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Serum Lipase and Alloxan Diabetes in Rats[1]

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It was found by Cantor, Tuba, and Capsey (*1*), that the production of alloxan diabetes in the adult male albino rat is paralleled by a pronounced increase in the activity of serum alkaline phosphatase. The development of the diabetic condition is usually accompanied by a marked transient lipemia, which appears about two days after the injection of alloxan and which then decreases after approximately a week. It seemed probable that the existence for several days of a severe lipemia might be reflected in altered serum lipolytic activity, and this paper presents our observations with the enzyme which hydrolyzes tributyrin. This enzyme, given a variety of names in the literature, will be called lipase, although it could equally well be called tributyrinase (*2*).

Twenty adult male rats were starved overnight and in the morning each was given a subcutaneous injection of 16 mg of alloxan monohydrate for each 100 g of body weight. The animals were fed Purina Fox Checkers and water ad lib. As a result of alloxan injection, diabetes was produced in fifteen rats; three of the animals died, while two recovered from the initial hyperglycemia.

Blood for glucose and lipase determinations was taken from the tails of the animals always at 8 A.M. Blood glucose was estimated by the method of Reinecke (*3*). Lipase activity was determined within 24 hr after the serum was obtained although the enzyme was found to be stable for several days in a refrigerator. The enzyme concentration of the sera was determined by a micromethod which will be described fully elsewhere. Essentially it consists of estimating by titration with NaOH the acid set free from tributyrin by the enzyme contained in 0.1 ml serum. Hydrolysis of the finely dispersed substrate proceeds for 30 min at 37° C in an aqueous digest buffered with sodium diethylbarbiturate at pH 7.9. The lipase activity of a serum in units is equivalent to the number of ml of 0.025 N NaOH required to neutralize the butyric acid which would be set free by the enzyme contained in 100 ml of serum under the above experimental conditions. One ml of 0.025 N NaOH is equivalent to one lipase unit. The normal mean value for serum lipase in 30 adult male albino rats was found to be 556 ± 70/100 ml, with a range from 390–690 units/100 ml. Activity of the enzyme in individual rats varies slightly from day to day, but levels are not appreciably altered by starvation for 5 days.

The effect on serum lipase activity of alloxan is indicated in Table 1. Results for the group of 15 diabetic rats are available for 7 days after the onset of hyperglycemia and this information is supplemented for longer periods of time as indicated in Table 1.

TABLE 1

EFFECT OF ALLOXAN ON SERUM LIPASE OF ADULT MALE RATS

Time after alloxan injection	Blood sugar (mg/100 ml)		Serum lipase (units/100 ml)	
	mean	range	mean	range
0	117	90–120	556 ± 70*	390– 690
1 day	369	315–450	536 ± 102	320– 720
3 days	544	230–800	541 ± 80	410– 750
5 days	434	260–670	686 ± 105	510– 890
7 days	429	314–630	800 ± 67	690– 880
21 days†	426	400–450	830 ± 92	760–1000
3 months‡	350	250–400	930 ± 238	930–1400

* The values following the ± sign represent the standard deviation.

† These values were obtained from seven of the group of 15 rats.

‡ These values were obtained from a group of five animals for which there are no normal values.

Blood sugars reach diabetic values within the first day in the usual way, but the effect on lipase follows a different pattern. Almost without exception, individual rats showed a temporary fall in serum lipase levels between the first and third day after injection with alloxan. By the end of the seventh day, however, there was in every diabetic animal a definite increase of the enzyme, with an average increase to about 40–45% above normal levels for the group of 15 rats. The results given in Table 1 for rats diabetic for periods of time up to 3 months indicate that the enhancement in lipase activity of rat serum is persistent and may reach concentrations as much as 65% above normal. The development of increased lipase levels roughly parallels the appearance of high alkaline phosphatase values in alloxan diabetic rats.

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