Influence of Hyperglycemic Factor (Glucagon) on 17-Hydroxysteroids in Plasma and Urine of Man. (21250)

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Abundant evidence indicates that hyperglycemic factor exerts its effect by stimulating hepatic glycogenolysis. Sutherland(1) has demonstrated that hyperglycemic factor accelerates resynthesis of active hepatic phosphorylase, the phosphorylase reaction being the limiting one in the enzymatic transformation of glycogen to glucose. Another method by which hyperglycemia may occur involves the accentuation of gluconeogenesis, and perhaps inhibition of glucose utilization, via adrenal stimulation(2). That this may be a minor, though significant, effect of glucagon has not been adequately investigated. Kirtley *et al.*(3) found an increase in urinary 17-hydroxycorticoid/creatinine excretion in 3 normal and 9 diabetic patients following the administration of hyperglycemic factor, and postulated the presence of adrenal stimulattion, perhaps secondary to the stress of administration rather than to an effect of hyperglycemic factor *per se*. The observations of Vuylsteke *et al.*(4) that hyperglycemic factor caused a decrease in eosinophils in the dog, also raised the possibility of adrenal stimulation.

It was our purpose to investigate several non-diabetic persons in an attempt to reproduce the findings of Kirtley's group, and in addition, to observe whether rises occurred in plasma 17-hydroxysteroid levels.

Method. Eight hospital patients with normal renal and hepatic function and no signs of congestive heart failure were chosen for study. These patients were fasted for 14 hours prior to onset of the experiment. In 4 patients, purified hyperglycemic factor† was given by intravenous infusion over a 30-min. period in a dose of 10 μg/kg. Blood glucose and plasma 17-hydroxysteroid determinations were performed on samples taken just before and at the end of infusion, and another 17-hydroxysteroid determination on plasma was made at the end of one hour following the start of infusion. The plasma 17-hydroxysteroid determinations were performed according to the method of Nelson and Samuels(5).‡ A 3-hour control urine specimen was collected prior to infusion, and 2-hour specimens were collected during and after the administration of hyperglycemic factor. Urinary 17-hydroxysteroid determinations were performed by the method of Glenn and Nelson(6). In the remaining 4 patients, hyperglycemic factor was given in similar doses, but injected intravenously over a 30-second period. Blood sugar responses were again noted, and plasma 17-hydroxysteroid determinations performed before, at 20 and 40 minutes and, in 2 cases, 80 minutes, following injection. Urinary steroids were performed on 3-hour, instead of

TABLE I. Effect of 30-Minute Infusion of Hyperglycemic Factor (Lilly Lot 208-158B-214) on 17-hydroxysteroid Levels on 4 Patients.

			17-hydroxysteroids			
				Plasma,	Urine,	
Age	Min.	Blood sugar, mg/100 cc	Min.	μg/100 cc	μg/hr	
15	0	98	0	8	B*	120
	30	165	30	4	0-2 hr	325
			60	4	2-4	135
16			0	8	B	120
		———	30	8	0-2 hr	140
			60	7	2-4	50
75	0	91	0	7	B	90
	30	165	30	8	0-2 hr	205
			60	5	2-4	205
67	0	110	0	15	B	323
	30	152	30	8	0-2 hr	<50
			60	8	2-4	469

* B = Baseline.

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TABLE II. Effect of 30-Second Injection of Hyperglycemic Factor (Lilly Lot 208-158B-34) on 17-hydroxysteroid Levels on 4 Patients.

Age	Min.	Blood sugar, mg/100 cc	17-hydroxysteroids Min.	Plasma, μg/100 cc	Urine, μg/hr	
60	0	110	0	13		
	30	157	20	12	———	
			40	16		
49			0	11		
	———		20	11	———	
			40	8		
80			0	14	B*	181
	———		20	13	0-3 hr	75
			40	13	3-6	146
			80	14		
49			0	0	B	108
	———		20	<2	0-3 hr	80
			40	0	3-6	70
			80	<2		

* B = Baseline.

2-hour, specimens, and on only 2 patients.

Results. Table I and II show the data obtained in the 2 groups of patients. We did not observe a significant rise in urinary steroid levels in those patients who received a rapid injection of hyperglycemic factor (Table II). In those receiving the intravenous infusion, urinary excretion of steroids rose significantly in patients 1 and 3. The low value for the 0-2-hour urine specimen of patient 4 is hard to understand; no difficulty in collecting the sample was experienced. The moderately elevated 2-4-hour specimen does not necessarily imply an elevated 0-2-hour sample, so no conclusions can be reached concerning the values for this patient. In none of the patients was a significant elevation of plasma 17-hydroxysteroids observed. The values for plasma steroids compared well with the normal values reported by Bliss *et al.*(7).

