Effects of Insulin on Excretion of Nitrogen in Normal, Depancreatized, and Steroid-Diabetic Rats

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SUMMARY

Sexually mature male rats in metabolism cages were adapted to the tube-feeding of a medium carbohydrate diet. In experiment 1, intact rats were fasted for 96 hrs. Injection of glucagon-free insulin caused an increase in urinary NPN. Cortisone caused a greater loss of NPN which was not significantly changed by addition of insulin. In experiment 2, intact tube-fed rats excreted amounts of NPN and glucose increased in proportion to the dose of cortisone. Glycosuria but not nitrogen loss was suppressed by insulin. In experiment 3, the spontaneous glycosuria of partially depancreatized rats was suppressed by insulin and the elevated urinary NPN was also suppressed. When the glycosuria of partially depancreatized rats was induced or aggravated by cortisone it could be suppressed by insulin but the level of urinary NPN remained high. DIABETES *16*:18-20, **January, 1967.**

These data confirm and extend earlier studies showing that insulin fails to suppress the catabolic effect of cortisone in either fasting or tube-fed intact rats or in tube-fed partially depancreatized rats. Although insulin will suppress the increase in urinary nonprotein nitrogen (NPN) in rats with pancreatic diabetes it fails to suppress the increase in urinary NPN accompanying steroid diabetes even when the glycosuria is decreased. Overdosage with insulin can cause increased nitrogen excretion.

METHODS

Male rats of the Sprague-Dawley strain at a weight of approximately 300 gm. were placed in metabolism cages. Some had been partially depancreatized at 250 to 260 gm. All animals were adapted to the tube-feeding of a medium carbohydrate diet[2] each morning and late afternoon (26 cc. per rat per day). Twenty-four-hour samples of urine (preserved with toluene and citric acid, 1 gm./sample) were collected each morning just prior to feeding. Urine glucose was determined by the method of Shaffer and Williams[3] and nonprotein nitrogen by the micro-Kjeldahl procedure.[4] The room temperature was 23 to 27° C. and humidity was approximately 50 per cent. Cortisone acetate (Upjohn) and glucagon-free insulin (Lilly) were injected subcutaneously in divided doses each morning and late afternoon just prior to the time of feeding. The rats were disease-free. Rats with diarrhea and those which had irregular values for urinary NPN during preliminary observations were not used.

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EXPERIMENTS AND RESULTS

Experiment 1 involved eighty-four intact rats which were adapted to tube-feeding and then fasted for 96 hrs. Hormone dosages and data on urinary NPN for each twenty-four-hour period are in table 1. None of the fasting rats excreted glucose during the administration of cortisone. Insulin at 0.1 U. daily caused some rise in urinary NPN and 1 U. daily caused a greater rise. Cortisone at 2 mg. daily caused a marked rise in urinary NPN and this was facilitated, not suppressed, by 1 U. insulin daily. Cortisone at 7 mg. daily caused a still greater loss of NPN and there was no significant effect of adding 1 U. insulin daily.

Experiment 2 (table 2) involved 180 intact, tube-fed rats given cortisone with and without insulin for seven days. Since there is a latent period of two days before the response to cortisone begins to reach a peak, the mean values for urinary glucose and NPN are based on the third to seventh days of response to the hormones. Rats given insulin without cortisone showed a small increase in urinary NPN. Cortisone at 1 mg. daily caused a small rise in urinary NPN which was aggravated by the addition of 10 U. and 50 U. insulin daily. None of these rats excreted glucose. When the dose of cortisone was 5 mg. daily, all of the rats excreted glucose and greater amounts of NPN. Similar rats given 5 mg. of cortisone with 10 and 50 U. insulin daily showed suppression of glycosuria without suppression of nitrogen loss. The administration of 10 mg. of cortisone

TABLE 1

Urinary NPN of normal fasted male rats treated with cortisone acetate and with glucagon-free insulin. Twelve rats per each of seven groups

Hours of fast	Cortisone (mg./24 hr.)	Insulin (U./24 hr.)	Urine NPN mg./24 hr. (mean and standard error)
0-24	0	0	205.0± 4.9
24-48	0	0	161.7± 7.2
48-72	0	0	166.4± 8.7
72-96	0	0	179.6±11.1
0-24	0	0.1	202.5± 6.2
24-48	0	0.1	184.0± 7.9
48-72	0	0.1	183.6± 9.9
72-96	0	0.1	186.8±12.9
0-24	0	1.0	210.5± 4.8
24-48	0	1.0	277.7± 8.1
48-72	0	1.0	295.3± 9.6
72-96	0	1.0	319.3±13.4
0-24	2	0	241.1± 4.3
24-48	2	0	236.9± 6.4
48-72	2	0	298.0± 9.0
72-96	2	0	348.2±10.7
0-24	2	1	256.9± 5.1
24-48	2	1	290.8± 8.1
48-72	2	1	335.8±13.9
72-96	2	1	374.3±17.6
0-24	7	0	265.0±11.3
24-48	7	0	299.7±11.9
48-72	7	0	347.7±15.0
72-96	7	0	362.5±12.8
0-24	7	1	260.1± 7.7
24-48	7	1	301.3± 9.2
48-72	7	1	352.9±17.1
72-96	7	1	364.4±12.7

daily caused a moderate glycosuria with greater loss of NPN. The glycosuria but not the nitrogen loss was reduced by treatment with insulin. Cortisone at 15 mg. daily caused a severe glycosuria which was markedly reduced but not abolished by 50 U. insulin daily. There was no significant reduction in the markedly elevated values for urinary NPN.

