95	33.10 ± 1.06	3.12 ± .46	8.06 ± .88	.759 ± .08
	Adrenal × + DCA (10)	11.58 ± 1.00	3.16 ± .22	33.75 ± 2.32	2.92 ± .26	11.50 ± .40	.996 ± .06
	Adrenal × + DCA + cortisone (12)	9.25 ± .73	3.55 ± .35	27.82 ± 2.16	3.03 ± .34	6.19 ± .81	.670 ± .07

* No. of animals.

anesthesia from the abdominal aorta into centrifuge tubes as soon as possible after the animal was anesthetized, since a rise in blood cholesterol may occur after 5 to 7 minutes of anesthesia(13). The blood was immediately centrifuged and the serum separated. The testes were quickly removed, and weighed to the nearest 5 mg; the tunica albuginea was stripped from each testis before weighing in an effort to eliminate contributions of tissue other than the tubule-interstitial cell complex. Each testis was then put in a metal cup and desiccated immediately. The livers were carefully dissected and weighed to the nearest 5 mg, divided into several weighed pieces of approximately 1 g each, and desiccated immediately in metal cups.

Total cholesterol and lipid phosphorus determination in serum. Total cholesterol was determined by the method of Bloor, Pulkan and Allen(14), using a Klett-Summerson photoelectric colorimeter. Lipid phosphorus was determined by the method of Youngburg and Youngburg(15) as modified by Hawk, Oser, and Summerson(16), using an Evelyn photoelectric colorimeter.

Total cholesterol and lipid phosphorus determination in testes and liver. The dry tissue was ground with C.P. sea sand in a mortar and then transferred to a 50 ml centrifuge tube. The mortar, pestle and cup which contained the tissue specimen were washed down with 25 ml of ethanol-ether mixture and the washings were collected in the centrifuge tube. The tube was attached to a vertical Graham coiled condenser for refluxing, and the contents boiled for 40 minutes. Tube was then raised out of the water bath and the condenser washed with 5 ml of ethanol-ether mixture. When the tube was cooled, it was centrifuged for a few minutes and the supernatants filtered using fat-free filters. The tissue was extracted a second and third time in the same way, the extracts combined with the initial filtrate and made to 100 ml in a volumetric flask with ethanol-ether mixture. Duplicate

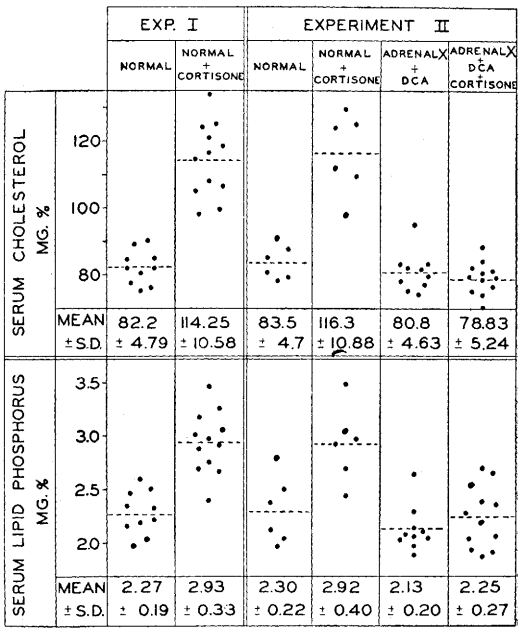

FIG. 1. Total cholesterol and lipid phosphorus in serum of the rats in different experimental groups. Dotted lines represent mean value in each group.

TABLE II. Testes Weight and Concentration in Total Cholesterol and Lipid Phosphorus. (Mean value ± stand. dev. of group.)

	Exp. groups	Testes wt, g — Total	Testes wt, g — Per 100 g B.W.	Total cholesterol, mg — Total	Total cholesterol, mg — Per g of testis	Lipid P, mg — Total	Lipid P, mg — Per g of testis
I	Normal	3.196±.118	.937±.164	5.861±.316	1.832±.090	1.158±.16	.363±.063
	Normal + cortisone	2.802±.254	1.231±.126	4.754±.505	1.702±.183	.887±.18	.317±.050
II	Normal	3.343±.157	.938±.089	6.180±.480	1.848±.112	1.069±.15	.319±.047
	Normal + cortisone	2.946±.241	1.146±.097	5.231±.630	1.775±.130	.985±.11	.338±.052
	Adrenal × + DCA	3.279±.222	.894±.075	5.696±.363	1.650±.16	1.087±.172	.332±.043
	Adrenal × + DCA + cortisone	2.974±.257	1.130±.175	5.120±.396	1.725±.144	.805±.133	.270±.041

TABLE III. Level of Significance of the Salient Differences in Results as Tested by "Students" T Test(20).

	Differences tested		t	D.F.*	P.†
Serum cholesterol	Normal against normal + cortisone	Exp. I	8.32	20	<.001
	" " "	" II	6.17	15	<.001
Serum lipid P.	" " "	" I	6.11	20	<.001
	" " "	" II	3.11	10	<.02
	Normal against adrenal × + DCA	" II	1.38	16	>.1 <.2
Liver lipid P.	" " "	" II	5.60	14	<.001
	Normal against adrenal × + DCA + cortisone	" II	3.05	16	<.01

* D.F. = degrees of freedom. † P = probability of the difference being due to chance.

aliquots of this stock solution were used for total cholesterol and lipid phosphorus determinations as described for serum.

