PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 147, 803-806 (1974)

Effects of Glucagon Upon Rat Liver Mitochondria *in Vivo* (38442)

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Glucagon stimulates the net uptake *in vivo* of inorganic phosphate into proteins of microsomes, mitochondria, and lysosomes probably by protein–phosphorylating processes involving ATP (1, 2). Glucagon has also been found to stimulate glycogenolysis, lipolysis, and gluconeogenesis (3, 4). Injections of glucagon also induce the synthesis of several amino acid-degrading enzymes such as serine dehydratase, tyrosine amino transferase, ornithine amino transferase, aspartate amino transferase (cytosol), alanine amino transferase (cytosol) (5), and histidine–pyruvate amino transferase (both mitochondrial and cytosolic) (6).

Since many of the reactions stimulated by glucagon are energy-dependent, the present study was intended to assess the effects of glucagon injections upon liver mitochondria, and more specifically upon mitochondrial cytochromes and protein.

Materials and Methods. Adult male Sprague–Dawley rats fed a standard laboratory chow were used as experimental animals. Prior to sacrifice, the animals were injected intraperitoneally with 0.25 mg glucagon (Sigma Chemical Co.) in 0.1 ml 0.9% NaCl/100 g body wt. They were injected at 3:30 PM on the first day and at 8:30 AM and again at 3:30 PM on the next day. At 8:00 AM on the following day the animals were sacrificed by decapitation without prior fasting, and mitochondria were isolated from 6 g of liver as previously described (7). The mitochondria were then suspended in 5 ml 0.25 *M* sucrose. Noninjected controls were run simultaneously with the experimental animals.

Mitochondrial cytochromes aa_3, *b*, c_1, and *c* were estimated by the spectrophotometric method of Williams (8) using the extinction coefficients for cytochromes aa_3 and *c* of van Gelder and Slater (9, 10). Cytochrome oxidase activity was estimated by observing the rate of oxidation of reduced cytochrome *c* using the appropriate amount of mitochondria suspended in 0.1 *M* sodium phosphate (pH 7.4) in a recording spectrophotometer at 550 nm. Protein was determined by the method of Lowry *et al.* (11). Since variation among groups of animals was observed from experiment to experiment, data were recalculated with the control mean for each experiment set equal to 100 and experimental values expressed as percent of control. Thus data could be averaged and Student's *t* test used as a measure of significance of difference.

Results and Discussion. Mitochondrial protein per g of liver decreased significantly in animals injected with glucagon as compared with controls (Table I). Values per g of liver were decreased by 31% ($P < 0.001$) and per 100 g of body wt, by 26% ($P < 0.005$) compared with values from control animals.

The decrease in concentrations of cytochromes aa_3, *b*, c_1, and *c* as compared with control values paralleled the decrease in mitochondrial protein (Table II). The decrease in the cytochromes compared with the controls became significant when values were expressed per gram of liver ($P < 0.001$) and per 100 g of body wt ($P < 0.005$). Concentration of cytochrome aa_3 showed the greatest decline, and cytochrome c_1, the least. The actual values for which 100 stands for the controls in Table II are presented in Table III.

Cytochrome oxidase activity declined significantly when compared with mitochondrial protein ($P < 001$) (Table IV). This decrease was even greater when expressed per gram of liver or per 100 g rat. Although there was a tendency for cytochrome aa_3 concentration per mg protein to decrease in the livers of the glucagon-injected animals (Table II), the difference was not statistically significant. However, cytochrome oxidase activity per milligram protein did decrease significantly (Table IV). The reason for this apparent discrepancy is not clear. Perhaps

TABLE I. Hepatic Mitochondrial Protein of Control and Glucagon-Injected Rats.

Experiment No.	mg protein/g of liver			mg protein/100 g rat		
	Control	Injected	% of control	Control	Injected	% of control
I	15.0 (2)	12.0 (4)	79	48.6	38.9	80
II	17.2 (4)	10.6 (4)	62	64.6	41.0	64
III	18.7 (4)	12.4 (4)	66	69.6	54.2	78
Averages	100 ± 3.72[a] (10)		69 ± 6.40 (12)	100 ± 3.84 (10)		74 ± 6.12 (12)
P values for significance of difference from controls			$P < 0.001$			$P < 0.005$

[a] SEM; ( ), number of animals.

the spectrophotometric method for cytochrome aa_3 measures an inactive form of cytochrome oxidase.

