Effects of Reserpine on Hepatic Glycogen and Plasma Free Fatty Acids of Thyroidectomized-Adrenodemedullated Rats.* (30771)

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Rats deprived of both their thyroid and adrenal medullas are unable to maintain body temperature or basal metabolic rate at normal levels after treatment with reserpine, and they succumb within 1 to 6 days(1). If either the thyroid or adrenal medulla remains intact or if animals without thyroid or adrenal medullas are treated with triiodothyronine, they do not succumb. The effects of reserpine on thyroidectomized-adrenodemedullated rats can be prevented by increasing the environmental temperature to 30°C(2). Pretreatment with reserpine has been shown to increase the oxygen consumption of heart and liver slices to a small extent when measured *in vitro,* indicating that cellular metabolic processes have not been directly depressed by the drug(2). A clue to the possible mechanism for these effects of reserpine arose from the observations that the hepatic glycogen content was increased in the reserpine-treated animals(2,13). This suggested that the inability of animals treated with reserpine to maintain body temperature might result from an impaired capacity to mobilize stored forms of energy. These experiments were carried out to evaluate the effect of reserpine on concentration of hepatic glycogen and on concentration of plasma free fatty acids in fasted rats after ablation of various endocrine organs.

Methods and materials. Animals. Hypophysectomized rats weighing 90-100 g were obtained from Charles River Laboratories and showed no gain in weight while maintained in the laboratory. One group of Charles River rats (adrenalectomy I) weighing 250-300 g and one group of Holtzman rats (adrenalectomy II) weighing 100-120 g were adrenalectomized and maintained on 1% sodium chloride in their drinking water for 5 days prior to the experiments. Thyroidectomy, adrenodemedullation, or the combined operation was performed on 100-200 g male Holtzman rats as previously described(1). All animals, including unoperated rats from Holtzman and Charles River, were maintained on Purina Lab Chow and tap water *ad libitum,* in a room with temperature maintained at 18°C.

Procedure. Each experiment lasted 6 days during which time all groups except "adrenalectomy I" were maintained at 30°C. This was found necessary to prevent fatalities among the reserpine-treated thyroidectomized-adrenodemedullated rats(2). The group "adrenalectomy I" was maintained at 18°C during the experiment. Rats received 25 μg of reserpine/100 g B. W. intramuscularly on the first and third day and 50 μg of reserpine/100 g B. W. on the fifth day. Half of the adrenalectomized rats in each group received 1.0 mg of cortisone/100 g B. W. daily during the experiment. Controls received an equivalent volume of the vehicle, which was either physiological saline (cortisone vehicle) or a 1:1 mixture of physiological saline and propylene glycol (reserpine vehicle). On the fifth day food was removed at 5:00 p. m. After an overnight fast, rats were anesthetized with ether, and blood was drawn from the abdominal aorta into heparinized syringes. 100 to 200 mg of liver were quickly removed, weighed and added to 30% KOH in a boiling water bath. The glycogen was precipitated with 95% alcohol and the residue quantitated by the method of Kahan(3). Plasma free fatty acids were determined on 0.5 ml aliquots of plasma by the method of Dole(4) and plasma glucose was determined by the glucose oxidase method. Free fatty acid release from epididymal fat pads was determined in Krebs-Ringer-bicarbonate buffer containing 4% bovine albumin (Armour) as previously described(5). Tissues were incu-

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TABLE I. Effect of Reserpine on Hepatic Glycogen of Rats.

Operative state	No.	Vehicle treated	No.	Reserpine treated	P
		Hepatic glycogen (μg/mg)			
Normal					
Fed	7	68.2 ± 4.6*	8	38.5 ± 5.2	<.01
Fasted	7	.98 ± .4	8	20.6 ± 10	=.05
Thyroidectomized					
Fasted	8	.46 ± .11	9	10.5 ± 2.8	<.01
Thyroidectomized-adrenodemedullated					
Fasted	10	.73 ± .41	9	5.5 ± 1.6	<.01
"	7	.35 ± .46	6	6.8 ± 1.4	<.001
Adrenodemedullated					
Fasted	4	.44 ± .06	5	6.1 ± .93	<.001
Hypophysectomized					
Fasted	10	.15 ± .012	10	.24 ± .016	<.001
Adrenalectomized (I)					
Fasted					
Cortisone	7	3.0 ± .64	6	7.1 ± 2.3	N.S.
Vehicle	7	.23 ± .012	7	.56 ± .13	<.05
Adrenalectomized (II)					
Fasted					
Cortisone	8	3.9 ± 1.4	7	4.2 ± 1.6	N.S.
Vehicle	8	.17 ± .003			

* Mean ± S.E.M.

All rats with exception of the adrenalectomy group I (kept at 18°C) were maintained at 30°C for 5 days during which time reserpine was given on the 1st and 3rd days (25 μg/100 g) and on the 5th day (50 μg/100 g). Statistical comparison is between corresponding vehicle- and reserpine-treated groups.

bated in the presence of glucose (1 mg/ml) and all values are expressed as μEq/g/hr.

