Effects of Insulin Deficiency and Cortisol Administration on Mobilization of Protein to Gluconeogenesis.* (31033)

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The proteins of the body are in a constant state of change and are continuously being broken down and resynthesized. This dynamic state suggests that nitrogen metabolism can be influenced by many factors—such as hormones, which are not necessarily essential but rather serve to stimulate or to inhibit a reaction. These hormones may be labeled as having a protein anabolic or catabolic action. It is well known that both insulin deficiency(1) and corticoids administration (2) lead to increased gluconeogenesis in intact animals within few hours after *in vivo* administration of cortisol or anti-insulin serum. It is therefore of intesest to see if the breakdown of tissue proteins contributes as substrates for gluconeogenesis. Thompson *et al* (3,4), and Swick(5) have shown that body proteins turn over at different rates and thus it may be expected that short-lived tissue protein may contribute to *in vivo* glucose formation. The present study reports conversion of short- and long-lived protein fraction into glucose in rats treated with anti-insulin serum and cortisol.

Experimental procedure. Animals. Male, albino rats of the Wistar strain, weighing between 100 g and 110 g were fed *ad libitum* on Purina laboratory chow and housed in individual cages. They were fed, by stomach tubing, 10 μc of C^{14} algae protein hydrolysate per day for a period of 10 days to label their tissue proteins. These rats were then used for various experimental studies.

Studies with anti-insulin serum and cortisol treated rats. Anti-insulin serum (AIS) was prepared(6) and acute insulin insufficiency was produced by administration of anti-insulin serum which neutralized the activity of 2 to 2.5 units of insulin per ml of serum. This (3.0) ml quantity of anti-insulin serum was sufficient to increase the blood sugar to 180 mg-220 mg/100 ml within 30 minutes after AIS administration. Rats fed C^{14} protein hydrolysate were injected with 3 ml of anti-insulin serum intraperitoneally and 6 hours later received another 3 ml. Another set of rats received 5 mg of cortisol subcutaneously. Twelve hours after administration of cortisol or anti-insulin serum, 0.2 ml of blood was collected from the severed end of the tail. The radioactivity of blood glucose, blood protein and blood protein free filtrate was determined. The results are given in Table I. At the end of 12 hours, animals were stunned by a blow on the head, exsanguinated and their livers removed for analysis of C^{14} activity in protein, glycogen and lipids (7,8). These results are given in Table II.

Studies with liver slices. Liver slices prepared as described previously(1,2) from normal, normal with anti-insulin serum and cortisol treated rats for 12 hours which were fed previously C^{14} protein hydrolysate were incubated in Ringer-bicarbonate medium equilibrated with 95%O_2-5%CO_2(7,8). Approximately 0.5 g of wet liver slices weighed on a Roller-Smith torsion balance was incubated in 6 ml of medium. After 90 minutes of incubation, tisses were analyzed for glycogen and protein and medium for glucose and CO_2. Glucose and glycogen were isolated as the phenylglucosazone and CO_2 as $BaCO_3$ and tissue protein by precipitation with trichloroacetic acid(7,8). The results of this study are given in Table III.

Results. Radioactivity found in blood, blood glucose and blood protein free filtrate of rats fed on C^{14} labeled amino acids is recorded in Table I. In rats killed 12, 24, and 36 hours after administration of C^{14} protein hydrolysate, little change in the radioactivity of these fractions was found; but, after 60 and 72 hours a reduction in total blood activity and blood C^{14} glucose was observed. Rats treated with anti-insulin serum or cortisol 24 hours after the dis-

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continuance of C^{14} amino acid feeding showed an increase in the radioactivity of blood glucose and protein free filtrate, whereas rats injected with AIS or cortisol 60 hours after withdrawal from the hydrolysate indicated no increase in blood glucose radioactivity of protein free filtrate.

Table II summarizes data on the radioactivity of liver protein and liver glycogen. A decrease in protein radioactivity was found when cortisol or AIS were administered to rats 24 hours after the withdrawal of C^{14} protein hydrolysate. Under these conditions there was observed a fall in glycogen radio-

TABLE I. Effect of Anti-Insulin Serum (AIS) or Cortisol Administration upon *in vivo* Blood Glucose Formation.

