TABLE I.
Effect of 4-amino-N10 Methyl-pteroylglutamic Acid and Pteroylglutamic Acid on Subline AM-resistant Lymphoid Leukemia L1210.

Experiment	Daily dosage, mg/kg	Transplant generation	No. of mice	Mean wt of tumor, mg*	Absolute No. lymphoblasts (peripheral blood)
AM-Resistant L1210					
4-amino-N10-methyl-pteroylglutamic acid	3.0		38	388.5	1430†
Pteroylglutamic acid	30	G7 to G11	34	159.0 } 176.7	—
Controls	—		20	207.7 }	—
Diff. of means = 211.8 ± 54.6 mg; t = 3.9; P >0.01‡					
4 amino-N10-methyl-pteroylglutamic acid	2.5	G12	12	750.2	
Controls	—	"	13	336.5	
Diff. of means = 413.7 ± 59.2 mg; t = 6.9; P >0.001					
Control L1210					
4 amino-N10-methyl-pteroylglutamic acid	3.0	G1 to G6	25	No growth	
Pteroylglutamic acid	30	G6	5	544.0	
Controls	—		5	494.3	
4 amino-N10-methyl-pteroylglutamic acid	2.5		5	28.6	300
Pteroylglutamic acid	30	G12	5	581.7	—
Controls	—		5	808.1	1750

* Wet wt obtained on a Roller-Smith micro torsion balance.
† The mean absolute number obtained from 6 mice, transplant generations 6, 7, and 8.
‡ Since the difference of the mean weights of the tumor mass in the pteroylglutamic acid group and the control group was not significant statistically, the mean weight of pooled data for these 2 groups (176.7) mg is compared with the mean weight of the 4-amino-N10-methyl-pteroylglutamic acid group.

4-amino-9, N^{10} dimethyl PGA and 4-amino-9 methyl PGA. All 3 sublines continue to be resistant in subsequent transplant generations following emergence of resistance. Subline AD-resistant leukemic cells grown in mice not given the folic acid antagonist for 7 successive transplant generations continue to be resistant to 4-amino-9, N^{10} dimethyl PGA. Resistance developed to one of these antivitamins was found not to be independent of resistance to the others. AM-resistant leukemic cells were found to grow better in animals receiving the MTD of 4-amino-N^{10} methyl PGA than in animals untreated with this antivitamin or given PGA parenterally.

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Diabetic State with Lipaemia and Hydropic Changes in the Pancreas Produced in Rabbits by Cortisone.* (17989)

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During the course of an experiment undertaken with the purpose of observing the effect of Cortisone on the tissue lesions produced by foreign serum proteins in rabbits living in a cold environment, a marked alteration of carbohydrate metabolism which resembled a diabetic state and abnormalities of the Islets of

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† This work was done during the tenure of a Life Insurance Medical Research Fellowship.

TABLE I.
Biochemical Findings in the Blood and Serum of Two Rabbits Treated with Cortisone in a Dose of 20 mg Per Day.

Rabbit No.	Day of cortisone	Blood sugar, mg %	Visible lipaemia	Lipid phosphorus, mg %	Cholesterol, mg %		Fatty acids of neutral fat, m.Eq./liter
					Free	Total	
K-33	2	180	0				
	5	203	++				
	8	310	++	25.4	65.0	90.0	43.99
	12	688	++++	30.6	50.0	250.0	69.57
K-37	—1	133	0				
	2	209	0				
	5	272	+	14.8	36.0	50.0	23.55
	8	367	++	29.0	100.0	330.0	57.23
	15	448	+++				
	22	1280	++++	61.2	425.0	500.0	742.8
Normal rabbit serum	Payne & Duff (2)	42 rabbits		4.4 S.D.* 1.7	19 S.D. 9.5	46 S.D. 17.5	7.3 S.D. 3.1

* S.D. = Standard deviation.

Langerhans were observed. These were accompanied by a profound effect on lipid metabolism. Since these changes did not occur in control animals kept under the same conditions but not given Cortisone, the abnormalities are attributable to the Cortisone. So far as we are aware, the occurrence of lipaemia and lesions of the islets have not heretofore been reported as resulting from the treatment of animals with Cortisone, although McLean(1) has observed the occurrence of visible lipaemia in rabbits treated with Cortisone and horse serum.

