

provision of the minimal daily requirements of the animal. Although mild to serious anemias have been reported to develop as a result of the infusions of the fat emulsions^{7,8} the latter complication failed to occur in the present investigation. This is believed to be due to the fact that phosphatide stabilizers were not used in the preparation of our emulsion. The disappearance of fat from the circulating blood determined either by the chylomicra count or by total serum lipids was interpreted to indicate that lipolysis commenced in the blood, the process being initiated by serum pancreatic lipase. Evidence to support the latter view was based on the increase of lipase activity of the blood and substantiated further by the report of Meng and Freeman⁹ that the fatty acids increased during the infusion of a fat emulsion. The assimilation of preformed fat infused intravenously may therefore be conceived as a possible biochemical function whereby the

neutral fat ultimately reached the liver for catabolism. An analysis of the slides revealed that in the acute experiments after an infusion all the organs were richly laden with fat. In the lung the fat did not permeate into the alveolar spaces but was contained in the capillaries. In the chronic experiments of Group II and Group III, the liver and the spleen were the only organs which contained evidences of fat.

Summary. (1) A combined emulsion of fat protein and glucose was infused intravenously in a series of 19 dogs.

(2). Toxic reactions such as secondary anemia and degenerative pathology in vital organs failed to develop after prolonged injections of the combined emulsion.

(3). An attempt was made to correlate the associated laboratory tests with the biochemical functions of hydrolysis and assimilation of fat.

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⁷ Johnson, V., Longini, J., and Freeman, L. W., *Science*, 1943, **97**, 400.

⁸ Dunham, L. J., and Brunschwig, A., *Arch. Surg.*, 1944, **48**, 395.

⁹ Meng, H. C., and Freeman, S., *J. Lab. and Clin. Med.*, 1948, **33**, 689.

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Effect of Alloxan Diabetes on Hyaluronidase Level of the Rat Testis.*

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Although hyaluronidase, an enzyme obtained from mammalian sperm and testes, can disperse follicle cells of ovulated mammalian ova, a normal role of this enzyme in

reproduction remains to be established.^{1,2} In line with the main problem of determining this role it was desired to learn if a physiological disturbance of the metabolism of the animal, without the destruction of the germinal elements, could alter the level of hyaluronidase within the testis. Experimental diabetes, therefore, was induced in male rats and after one month the amount of hyaluronidase was measured in the control and diabetic rat testes.

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¹ Chang, M. C., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 51.

² Leonard, S. L., Perlman, P. L., and Kurzrok, R., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 517.

TABLE I.
Hyaluronidase Concentration in Normal and Diabetic Rat Testis. (Turbidimetric Method).

Testes	Normal		Diabetic		Significance of Diff. of means
	No. of rats	TRU/g*	No. of rats	TRU/g	
Chilled, homogenate	10	10.1 $\pm$ 1.5†	10	10.4 $\pm$ 2.8	P = .90
Incubated, homogenate	9	46.6 $\pm$ 6.6	9	52.2 $\pm$ 17.4	P = .75
+ Sem. ves. incubated	5	68.1 $\pm$ 3.8	5	68.6 $\pm$ 4.3	P = .90

* TRU: turbidity reducing unit.

† Standard deviation.

TABLE II.
Hyaluronidase Concentration in Normal and Diabetic Rat Testes. (Rat-Ova Test).

Testes	Normal		Diabetic		Significance of Diff. of means
	No. of rats	Time to denude ova (min)	No. of rats	Time to denude ova (min)	
Chilled, homogenate	5	46 $\pm$ 8.2*	5	66 $\pm$ 15.2	P = .30
Incubated, homogenate	5	30 $\pm$ 14.6	5	24 $\pm$ 12.7	P = .75
+ Sem. ves. incubated	5	35 $\pm$ 9.3	5	46 $\pm$ 10.8	P = .65

* Standard deviation.

Methods. Adult male rats were made diabetic by injecting alloxan intraperitoneally (175 mg/kg body weight). Qualitative determinations for glycosuria in the alloxan treated rats were made. In some cases quantitative blood sugar determinations³ were performed because it was thought that hyaluronidase concentration might vary with the degree of hyperglycemia. Body weights were obtained before and after alloxan administration; after 30 days the testes of each rat were removed and assayed for hyaluronidase.

One of the two assay procedures used was the turbidimetric method as previously described for chilled testis homogenates.⁴ Since it has been reported that incubation of the testis homogenate increases the yield of the enzyme,⁵ a series of experiments was performed with the additional step of incubation. The amount of enzyme is reported as "Turbidity reducing units" (T.R.U.). The other assay procedure, the rat-ova test, was modified

from the original procedure⁶ in that comparisons were made of the time required for denudation of rat ova of their follicle cells. Saline extracts of testes from normal and diabetic rats were prepared in concentrations which were the same for both the experimental and control testes in each assay.

An additional test was included in this study. Comparisons were made in the yields of enzyme produced in the experimental and control groups when macerated seminal vesicles were mixed with the testis homogenates before extracting. It was shown that increased yields of enzyme could be obtained when the seminal vesicles were added.⁷

Results. The hyaluronidase concentration of the testes of normal and diabetic rats are presented in Tables I and II. In no case was the enzyme concentration in the testes of the experimental and control rats significantly different. When the testis homogenate was incubated before extraction an increase in enzyme concentration was obtained in both groups. In the rat ova test, the amount of extract in gram-equivalents of testis material was purposely lowered when the testis homogenate was incubated, otherwise the reaction would have proceeded so rapidly that an ac-

³ Sumner, J. B., *J. Biol. Chem.*, 1925, **65**, 393.

⁴ Leonard, S. L., Perlman, P. L., and Kurzrok, R., *Endocrinology*, 1946, **39**, 261.

⁵ Perlman, P. L., Leonard, S. L., and Kurzrok, R., *Endocrinology*, 1948, **42**, 26.

⁶ Leonard, S. L., and Kurzrok, R., *Endocrinology*, 1946, **39**, 85.

⁷ Leonard, S. L., Perlman, P. L., and Kurzrok, R., *Endocrinology*, 1947, **40**, 199.

curate comparison of the time of denudation of the ova would be impossible. With the chilled testis homogenates equivalents of .05 g of tissue were employed and with the incubated testes .01 g of tissue.

In the experiments where the seminal vesicle material augmented the yield of hyaluronidase no significant difference was observed between the amount of enzyme obtained from normal and diabetic animals (Tables I and II). The amount of enzyme present in the seminal vesicle was practically negligible. In the rat-ova test .005 g of tissue equivalent were required to denude the ova in the time indicated.

Although the diabetic rats exhibited hyperglycemia during the period of the experiments no correlation was observed between blood sugar levels and the enzyme concentrations of the testes. No histological changes were observed in the testes of the experimental rats, and there was no altered breeding per-

formance as measured by the size of the litters sired.

In contrast to these results destruction of the germinal epithelium by hypophysectomy or cryptorchidism is followed by a marked decrease in hyaluronidase levels.⁸ Whether the enzyme level of the germinal cells of the testes can be altered by physiological changes which do not destroy these germ cells remains to be shown.

Summary. 1. Alloxan diabetes in the male rat had no effect on the hyaluronidase concentration of the testes.

2. Augmentation of the yield of extractable hyaluronidase from testis homogenates by seminal vesicle homogenates was confirmed. No difference in augmentation was noted between the normal and diabetic rat testes.

⁸ Leonard, S. L., Perlman, P. L., and Kurzrok, R., *Endocrinology*, 1948, 42, 176.

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