Animals	No. of hours after C^{14} withdrawal	Blood glucose, mg %	Blood, cpm/ml	Blood glucose, cpm/ml	Blood (protein free), cpm/ml
Control	12	91.0 ± 11	34,000 ± 3200	2200 ± 140	4300 ± 160
"	24	88.5 ± 9	32,300 ± 3050	2360 ± 170	4460 ± 180
"	36	86.0 ± 10	30,800 ± 2800	2080 ± 180	4100 ± 120
"	60	85.6 ± 8	28,600 ± 2760	1960 ± 150	3820 ± 160
"	72	89.5 ± 8	26,380 ± 2200	1800 ± 160	4000 ± 140
" + AIS	36*(12)	240 ± 20	38,000 ± 4200	6800 ± 450	14,600 ±1800
" + "	72*(12)	230 ± 23	28,380 ± 4600	2350 ± 480	5400 ± 280
" + cortisol	36*(12)	220 ± 15	36,600 ± 2800	8340 ± 680	16,800 ±1300
" + "	72*(12)	208 ± 16	22,380 ± 3200	2600 ± 320	4680 ± 240

* Total No. of hours after C^{14} withdrawal, but treated with cortisol or AIS 12 hr prior to bleeding. Each figure is an average of 4 values.

TABLE II. Effect of Anti-Insulin Serum (AIS) or Cortisol on *in vivo* Glucose Formation and Protein Catabolism.

Treatment	No. of hours after C^{14} withdrawal	Liver protein, cpm/mg	Liver glycogen		Liver lipids, cpm/g tissue
			μmoles/g tissue	cpm/g tissue	
Control	12	320 ± 40	128 ± 12.0	4830 ± 380	12,300 ± 1280
"	24	295 ± 32	115 ± 10.0	5200 ± 280	13,400 ± 1260
"	36	280 ± 30	118 ± 9.0	5260 ± 180	14,320 ± 1060
"	60	202 ± 26	118 ± 9.5	4830 ± 300	11,800 ± 1300
"	72	180 ± 23	112 ± 10.0	4630 ± 220	11,800 ± 1250
" + AIS	36*(12)	162 ± 13	40 ± 4.5	800 ± 120	12,100 ± 1250
" + "	72*(12)	146 ± 16	34 ± 3.0	900 ± 112	11,800 ± 1460
" + cortisol	36*(12)	173 ± 12	230 ± 18.0	10,380 ± 820	11,600 ± 1100
" + "	72*(12)	159 ± 18	220 ± 21.0	6320 ± 620	9000 ± 860

* Total No. of hours after C^{14} withdrawal, but treated with cortisol or AIS 12 hr prior to killing. Each figure is an average of 4 values.

TABLE III. Incorporation of C^{14} Label from Tissue Protein into Glucose, and CO_2 by Liver Slices Prepared from Rats Fed C^{14} Protein Hydrolysate and Treated with Anti-Insulin Serum (AIS) or Cortisol.*

Animals	No. of hours after C^{14} withdrawal	Medium glucose		CO_2, cpm/g tissue
		μmoles/g tissue	cpm/g tissue	
Control	12	88 ± 6.8	5820 ± 380	6680 ± 600
"	36	92 ± 7.6	6200 ± 460	5820 ± 510
"	72	82 ± 6.6	2900 ± 240	3800 ± 400
" + AIS	36†(12)	96 ± 7.3	10,220 ± 980	5300 ± 420
" + "	72†(12)	90 ± 8.0	3200 ± 260	5050 ± 380
" + cortisol	36†(12)	83 ± 6.9	11,800 ±1200	4800 ± 460
" + "	72†(12)	89 ± 7.9	2360 ± 210	3260 ± 320

* Approximately 500 mg of liver slices were incubated for 90 min in 6 ml of Ringer-bicarbonate medium.

† Total No. of hours after C^{14} withdrawal, but treated with cortisol or AIS 12 hr prior to killing. Each figure is an average of 4 values.

activity in AIS treated rats, whereas cortisol treated rats showed an increase in C^{14} glycogen. Rats treated with cortisol or AIS 60 hours after the withdrawal of C^{14} feeding showed no change in radioactivity between control and experimental rats. Lipid radioactivity was unchanged by administration of cortisol or AIS for 12 hours. Table III summarizes the data obtained with liver slices from experimental and control animals. An increase in the radioactivity of glucose is observed in rat liver slices treated with anti-insulin serum or cortisol 24 hours after withdrawal of C^{14} protein hydrolysate. A similar increase was not observed in rats treated with AIS or cortisol 60 hours after C^{14} amino acid hydrolysate.

Discussion. The effects of adrenal glucocorticoids and insulin deficiency upon hepatic glucose production are well recognized. In early experiments it was inferred, primarily from urinary D:N ratios, that glucose formation was at the expense of protein catabolism. The present results are in agreement with these observations. However, it appears that the body protein constituents with short half-life are the primary contributors of substrates for gluconeogenesis. Previous studies from this laboratory(7) have shown increased conversion of amino acids into glucose in diabetic liver preparations. The present study indicates that necessary substrates *in vivo* are provided for the increase in glucose synthesis by the catabolism of short-lived tissue protein components.