After a suitable period of observation during which time it was established that the animals did not manifest a significant glycosuria, albuminuria, elevation of the blood sugar or visible lipaemia, 2 rabbits were treated with 2 intramuscular injections of 10 mg of Cortisone acetate (Merck) each day. On the third day of treatment with Cortisone, the animals were placed in a cold room in which the temperature varied between 8 and 30°F, remaining near the lower limit for most of the day. On the sixth day, each animal received an intravenous injection of 10 ml of normal horse serum per kilo of body weight. One animal (K-33) died on the 12th day and the other (K-37) was killed on the 22nd day after the commencement of the Cortisone treatment. Pairs of controls receiving no treatment or intravenous horse serum only, were kept in the cold room at the same time. Food and water were given *ad libitum*. Though the conditions of the experiment are detailed, it appears from our observations on animals presently under study, that the lipaemia, hyperglycemia and glycosuria appear even if the animals are kept at ordinary room temperature and no serum is administered.

Five days after the commencement of Cortisone, a definite cloudiness of the serum was evident to the naked eye. On chemical analysis, the serum manifested an increase in all of the lipid fractions which was similar in pattern to that reported from this laboratory by Payne and Duff(2) in alloxan diabetes in the rabbit, but reaching somewhat higher values. The findings in the serum are detailed in Table I. The blood sugar was markedly elevated and the rise in the lipid content of the serum paralleled the rise in the blood sugar (Table I). A marked glycosuria occurred corresponding to the hyperglycemia.

The islets of the animal that died on the 12th day of the treatment (K-33), showed some swelling of the cells with an occasional one showing fragmentation of the cell border.

1. McLean, C. R., unpublished observations.

2. Payne, T. P. B., and Duff, G. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 332.

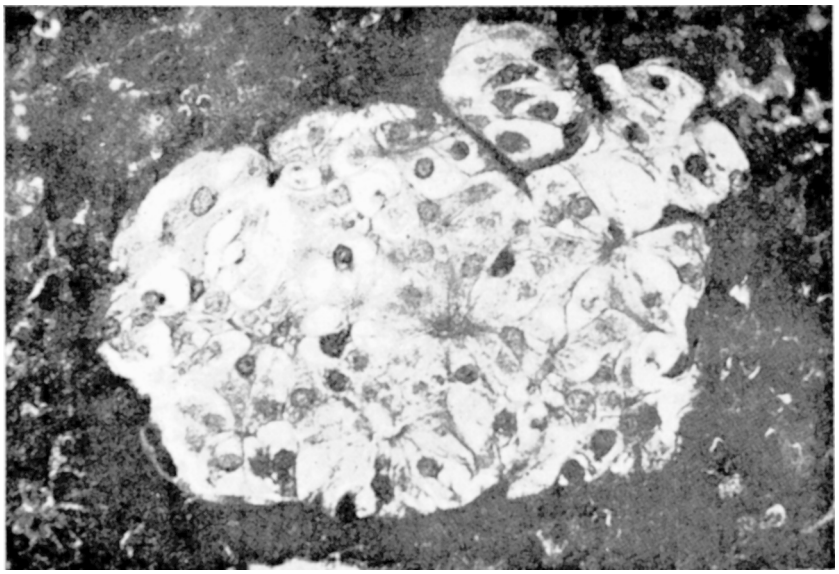

FIG. 1.
Islet of Langerhans from animal K-37 showing marked "hydropic change" of the beta cells. Iron haematoxylin, phloxine × 400.

It is possible that these changes were due to postmortem autolysis, since the animal died and was autopsied 18 hours after death. However, autolysis does not usually occur if animals die in the cold room, even if 24 hours elapse between death and autopsy. The animal that was killed on the 22nd day (K-37) and autopsied immediately, showed a marked vacuolation or "hydropic change" of the cells of the islets and ductules of the pancreas (Fig. 1). As first demonstrated by Toreson (3) in this laboratory in experimental diabetes produced by anterior pituitary extract, partial pancreatectomy or alloxan, and in human diabetes, the so-called hydropic cells contained glycogen stainable with Best's carmine, but not stainable in diastase-treated control sections. Gomori's stain showed that the vacuolation was chiefly in the beta cells sparing the alpha cells.