Deter (12) and Rosa (13) have observed by electron microscopy of liver tissue from glucagon-injected animals that lysosomes can be seen that have swollen up to 800% of their normal volume. Moreover, mitochondria located near the swollen lysosomes are apparently digested by the swollen lysosomes. This could account for the loss of mitochondrial cytochromes and protein observed by us. It is of interest that the concentration of cytochrome *c* did not increase with glucagon injection since it is a soluble mitochondrial enzyme which is presumably made in the microsomes and then incorporated into the mitochondria. Many cytosolic enzymes, also presumably made in the microsomes, increase dramatically after glucagon injections. In experiments not reported in this paper in which we estimated cytochrome *c* in a mixture of microsomes and cytosol, there was also no indication that cytochrome *c* was increased after glucagon injection. Similarly, in other experiments not reported here using animals injected with allylisopropylacetamide (AIA), which induces the formation of δ-aminolevulinic acid sythetase (an enzyme di-

TABLE II. Values for Cytochromes aa_3, *b*, c_1, and *c* in Control and Glucagon-Injected Rats. Values from Injected Animals Are Averages Expressed as Percent of Control Averages. These Values Were Obtained From the Same Animals as in Table I.

	(aa_3)	(*b*)	(c_1)	(*c*)
		per mg protein		
Control	100 ± 4.95[a]	100 ± 3.22	100 ± 1.38	100 ± 2.63
Injected	88 ± 4.60	93 ± 8.99	108 ± 5.00	107 ± 4.99
	NS	NS	NS	NS
		per g of liver		
Control	100 ± 5.21	100 ± 7.05	100 ± 3.14	100 ± 4.11
Injected	66 ± 5.45	70 ± 4.28	79 ± 4.66	75 ± 5.19
	$P < 0.001$	$P < 0.001$	$P < 0.005$	$P < 0.005$
		per 100 g body wt		
Control	100 ± 4.95	100 ± 1.80	100 ± 2.59	100 ± 3.42
Injected	70 ± 5.16	74 ± 3.74	84 ± 4.26	80 ± 5.58
	$P < 0.001$	$P < 0.001$	$P < 0.005$	$P < 0.001$

[a] SEM; NS, difference is not significant.

TABLE IV. Cytochrome Oxidase Activity (C.O.) Expressed as $-\Delta A_{550}$/Min.

Expt. No.	C.O./mg protein			C.O./g liver			C.O./100 g rat		
	Control	Injected	% of control	Control	Injected	% of control	Control	Injected	% of control
I	13.0 (2)	9.10 (4)	70	195 (2)	109 (4)	56	630 (2)	353 (4)	56
II	7.56 (2)	6.26 (3)	82	128 (4)	69 (4)	54	481 (4)	309 (3)	64
III	5.59 (4)	4.63 (4)	83	105 (4)	56 (4)	54	414 (4)	245 (4)	59
Averages	100 ± 0.90[a] (8)		78 ± 2.78 (11)	100 ± 3.45 (10)		54 ± 3.90 (12)	100 ± 3.46 (10)		60 ± 2.77 (11)
P values for significance of difference from controls			$P < 0.001$			$P < 0.001$			$P < 0.001$

[a] SEM; ( ), number of animals.

TABLE III. Actual Values for Cytochromes aa_3, b, c_1, and c in Liver Mitochondria of Control Rats. The Values in Table II Are Based Upon the Values Below.

	(aa_3)	(b)	(c_1)	(c)
	(nmoles)			
per mg protein				
Expt I	0.195	0.098	0.100	0.149
Expt II	0.173	0.086	0.093	0.112
Expt III	0.152	0.082	0.087	0.111
per g liver				
Expt I	2.93	1.47	1.49	2.25
Expt II	2.90	1.45	1.40	1.90
Expt III	2.83	1.52	1.63	2.06
per 100 g body weight				
Expt I	9.36	4.76	4.82	7.23
Expt II	10.9	5.46	4.33	7.13
Expt III	10.5	5.65	6.07	7.70

rectly involved in porphyrin synthesis), we found no indication that cytochrome *c* was increased in any subcellular fraction. This was true whether AIA was injected alone or with glucagon simultaneously. Thus cytochrome *c* appears to be very refractory to induction.

Summary. After intraperitoneal injections of glucagon into adult male rats it was found that hepatic mitochondrial protein as well as cytochromes aa_3, b, c_1, and c were significantly reduced when expressed per gram liver or per 100 g body wt. The concentrations of the cytochromes paralleled the loss of mitochondrial protein. However, enzymatic activity of cytochrome oxidase, which is presumably a measure of cytochrome aa_3, decreased more than mitochondrial protein indicating that spectrophotometric assays of cytochrome aa_3 may measure an inactive form of this enzyme.

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Received July 1, 1974. P.S.E.B.M., 1974, Vol. 147.