Body protein turnover occurs at different rates(3-5). A rapidly mobilized fraction amounting to 2-5% of body protein turnover occurs in a matter of 2 to 5 days while a much larger fraction, presumably comprising a "structural protein" is turned over at the rate of 80-90 days. Protein catabolism and gluconeogenesis initiated by insulin deficiency or excessive amounts of cortical hormones presumably involve the more rapid turnover of protein fraction in the body. *In vitro* studies with liver slices suggest that 20-25% of the radioactivity lost from hepatic protein appears in glucose. It is suggested that the remaining 75-80% may be present in tricarboxylic acid or other intermediates and partly oxidized to CO_2. Thus these studies suggest that the rapid turnover of hepatic protein fractions might be the principal contributor of substrates to gluconeogenesis in insulin deficiency or excessive amounts of corticoids.

Increases in various enzymes involved in gluconeogenesis have been reported(9-14). Both pyruvate carboxylase(11-14) and PEP carboxykinase(9,10) are increased 6 to 12 hours after administration of cortisol, alloxan or anti-insulin serum, whereas glucose-6-phosphatase is unchanged(9). These results suggest that the primary action of insulin deficiency or glucocorticoids is to establish conditions leading to increased metabolites such as amino acids, pyruvate and glutamate needed for carbohydrate formation. If the substrate induction is the primary effect of insulin deficiency or excessive corticoids, then net glucose synthesis should be observed in liver slices incubated with a mixture of substrates. This indeed has been found to be true in recent studies(15). Ray *et al*(16) and Segal and Lopez(17) suggested that stimulated gluconeogenesis is independent of *de novo* synthesis of gluconeogenic enzymes. Present results also indicate that under conditions of increased glucose synthesis, the primary effect is to increase the substrates necessary for glucose formation.

Summary. The effects of insulin deficiency and cortisol on the mobilization of protein to glucose have been studied. Rats were fed on a diet supplemented with C^{14} labeled hydrolyzed algae protein for 10 days. After stopping the intake of labeled amino acids, they were treated with anti-insulin serum or cortisol and the radioactivity of tissue protein, blood glucose and glycogen was studied. Rats treated with anti-insulin serum or cortisol 60 hours after C^{14} administration showed an increase in blood glucose and liver glycogen (only in cortisol treated animals) but no increase in incorporation of C^{14} into glucose was observed. When anti-insulin serum or cortisol was given 24 hours after C^{14} administration, an increase both in radioactivity of blood glucose and liver glycogen (only in cortisol treated animals) was observed. These studies indicate that short-lived protein com-

ponents contribute to increased gluconeogenesis whereas proteins with slower turnover rates were not as effectively catabolized. Under these conditions lipid activity was found to be unchanged.

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Inability of Neonatal Rabbit Thymus to Induce Antibody Producing Capacity in Bursectomized Chickens.* (31034)

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The thymus has been shown to elaborate a humoral or hormone-like substance which is essential in the establishment of immunological reactivity in newborn mammals. Ablation of the thymus at a time before thymic development is complete results in an impairment of antibody production, prevents homograft rejection and causes hypoplasia of lymphoid tissue and lymphopenia. Implantation of thymic tissue enclosed in cell impermeable diffusion chambers restores the lymphoid tissue, the antibody capacity and homograft immunity in these animals(1-4). In birds, 2 separate organs are essential for the development of immunological reactivity. The thymus is involved primarily with delayed hypersensitivity and homograft immunity while the bursa of Fabricius is concerned with the antibody producing capabilities(5). The formation and development of the bursa of Fabricius can be completely prevented by the *in ovo* administration of testosterone(5-7). Birds hormonally bursectomized *in ovo* or surgically bursectomized at hatching are incapable of evoking an antibody response to most antigens(5,7-10). Implantation of bursal tissue enclosed in cell impermeable diffusion chambers acts to restore significant antibody producing capacities in bursectomized birds(9-11). Experimental evidence indicates that the bursa *per se* is not involved in the direct elaboration of antibody(9,12).

The mammalian thymus, avian bursa and probably the avian thymus function at least in part by the elaboration of a humoral substance which induces antibody formation and homograft responses in these animals. Since the avian thymus cannot induce the ability of bursaless birds to form circulating antibody, 2 independent humoral substances ap-

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