The full significance of the observations cannot be appreciated at this time, since they are not complete. However, it appears that a state very similar to a true diabetic state has been produced in rabbits with Cortisone. This state is accompanied by a lesion of the islets similar to that seen in other forms of diabetes, both experimental and human(3). Whether the lipaemia is a derangement of lipid metabolism accounted for by the direct effect of Cortisone, or whether it is secondary to the disturbance of carbohydrate metabolism and the diabetic state cannot be decided from consideration of the data at hand.

The fact that the metabolic disturbances here reported occur in rabbits under Cortisone therapy provides another possible experimental approach to the study of the diabetic state. The occurrence of lipaemia in association with a disturbance of carbohydrate metabolism simulating diabetes suggests that Cortisone may have similar effects on experimental atherosclerosis to those recently observed by Duff and McMillan(4) who demonstrated that alloxan diabetes has an inhibitory effect on cholesterol arteriosclerosis. Duff and Payne(5) have since shown that this effect is correlated with a marked elevation of the

3. Toreson, W. E., Abstract, *Am. J. Path.*, 1950, v26, in press.

4. Duff, G. L., and McMillan, G. C., *Exp. Med.*, 1949, v89, 611.

5. Duff, G. L., and Payne, T. P. B., Abstract, *Am. Heart J.*, 1949, v38, 455.

neutral fats and phospholipid fractions in the serum.

The appearance of these deleterious effects in rabbits would seem to warrant a search for similar effects in other animal species including man.

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Alterations in Rate of Influenza Virus Proliferation Produced by Growth Hormone and Testosterone.* (17990)

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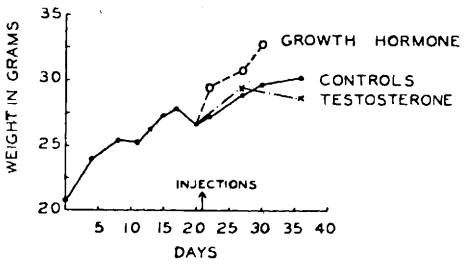
Recent studies have revealed marked differences in the susceptibility of mice of different ages to influenza virus infection. With advancing age there is a progressive increase in the amount of virus necessary to produce a lethal infection(1). These observations led to the development of the hypothesis that the rate of virus proliferation is, in part, dependent upon the rate of protein anabolism in the tissues of the host. It was, therefore, decided to study the effect on virus proliferation of deliberate alterations in the protein anabolic activity of the host. Since it is well established that the administration of pituitary growth hormone and testosterone results in increased rates of protein synthesis in the host(2,3), these substances were selected for use. The following report, then, is concerned with the rates of virus proliferation in growth hormone-treated and testosterone-treated mice as compared with appropriate controls.

Materials and methods. Female Swiss mice were employed throughout these experiments. They were obtained from the same source at the age of approximately 8 weeks and maintained in the laboratory on Purina dog chow and water *ad libitum*. The mice were weighed 3 times weekly, and, after a period of about 20 days, their weight curves "plateaued."

Both hormones were administered by subcutaneous injection. The growth hormone‡ was given for a period of 10 days, each mouse receiving 0.2 mg in a single injection per day. Testosterone‡ was given in 3 weekly injections, each mouse receiving 5.0 mg per week of a commercially prepared aqueous suspension. Control mice received appropriate injections of the vehicle in which the hormone was dissolved. The day after the last hormone injection, approximately 35-40 mice of both treated and control groups were inoculated intranasally with a 10^{-5} dilution of mouse-adapted PR 8 influenza virus. This inoculum was equivalent to approximately



THE EFFECT OF GROWTH HORMONE AND TESTOSTERONE ON THE WEIGHT OF MATURE FEMALE MICE.

FIG. 1.

* Aided by a grant from the Hendricks Research Fund.

† Submitted in partial fulfillment of the requirements for the degree of Master of Science.

1. Kalter, S. S., *J. Immunol.*, 1949, v63, 17.

2. Li, C. H., *Growth*, 1948, v12 suppl., 47.

3. Kochakian, C. D., In R. S. Harris and K. V. Thimann, Vitamins and Hormones, 1946, v4, 255.

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