Experiment 3 (table 3) involved thirty-three partially depancreatized, tube-fed rats. The animals of groups 1 and 3 were selected as having a mild but stable glycosuria, greater in group 1 than in group 3. Each experimental period was seven days, but the data in the table represent the third to seventh days of response for each period as in Experiment 2. In group 1 the glycosuria of the rats was almost abolished by 10 U. insulin daily and there was suppression of the urinary NPN to the normal range. The partially depancreatized rats of group 2 had no spontaneous glycosuria but developed mild glycosuria with increased urinary NPN when given 5 mg. of cortisone daily. Treatment with

TABLE 2

Urinary NPN and glucose of normal force-fed male rats treated with cortisone acetate and glucagon-free insulin. Means and standard errors based on average daily excretion for individual rats during the third to seventh day of injections for seventeen groups

Number of rats	Cortisone (mg./ 24 hr.)	Insulin (U./ 24 hr.)	NPN (mg./ 24 hr.)	Glucose (mg./ 24 hr.)
10	0	0	260± 5.3	0
10	0	1	270± 6.4	0
11	0	10	270± 7.0	0
12	0	50	281±10.1	0
10	1	0	276± 6.7	0
10	1	1	273± 6.9	0
10	1	10	296± 8.2	0
10	1	50	315±12.6	0
11	5	0	367± 9.1	2,163±470
10	5	1	373± 8.6	1,995±395
10	5	10	359±10.7	530±230
10	5	50	361±11.0	260± 92
12	10	0	390±12.2	4,465±577
12	10	10	411±12.8	110± 90
12	10	50	396± 9.9	283±147
10	15	0	481±12.4	8,035±958
10	15	50	469±14.7	1,456±123

TABLE 3

Urinary NPN and glucose of partially-depancreatized, tube-fed male rats treated with cortisone and glucagon-free insulin. Means and standard errors based on average daily values for five-day experimental periods beginning two days after starting injections

Group	Number of rats	Cortisone (mg./ 24 hr.)	Insulin (U./ 24 hr.)	NPN (mg./ 24 hr.)	Glucose (mg./ 24 hr.)
1	10	0	0	304±11.2	2,028±271
		0	10	251± 5.2	70± 48
2*	11	0	0	267± 7.0	0
		5	0	386±11.5	2,265±563
		5	10	399±12.0	1,315±509
		5	50	422±15.4	20± 37
3	12	0	0	278± 4.8	930±187
		1	0	303± 9.1	1,830±360
		1	1	312±10.6	1,440±274
		1	10	306±10.2	870±211
		1	30	301±13.7	27± 43
		2	0	354±16.6	2,076±425
		2	10	382±14.1	1,220±403
		2	30	348± 8.9	34± 56

*No spontaneous glycosuria.

insulin suppressed the glycosuria but not the level of urinary NPN. The rats of group 3 each had a very mild spontaneous glycosuria which was exacerbated by cortisone. Treatment with insulin reduced the glycosuria but not the elevated level of urinary NPN.

DISCUSSION

Under many laboratory conditions insulin stimulates or supports the synthesis of protein[5] and insulin deficiency is characterized by an increased excretion of NPN. The data on group 1 in table 3 illustrate the increased loss of NPN during pancreatic diabetes and suppression of the rise by insulin.

An excess of insulin, especially during fasting, can cause a rise in urinary NPN rather than a decrease. The catabolic effect of insulin may be secondary to hypoglycemia but this is uncertain. The possibility that physiologically important amounts of glucagon remain in "glucagon-free" insulin must be considered. Insulin did not suppress the rise in urinary NPN induced by cortisone even when it suppressed glycosuria and, under some conditions, it aggravated the loss of nitrogen. These data support and extend the studies of Ingle et al.[6] and further illustrate the well documented fact[5] that the effects of insulin upon nitrogen balance are not always clearly related to changes in the utilization of glucose. The data of experiments 2 and 3 also illustrate the fact that rats with steroid diabetes are more resistant than are rats with pancreatic diabetes to the effect of insulin on the level of urinary glucose.

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Glaucoma and Phenylthiourea Taste Sensitivity

Glaucoma is a disease resulting in an increase in intraocular pressure to levels which are pathologic. It is true that there is a statistical distribution of intraocular pressures in normal eyes resulting in an overlap with some cases of glaucoma.

There are other characteristics of the glaucomatous eye, however, that usually permit a diagnosis to be made. The finding of a loss of visual field accompanied by cupping of the optic disc is supporting evidence that intraocular pressures have resulted in pathologic changes.

Glaucomatous eyes also show an impairment of outflow facility. A valuable provocative test involves the measurement of intraocular pressure after the consumption of one liter of water. Normal eyes show essentially no rise in pressure, whereas many glaucomatous eyes have a rise of 8 mm. Hg or more (W. Leydhecker, in *Glaucoma,* p. 205, W. S. Duke-Elder, Editor, Thomas Company, Springfield, Illinois, 1955).

Early detection with treatment is so successful in the prevention of blindness that much publicity is being directed toward urging persons, especially those over forty, to have their intraocular pressure tested. The aim is to discover the abnormality while the patient is still in the preclinical state. Of aid in these surveys has been the confirmation that glaucoma is frequently an hereditary condition.

Studies have demonstrated the high incidence of glaucoma in the relatives of patients with the condition (L. Kellerman and A. Posner, *Amer. J. Ophth.* 40:681, 1955). From 13 to 25 per cent of glaucoma patients have a family history of glaucoma. Most pedigrees demonstrate a pattern compatible with dominant heredity, although it is also likely that some glaucomas are transmitted as a recessive characteristic.

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