All data have been expressed as the mean ± standard error of the mean and statistical comparison has been made by using Student's "t" test. The data from 2 experiments on thyroidectomized-adrenodemedullated rats and 2 experiments on adrenodemedullated rats have been tabulated separately.

Results. Table I shows the effect of reserpine on hepatic glycogen content in 11 groups of rats. Reserpine increased hepatic glycogen in all groups except the normal, fed rats and the cortisone-treated, adrenalectomized rats. In the thyroidectomized, adrenodemedullated and thyroidectomized-adrenodemedullated animals this difference was more than 7-fold. This effect of reserpine was reduced by hypophysectomy or adrenalectomy, but not completely abolished.

The effects of fasting and reserpine on the level of blood sugar are shown in Table II. The high values for blood sugar in the fed normal rats may have resulted from the increased ambient temperature at which the rats were maintained. Reserpine significantly increased glucose concentration in thyroidectomized, adrenodemedullated and thyroidectomized-adrenodemedullated rats, but not in the other groups. Levels of blood glucose were lowest in the hypophysectomized rats and the adrenalectomized rats which had received no cortisone.

Tables III and IV show the effect of reserpine on the plasma free fatty acids and on the release of free fatty acids from adipose tissue incubated *in vitro*. Reserpine significantly reduced the level of plasma free fatty acids in thyroidectomized-adrenodemedullated and adrenodemedullated rats but was without effect in any of the other groups. The adipose tissue from the adrenodemedullated rats pretreated with reserpine released significantly less free fatty acids into the medium than their controls. The mean release of the thyroidectomized-adrenodemedullated rats pretreated with reserpine was likewise less than their vehicle-treated controls but due to one high value this difference was not statistically significant. Reserpine was without effect on the release of free fatty acids from adipose tissue of thyroidectomized, hypophysectomized or normal rats.

Discussion. The fact that the levels of hepatic glycogen in thyroidectomized, normal and thyroidectomized-adrenodemedullated rats were increased to a similar extent by pretreatment with reserpine would suggest that the effect of reserpine on survival of animals without their thyroid and adrenal medullas was not related to the effects on glycogen. These studies have, however, clearly demonstrated

TABLE II. Effect of Reserpine on Blood Glucose.

Blood glucose (mg %)

Operative status	No.	Vehicle treated	No.	Reserpine treated	P
Normal					
Fed	7	177 ± 4*	6	174 ± 5	N.S.
Fasted	5	118 ± 4	6	132 ± 6	N.S.
Thyroidectomized					
Fasted	9	98 ± 10	8	160 ± 7	<.001
Thyroidectomized-adrenodemedullated					
Fasted	10	95 ± 4	9	114 ± 8	<.05
Adrenodemedullated					
Fasted	4	113 ± 7	5	149 ± 4	<.01
Hypophysectomized					
Fasted	10	60 ± 3	10	56 ± 6	N.S.
Adrenalectomized (II)					
Fasted					
Cortisone	8	98 ± 10	6	107 ± 11	N.S.
Vehicle	8	56 ± 3			

* Mean ± S.E.M.

All rats in this experiment were maintained at 30°C for 5 days during which time reserpine was given on the 1st and 3rd days (25 μg/100 g) and on the 5th day (50 μg/100 g). Statistical comparison is between corresponding vehicle- and reserpine-treated groups.

TABLE III. Effect of Reserpine on Plasma Free Fatty Acids.

Plasma Free Fatty Acids (μEq/l)

Operative status	No.	Vehicle treated	No.	Reserpine treated	P
Normal					
Fed	4	269 ± 27*	4	312 ± 17	N.S.
Fasted	4	715 ± 66	4	640 ± 54	N.S.
Thyroidectomized					
Fasted	9	687 ± 91	8	639 ± 28	N.S.
Thyroidectomized-adrenodemedullated					
Fasted	9	661 ± 51	9	438 ± 30	<.01
Fasted	4	688 ± 63	8	482 ± 51	<.05
Adrenodemedullated					
Fasted	4	639 ± 86	4	386 ± 32	<.02
Fasted	7	746 ± 32	7	412 ± 24	<.001
Hypophysectomized					
Fasted	9	726 ± 48	10	649 ± 22	N.S.
Adrenalectomized (II)					
Fasted					
Cortisone	8	829 ± 77	7	818 ± 48	N.S.
Vehicle	8	726 ± 57			

* Mean ± S.E.M.

All rats in this experiment were maintained at 30°C for 5 days during which time reserpine was given on the 1st and 3rd days (25μg/100 g) and on the 5th day (50 μg/100 g). Statistical comparison is between corresponding vehicle- and reserpine-treated groups.

TABLE IV. Effect of Pretreatment with Reserpine on Release of Free Fatty Acids from Epididymal Fat Pads *in vitro*.

Free fatty acids in medium (μEq g^{-1} h^{-1})

Operative status	No.	Vehicle treated	No.	Reserpine treated	P
Normal	3	.66	3	.49	—
Thyroidectomized	6	−.11 ± .19*	7	−.08 ± .18	N.S.
Thyroidectomized-adrenodemedullated	7	.41 ± .19	5	−.14 ± .22†	N.S.
Adrenodemedullated	4	.22 ± .15	5	−.22 ± .18	<.05
Hypophysectomized	6	−.47 ± .10	6	−.33 ± .08	N.S.