Discussion. The lack of rise of plasma steroid in response to hyperglycemic factor makes it very unlikely that any of the metabolic effects of this material can be attributed to adrenal gland stimulation. Although the recent studies of Bongiovanni(8) have shown a much larger yield of 17-hydroxysteroids from plasma after glucuronidase hydrolysis, there is as yet no evidence that adrenal stimulation can produce a rise in conjugated steroids in the absence of an elevation in unbound steroids.

In those 4 patients who received a 30-minute infusion of hyperglycemic factor, an increase in urinary excretion of steroids occurred in 2. These changes were similar to those reported by Kirtley *et al.*(3). These observations, being variable from patient to patient, and not apparent in the group subjected to rapid injection, support the proposal that the increased steroid excretion was a response to stress(3). The variability in our findings also makes it improbable that the hyperglycemic factor has any action on the renal mechanism responsible for steroid excretion. In the patients who showed a rise in urinary steroid excretion, apparently due to stress, one might question why the plasma levels did not also rise. Some elevation in these levels might be expected during the second hour but unfortunately we did not measure plasma levels in this period to clarify this point. However, a response occurring this long after the administration of hyperglycemic factor should have little relation to its metabolic effect, which is almost entirely dissipated within one-half hour after administration(3,9).

The alternate postulation raised by Vuylsteke's group, that the eosinophil drop after hyperglycemic factor is independent of the adrenal cortex, and thus similar to that produced by epinephrine or intravenous glucose, is also more compatible with our observations.

Summary. 1. Plasma 17-hydroxysteroid levels and urinary 17-hydroxysteroid excretion rates were observed in humans before, during, and after the intravenous administration of purified hyperglycemic factor. There were no significant changes in plasma values, but some patients showed elevation in urinary excretion of steroids, apparently related to the degree of stress incurred. 2. By these methods, we could observe no direct action of hyperglycemic factor on the adrenal cortex.

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Chelating Agents, Barbiturates, and Stimulation of Rat Brain Adenosinetriphosphatase. (21251)

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Recent studies have indicated that barbiturates may, *in vitro*, interfere with oxidative phosphorylation(1). The significance of this interference is difficult to assess, since many compounds of apparently unrelated biological activity and structure exhibit the same effect. However, further understanding of the manner in which oxidative phosphorylation is interrupted may reveal points of departure among the various compounds involved. In a previous discussion(2), it was pointed out that certain broad-spectrum antibiotics, as well as 2,4-dinitrophenol(3), increased the adenosinetriphosphatase (ATPase)* activity of rat liver homogenates and mitochondria. It was also observed(2) that the ATPase activity of rat brain homogenates was not stimulated by either the antibiotics or dinitrophenol. The results obtained in the present study indicate a possible role of chelation effects in ATPase stimulation.

Materials and methods. The purities of Sigma sodium adenosinetriphosphate (ATP), Sigma sodium adenosinediphosphate (ADP), and Pabst sodium uridinetriphosphate (UTP) were confirmed by analyses of labile and total phosphorus. The barbiturates† and other chemicals used were the purest obtainable from various commercial sources. Ten per cent homogenates of rat liver or brain (frontal lobes) were made in 0.25 M sucrose at 0°C by use of a glass homogenizer(5). Tubes were set up containing the following: 0.04 M KCl, 0.0001 M $MgSO_4$, 0.05 M tris(hydroxymethyl)amino-methane buffer (pH 7.4), 0.003 M ATP (or other substrate), 0.1 ml homogenate, barbiturate solution or other additions if desired, total volume 1.0 ml. All solutions were made in glass-redistilled water and adjusted to pH 7.4. Substrate was added after the other components of the reaction mixture had been equilibrated in the water bath for 5 minutes. Following 10 minute incubation at 30°C, proteins were precipitated by addition of 0.1 ml 50% trichloracetic acid. Orthophosphate was determined(6) in the supernatant after centrifugation. Corrections were made for phosphorus liberation in the absence of homogenate and in the absence of substrate. The level of magnesium ion used in these experiments was purposely set far below the optimum in order to allow for maximum expression of stimulatory and chelation effects.

Results. In these experiments, the reproducibility of results when duplicate determinations were made with the same homogenate was quite satisfactory, a variation of more than ±5% being unusual. However, the activities and stimulatory responses of tissues from different animals were often subject to considerable quantitative variation. For this reason, comparisons of stimulatory activity

* This term is used to identify enzyme(s) catalyzing release of inorganic phosphate from ATP. The exact mechanism involved may be more complex than that of a direct hydrolysis of substrate(4).

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