Effects of Cortisone and Insulin on Tumor Weights, Urinary Glucose and Nitrogen in Rats.* (32004)

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Walker Carcinoma 256 grows at a subnormal rate in rats made severely diabetic by pancreatectomy, but the growth of the tumor can be normalized by treatment with insulin(1). The present study has tested the possibility that insulin will normalize the subnormal tumor growth, elevated nitrogen excretion and other signs of hypercorticalism in intact rats having steroid diabetes induced by cortisone. The data show that only glycosuria was suppressed; the other changes from normal were not corrected by insulin.

Methods. Male rats (300 g) of the Sprague-Dawley strain were placed in metabolism cages and adapted to the tube-feeding of a medium carbohydrate diet(2) (26 ml per rat per day) each morning and late afternoon. Twenty-four-hour samples of urine (preserved with toluene and citric acid, 1 g per sample) were collected each early morning just prior to feeding. Urinary nitrogen and glucose were determined with the Technicon AutoAnalyzer. This requires measuring the creatinine and uric acid of urine and subtracting the values of these reducing substances from total reducing substances to give approximate values for glucose. The room T was 23-27°C and humidity was approximately 50%. Cortisone acetate (Upjohn) and glucagon-free insulin (Lilly) were injected subcutaneously in divided doses each morning and late afternoon. The rats were disease-free. Sterile undiluted homogenates of either Walker Carcinoma 256 or Jensen Sarcoma were injected into the upper thigh of each hind leg. At the end of the experiment each rat was exsanguinated under deep ether anesthesia and tumor, adrenals, thymus and spleen were weighed.

Experiments and results. Experiments 1, 2, 3 and 4 (Fig. 1, 2, 3, 4) involved rats given cortisone and insulin, separately and in combination and with and without implantation of Jensen Sarcoma. All rats had a control period of 10 days, but data for 3 days only are given in the figures. Standard errors were calculated for all averages, but only those for

TABLE I. Organ Weights (mg) from Experiments 2 and 3. Averages and standard errors.

Treatment	Exp	Thymus	Spleen	2 adrenals
No tumor, no treatment	2	489 ±30	667 ± 35	56 ± 2.1
" " , 10 u insulin daily		433 ±62	577 ± 34	54 ± 3.9
" " , 10 mg cortisone daily		26 ± 3.3	288 ± 12	25 ± 2.8
" " , 10 mg cortisone + 10 u insulin daily		29 ± 4.5	304 ± 20	29 ± 1.7
Tumor, no treatment		192 ±23	4113 ±282	241 ±35
" , 10 u insulin daily		251 ±30	3203 ±171	286 ±23
" , 10 mg cortisone daily		27 ± 6	1063 ± 86	34 ± 2.3
" , 10 mg cortisone + 10 u insulin daily		44 ± 3.1	1650 ±149	43 ± 4.2
No tumor, no treatment	3	455 ±34	590 ± 29	53 ± 2.5
" " , 20 u insulin daily		307 ±42	573 ± 36	60 ± 2.9
" " , 10 mg cortisone daily		32 ± 3.4	316 ± 22	29 ± 2.0
" " , 10 mg cortisone + 20 u insulin daily		30 ± 3.8	259 ± 68	26 ± 2.1
Tumor, no treatment		201 ±27	3720 ±253	203 ±26
" , 20 u insulin daily		156 ±24	3500 ±156	167 ±25
" , 10 mg cortisone daily		28 ± 4.2	1048 ±137	33 ± 2.7
" , 10 mg cortisone + 20 u insulin daily		34 ± 2.8	1047 ± 98	38 ± 2.5

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tumor weights are included in the figures.

Urinary glucose. Glycosuria was induced in amounts proportional to the dose of cortisone. The glycosuria was markedly reduced by injection of insulin, a divided dose of 10 units daily having a maximal effect. The glycosuria was also reduced by the growing tumor as first noted by David Ingle(3).