* Mean ± S.E.M.

† Individual values (−.09, −.31, −.28, −.67, +.65).

All rats in this experiment were maintained at 30°C for 5 days during which time reserpine was given on the 1st and 3rd days (25 μg/100 g) and on the 5th day (50 μg/100 g). All rats were fasted for 18 hours after the last dose of reserpine. Statistical comparison is between corresponding vehicle- and reserpine-treated groups.

that the increase in hepatic glycogen of rats pretreated with reserpine is dependent upon an intact pituitary-adrenal axis. Both adrenalectomy and hypophysectomy markedly reduced the effect of reserpine on hepatic glycogen. Moreover, adrenalectomized rats pretreated with cortisone showed little or no effect of reserpine on their hepatic glycogen. This implies that the effects of reserpine on hepatic glycogen may be predominantly mediated through a release of corticosteroids from the adrenal gland. Such an interpretation of the data is in accord with a considerable body of experimental data which shows that reserpine can deplete pituitary corticotropin(5-7), deplete adrenal ascorbic acid and raise the plasma concentration of corticosterone(8). In addition, however, reserpine would appear to produce a small but significant increase in hepatic glycogen of fasted hypophysectomized and adrenalectomized rats. The basis for this effect is unknown. In extensive studies on the effect of reserpine on glycogen, Balzer and Palm(9) demonstrated an increase in concentration of glycogen in liver, heart and brain and showed an increase in rate of incorporation of radioactivity from C^{14}-alanine into glycogen. However, it is difficult to know whether their data result from a direct action of reserpine on gluconeogenesis or are the result of stimulating gluconeogenesis through the release of adrenal steroids since they used animals with intact adrenal glands.

The plasma levels of free fatty acids were lower in adrenodemedullated or thyroidectomized-adrenodemedullated rats after an 18-hour fast than in any of the other groups studied. This decrease in free fatty acid concentration in the plasma could result from a decrease in mobilization or a decrease in peripheral utilization of fatty acids or a combination of both. In previous studies pretreatment with reserpine did not change the oxygen consumption of diaphragm when incubated *in vitro* with palmitate as the substrate(2). This would suggest that the depressed concentration of free fatty acids resulted from decreased mobilization from adipose tissue stores. This interpretation is strengthened by the experiments of Edmonson and Goodman(10), who showed that reserpine can reduce the release of free fatty acids from adipose tissue of normal rats, and by the *in vitro* experiments presented here. These authors also observed higher blood sugar concentration in rats pretreated with reserpine and suggested the decreased release of free fatty acids resulted from the inhibitory effects of increased blood sugar and insulin on lipolysis. This interpretation is not supported by the present data. Levels of blood glucose were significantly increased in both the thyroidectomized and adrenodemedullated rats pretreated with reserpine, but only in the adrenodemedullated group was the release of free fatty acids decreased.

The present experiments were conducted at 30°C since previous observations had shown that reserpine was lethal to thyroidectomized-adrenodemedullated rats at room temperature(2). The defect in mobilization of free fatty acids was demonstrated in all groups of adrenodemedullated rats whether they had thyroid glands or not, yet it is only the thyroidectomized-adrenodemedullated rats that succumb to reserpine. Indeed, Stern and Maickel(11) failed to show any effect of reserpine on plasma free fatty acids of adrenodemedullated rats housed at room temperature. Thus, temperature and thyroid status both play an important role in the response to reserpine. An explanation for the effect of thyroidectomy in the adrenodemedullated rat may come from the observation in several laboratories that thyroidectomy significantly reduces the lipolytic effect of epinephrine on adipose tissue(12,13). The fact that adrenodemedullated rats with intact thyroid glands survive pretreatment with reserpine while thyroidectomized-adrenodemedullated rats died may reflect the relatively severe impairment in the latter of the capacity to mobilize free fatty acids. The similarity of response of adrenodemedullated rats with and without thyroid glands to reserpine at 30° may well reflect the fact that as the temperature rises, thyroid function is diminished (14). At 30° the adrenodemedullated rats may be behaving like functionally thyroidectomized rats.

Summary. Reserpine increased hepatic glycogen in normal, hypothyroid, and adrenodemedullated rats but not in adrenalectomized or hypophysectomized rats. The elevation in plasma free fatty acids in fasted rats was impaired in reserpinized rats without adrenal medullas.

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Development and Growth of L Forms of Bacteria and PPLO on Membrane Filters.* (30772)

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Dr. J. R. Ward of Salt Lake City and Dr. C. W. Molander of Los Angeles called to our attention that the L forms of bacteria grow well on Millipore® membrane filters placed on appropriate solid media. Molander *et al* found that L cultures of staphylococcus grow through filters with pore sizes given by the manufacturer as 0.1 μ and 0.05 μ(1). We confirmed these observations and also observed that L forms develop from the bacteria on the filters. Thus far the development of L type growth from bacteria has been observed only on media solidified with agar, and not, with rare exceptions, on solid media of other type or in liquid media. The study of the growth in membrane filters

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