Results. 1) The results of total cholesterol and lipid phosphorus analyses of the sera are shown in Fig. 1. Cortisone therapy was associated with an increased total cholesterol in intact rats, but did not increase that of adrenalectomized rats maintained with DCA. Cortisone administration increased serum lipid phosphorus of intact rats but had no effect on the adrenalectomized rats maintained with DCA. A slight decrease of serum lipid phosphorus was noted in adrenalectomized rats maintained with DCA, compared with the values for intact rats. This change was not significant (Table III).

2) *Effect of cortisone therapy on the liver.* The results are shown in Table I. Cortisone administration did not modify liver weight or total cholesterol concentration in either intact or adrenalectomized rats. Cortisone therapy did not change lipid phosphorus concentration in the liver of intact rats. The adrenalectomized rats maintained with DCA had a significant increase in liver lipid phosphorus, compared with intact rats. Cortisone treatment slightly decreased liver lipid phosphorus in adrenalectomized rats, compared with intact rats. This decrease was not significant (Table III).

3) *Effect of cortisone therapy on testes.* Cortisone therapy decreased the weight of the testes, but the weight per 100 g of body weight was normal or slightly increased over control values (Table II). Total cholesterol and lipid phosphorus content of the testes decreased on cortisone therapy in both intact and adrenalectomized rats, but the changes were not significant.

Discussion. The administration of cortisone did not increase total cholesterol and lipid phosphorus of serum in adrenalectomized rats maintained with DCA, although these were increased in intact animals. Other workers had noted that administration of ascorbic acid

(10-100 mg intramuscularly) increased serum cholesterol of the intact rat but had no effect on the adrenalectomized rat(17). Our findings cannot be explained by a difference in food intake, as the intact rats and adrenalectomized rats with DCA, both showed a decrease in food intake during cortisone therapy (18). Since serum sodium and potassium levels were within normal limits in every group, the results cannot be explained by a change in the dilution of plasma.

The treatment of adrenalectomized dogs with DCA lowered their blood phospholipids (19). In our experience, the serum lipid phosphorus of adrenalectomized rats maintained with DCA was slightly but not significantly lower than normal.

Although the cholesterol concentration of the liver was not changed in any group, the liver lipid phosphorus concentration was increased in the adrenalectomized rats maintained with DCA. This finding is difficult to interpret.

Summary. 1. Cortisone therapy increased serum cholesterol and lipid phosphorus of intact rats but did not have this effect in adrenalectomized rats maintained with DCA. 2. Adrenalectomized rats maintained with DCA showed a significant increase in liver lipid phosphorus. This was restored to normal by a dosage of cortisone which was without effect on the liver lipid phosphorus of the intact rat. 3. Cortisone therapy decreased testicular weight of intact and adrenalectomized rats, but caused no change in the concentrations of cholesterol and lipid phosphorus of the testes.

1. Adlersberg, D., Schaefer, L. E., and Drachman, S. R., *J. Clin. Endocrinol.*, 1951, vII, 67.

2. Conn, J. W., Vogel, W. C., Louis, L. H., and Fajans, S. S., *J. Lab. Clin. Med.*, 1950, v35, 504.

3. Winter, C. A., Silber, R. H., and Stoerk, H. C., *Endocrinology*, 1950, v47, 60.

4. Hoffmeyer, J., *Acta Physiol. Scand.*, 1945, v10, 31.

5. Kobernick, S. D., and More, R. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 602.

6. Rich, A. R., Cochran, T. H., and McGoon, D. C., *Bull. Johns Hopkins Hosp.*, 1951, v88, 101.

7. Diczfaluzy, *et al.*, *Acta Endocrinologica*, 1950, v5, 43.

8. Salva, G., *et al.*, *Lancet*, 1951, v1, 641.

9. Best, C. H., and Campbell, J., *J. Physiol.*, 1936, v86, 190.

10. Levin, L., and Farber, R. K., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 758.

11. Friedman, S. M., Friedman, C. L., and Nakashima, M., *Am. J. Physiol.*, 1950, v163, 319.

12. Baker, B. L., Schairer, M. A., Ingle, D. J., and Li, C. H., *Anat. Rec.*, 1950, v106, 345.

13. Mahler, A., *J. Biol. Chem.*, 1926, v69, 653.

14. Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, v52, 191.

15. Youngburg, G. E., and Youngburg, M. V., *J. Lab. and Clin. Med.*, 1930, v16, 158.

16. Hawk, L. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, 12th Ed., The Blakiston Co. Ed., Philadelphia, p541.

17. Booker, W. M., Dent, F. M., and Hayes, R. L., *Fed. Proc.*, 1951, v10, 17.

18. Migeon, C. J., *et al.*, in preparation.

19. Zilversmit, D. B., Stern, T. N., and Overman, R. R., *Am. J. Physiol.*, 1951, v164, 31.

20. Snedecor, G. W., *Statistical Methods*, Iowa State College Press, Ames, Iowa, 1948, p82.

Received May 26, 1952. P.S.E.B.M., 1952, v80.

Oncolytic Effect of Viruses in Tissue Cultures.* (19695)

MORRIS POLLARD AND ROBERT H. BUSSELL.

From the University of Texas Medical Branch, Galveston, Texas.

When Levaditi and Nicolau(1) described the propagation of herpes virus in mouse epitheliomas they were seeking a substrate on which to propagate the virus. These authors and others(2-4) later demonstrated that when avian pest virus localized and propagated in mouse tumor tissue, it exerted therein a cytolytic effect. This initiated similar studies with other viral agents such as neurotropic

* This study was supported by funds provided under Contract AF 41-607-42 with the USAF School of Aviation Medicine, Randolph Field, Texas.