Urinary nitrogen. There was a tendency for urinary nitrogen to increase slightly for a few days following implantation of the tumor or during the administration of insulin. Cortisone caused a marked increase in urinary nitrogen that was not suppressed by insulin

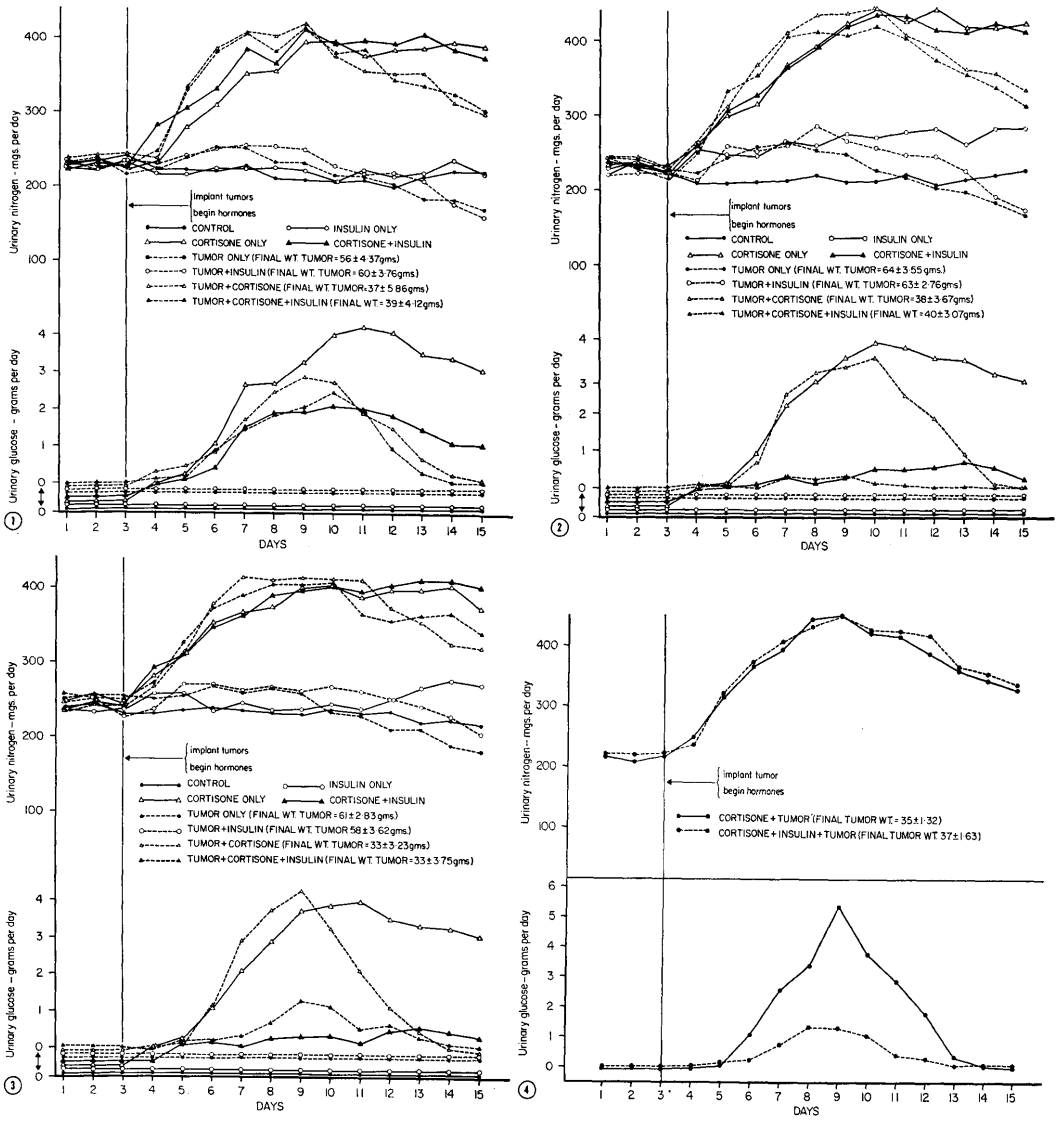

FIG. 1. Urine glucose and nitrogen and weights of Jensen sarcoma in intact tube-fed rats. Effects of cortisone and insulin. Averages for 6 rats per group.

FIG. 2. Urine glucose and nitrogen and weights of Jensen sarcoma in intact tube-fed rats. Effects of cortisone and insulin. Averages for 6 rats per group.

FIG. 3. Urine glucose and nitrogen and weights of Jensen sarcoma in intact tube-fed rats. Effects of cortisone and insulin. Averages for 6 rats per group.

FIG. 4. Urine glucose and nitrogen and weights of Jensen sarcoma in intact tube-fed rats. Effects of cortisone and insulin. Averages for 18 rats per group.

TABLE II. Urine Glucose and Nitrogen and Weights of Walker Carcinoma 256 in Intact Tube-Fed Rats. Effects of cortisone and insulin. Averages.

