beta-carotene treated animals are similar to those which occur in the vagina of the rat after topical application of Vit. A(4,5). Our results, following beta-carotene treatment are similar to those of Sherwood, Brend and Roper(6) who observed nucleated cells in vaginal smears of rats following oral treatment with beta-carotene.

Since rats receiving cortisone alone gave no indication of alterations in the vaginal smear, it seems clear that changes observed in the beta - carotene or beta - carotene - cortisone treated animals must have been the result of conversion of beta-carotene to Vit. A.

No estrogenic activity follows local or subcutaneous treatment with cortisone(7,8), therefore, persistence of cornified epithelium in the vagina of deficient animals treated with cortisone was due to Vit. A deficiency, and not to treatment with the hormone. Under our conditions, therefore, cortisone had no demonstrable effect on conversion of beta-carotene to Vit. A.

The present observations do not completely contradict the work of Clark and Colburn(2), who found a reduction in the quantity of Vit. A stored in liver and kidneys. The possibility of cortisone having an effect on storing of Vit. A, and not on conversion of beta-carotene to Vit. A, must be recognized.

Summary. Abnormally cornified epithelium of the vagina induced by Vit. A deficiency disappeared when beta-carotene alone or beta-carotene and cortisone was administered. Cortisone alone did not modify the cornified epithelium of the vagina. The results demonstrate, therefore, that cortisone has no demonstrable effect on conversion of beta-carotene to Vit. A.

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Failure of Blood Glucose Levels to Reflect Hepatic Glycogenolysis; Experiences with Glucagon.* (24215)

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It is common practice in some experimental situations to measure hepatic glycogenolysis indirectly through changes in blood glucose levels. Accurate blood glucose determinations can be performed rapidly and blood samples can be obtained conveniently at any desired interval. Direct determination of liver glycogen, on the other hand, requires liver biopsy or sacrifice of the experimental animal, and it is not practicable to perform repeated determinations on the same subject during an acute experiment. On the basis of serial blood sugar determinations, it has been stated that glucagon is inactive when administered subcutaneously(1,2). In the course of our studies of glycogen metabolism, we have in several instances drawn erroneous conclusions from such indirect evidence. It is the purpose of this communication to call attention to the hazards of such indirect "measurement" of glycogenolysis and to emphasize the necessity of performing glycogen analyses.

Methods. Normal and adrenodemedullated male Sprague-Dawley rats, weighing 200 to 260 g, were used. Blood glucose was

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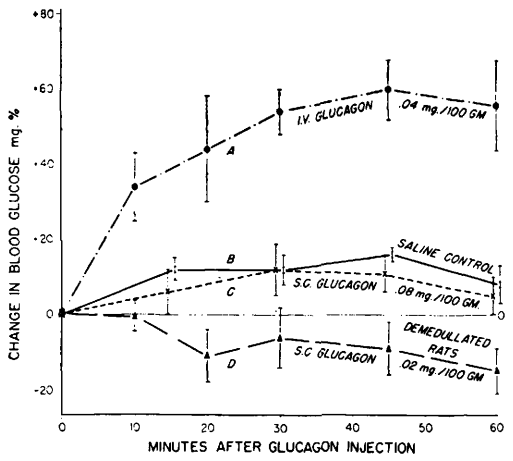

FIG. 1. Blood glucose curves after glucagon inj., illustrating range of variation encountered. Vertical lines represent stand. error.

determined by the Nelson(3) method, using tail blood. Liver specimens were obtained under nembutal anesthesia. Glycogen was determined by the method of Good, Kramer and Somogyi(4) and expressed in terms of glucose (Nelson method). Glucagon was supplied by the Eli Lilly Laboratories as a powder, lot No. 258-234B-54-2. This material has negligible insulin activity. It was dissolved in slightly alkaline distilled water, then diluted with physiological saline. Rats were fasted 24 hours, then given a standard feeding of 0.60-0.75 g of glucose/100 g body weight, by stomach tube. Four and one-half to 5 hours were allowed for deposition of liver glycogen. Glucagon was then injected intravenously, intraperitoneally, or subcutaneously, in doses ranging from 5 to 80 μg 100 g body weight. Controls received slightly alkaline physiological saline. Blood samples for glucose determinations were obtained at 10- to 15-minute intervals. Animals were sacrificed one hour after glucagon injection, and glycogen determination was performed.

Results. Considerable variation was observed in blood glucose curves after injection of glucagon. Rises in blood glucose of 24 to 104 mg % were observed, after intravenous administration of 20 to 40 μg of glucagon/100 g to normal animals. When similar doses were injected intraperitoneally or subcutaneously, distinct, but smaller rises in blood glucose levels usually resulted. In some experiments, however, no significant rise could be demonstrated. In the adrenodemedullated rats, even intravenous glucagon injection produced only slight increases, and subcutaneous administration regularly failed to raise blood glucose level. Degree of variation observed in this study is shown in Fig. 1, which presents data from 3 experiments, involving 17 rats.