Exp	Group	No. rats		Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
5	1	13	Cortisone, mg	0	0	10	10	10	10	10	10	10	10	10	10	10	10	
			Urine glucose, mg	0	0	0	0	218	404	932	1647	2681	2083	1816	1234	581	365	349
			Urine N, mg	250	245	252	282	362	402	418	415	415	414	412	398	396	349	316
			Tumor wt, g															40±3.84
	2	13	Cortisone, mg	0	0	10	10	10	10	10	10	10	10	10	10	10	10	
			Insulin, units	0	0	10	10	10	10	10	10	10	10	10	10	10	10	
			Urine glucose, mg	0	0	0	0	75	351	267	315	378	322	221	165	140	38	44
			Urine N, mg	243	250	253	317	382	432	434	418	416	392	385	374	366	323	289
			Tumor wt, g															46±2.96
6	1	12	Cortisone, mg	0	0	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	
			Urine glucose, mg	0	0	0	0	88	916	1996	3130	3770	3593	2741	1423	533	193	47
			Urine N, mg	251	254	258	291	372	422	426	450	446	454	412	424	397	374	325
			Tumor wt, g															32±2.56
	2	11	Cortisone, mg	0	0	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	
			Insulin, units	0	0	10	10	10	10	10	10	10	10	10	10	10	10	
			Urine glucose, mg	0	0	0	0	49	190	396	835	1030	632	314	310	143	98	41
			Urine N, mg	252	262	257	289	377	445	436	440	428	419	422	411	394	355	305
			Tumor wt, g															37±2.29
7	1	16	Cortisone, mg	0	0	15	15	15	15	15	15	15	15	15	15			
			Urine glucose, mg	0	0	0	0	533	1220	4826	5795	5500	4795	4187	2450	1574		
			Urine N, mg	249	242	242	277	348	389	429	479	477	480	471	465	411		
			Tumor wt, g													19±2.74		
	2	17	Cortisone, mg	0	0	15	15	15	15	15	15	15	15	15	15			
			Insulin, units	0	0	20	20	20	20	20	20	20	20	20	20			
			Urine glucose, mg	0	0	0	0	188	250	666	1082	775	672	471	373	159		
			Urine N, mg	255	253	254	295	362	396	420	456	465	466	450	449	412		
			Tumor wt, g													17±1.65		

in either the presence or absence of tumor. The tumor caused some suppression of urinary nitrogen toward the end of the experimental period. Calculation of standard errors of meaus and of differences in mean values between groups with and without insulin show that insulin failed to significantly suppress urinary nitrogen.

Tumor weights. Cortisone significantly inhibited the growth of the tumor, but insulin failed to significantly affect the average weight of tumors in the presence or absence of cortisone.

Organ weights. Data on weights of adrenals, thymus and spleen illustrate the well-known atrophy of the adrenal cortices, thymus and spleen during administration of cortisone and the hypertrophy of adrenal cortices and spleen and some regression of thymus in tumor hosts. The hypertrophy of adrenal cortices in tumor hosts is blocked and hypertrophy of spleen is partially blocked by treatment with cortisone. The administration of insulin did not significantly affect weights of these organs under conditions studied here. The data in Table I are from Experiments 2 and 3.

Experiments 5, 6 and 7 were identical in design to those described above except that Walker Carcinoma 256 was used. The results (Table II) are very similar to those obtained with Jensen Sarcoma; insulin failed to normalize tumor growth or to suppress nitrogen loss in rats with or without cortisone.

Discussion. The changes from normal in metabolism and growth in animals with pancreatic diabetes are corrected by treatment with insulin. Steroid diabetes is different. Although glycosuria of steroid diabetes in rats is not abolished by treatment with insulin, it is markedly reduced. Insulin either fails to suppress the abnormally high level of urinary nitrogen in rats with steroid diabetes or the effect is small. Insulin does not prevent the effects of cortisone over-dosage on adrenals, thymus and spleen. Similarly, insulin either fails to stimulate the growth of the Walker and Jensen tumors in the presence or absence of cortisone or the effect is small. This is a further example of a dissociation between the overt effects of insulin upon the utilization of glucose and effects on nitrogen balance and growth(4).

Summary. Sexually mature male rats in metabolism cages were adapted to the tube-feeding of a medium carbohydrate diet. Some rats were treated with cortisone acetate and with glucagon-free insulin, separately and in combination and in the presence and absence of implanted tumor. The severity of the glycosuria was proportional to the dose of cortisone. Glycosuria was markedly suppressed by insulin, but there was no suppression of the level of urinary nitrogen in the presence or absence of cortisone. Cortisone suppressed the growth of Walker Carcinoma 256 and of Jensen Sarcoma and caused atrophy of the adrenals, thymus and partial atrophy of spleen. None of these effects of cortisone overdosage were prevented by insulin.

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