The effect of glucagon on liver glycogen, on the other hand, was consistent and reproducible. Marked glycogenolysis was induced in every one of 104 animals injected with glucagon. The results of these glycogen determinations are presented in Table I. The values corresponding to the blood glucose curves in Fig. 1 are identified by the same code letters. At all dose levels investigated, the glycogen depletion at one hour after subcutaneous injection was at least as intense as that observed after other routes of administration.

Discussion. Blood glucose level reflects the operation of many well known metabolic processes which require no discussion here. Of importance, however, is the fact that the en-

TABLE I. Liver Glycogen One Hour after Glucagon Injection.

Glucagon dose and route of administration			Liver glycogen ± S.E. (%)	
Normal rats				
Control, saline			3.49	.12
.005 mg/100 g	Intraper.		1.10	.15
Idem	Subcut.		.40	.03
Control, saline			2.71	.12
.01 mg/100 g	Intraper.		.80	.11
Idem	Subcut.		.76	.14
Control, saline			2.51	.18
.02 mg/100 g	Intraper.		.10	.03
Idem	Subcut.		.10	.06
Control, saline			1.98	.18
.02 mg/100 g	Intrav.		.50	.30
.04 mg/100 g	"	(A)	.06	.01
Control, saline			2.78	.23
.04 mg/100 g	Intraper.		.63	.38
Idem	Subcut.		.39	.15
Control, saline		(B)	2.51	.51
.08 mg/100 g	Subcut.	(C)	.13	.12
Adrenodemedullated rats				
Control, saline			2.92	.22
.02 mg/100 g	Subcut.	(D)	.24	.09
Control, saline			1.89	.21
.04 mg/100 g	Intraper.		.16	.07
Idem	Subcut.		.13	.09

tire hepatic glycogen content represents only a small store of glucose, when compared to capacity of the glucose disposal mechanisms. Calculations from oxygen consumption data (5) and standard metabolic tables indicate that at a respiratory quotient of 0.85, the rat can completely oxidize within one hour the glucose represented by a 3% liver glycogen level. The data of Stetten and Boxer(6) indicate that this amount of glucose is converted into fatty acids within 2 hours by rats on a high carbohydrate diet. A barely detectable rise in muscle glycogen—0.1%, can account for one-third of this glucose reserve. Thus, relatively small increases in activity of these disposal mechanisms, operating together, can rapidly remove from the blood the glucose produced by relatively intense hepatic glycogenolysis. It is noteworthy that a single hormone, insulin, accelerates all 3 of these mechanisms. Other physiologic processes which may be of importance in this regard include gluconeogenesis, the rate of which can also vary greatly.

When glucagon is administered intravenously, hepatic glycogenolysis is initiated very rapidly and the activity of the glucose disposal mechanisms does not increase sufficiently to prevent a transient hyperglycemia. Subcutaneous injection, on the other hand, may fail to induce a rise in blood glucose, although glycogenolysis is of the same order of magnitude. Presumably, in such experiments, the onset of glycogenolysis is not as rapid, and glucose disposal mechanisms are stimulated in time to handle the increased glucose load. Even with intravenous injection, however, the rise in blood glucose accounts for only a fraction of the glucose liberated from the liver.

The adrenodemedullated rats showed little or no rise in blood glucose after glucagon administration. Such animals are more sensitive to insulin than are normal animals(7). The earlier stimulation of glucose disposal mechanisms in these rats is consistent with the hypothesis that endogenous insulin release is responsible for the prevention of hyperglycemia during glucagon glycogenolysis.

The experience reported here is not unique for glucagon. Similar findings have been observed with a glycogenolytic liver extract(8) and, more recently, with the synthetic agent, mercaptoethylamine (unpublished). Other examples of discrepancy between changes in liver glycogen and blood glucose are better known. Liver glycogen declines concurrently with a falling blood glucose after insulin administration(9). Epinephrine administered to a fasted animal causes hyperglycemia and liver glycogen deposition(10). Thus, in any experiment where stimulation of insulin or epinephrine secretion is possible, great caution should be exercised in drawing inferences regarding hepatic glycogenolysis or glycogenesis from the blood glucose values. Direct determination of liver glycogen must be performed, at least in representative animals.

Summary. 1) Glucagon is as active a glycogenolytic agent when administered subcutaneously as it is by intraperitoneal or intravenous route. However increases in blood glucose may not be observed following subcutaneous administration of glucagon, even though liver glycogen is almost completely mobilized. In adrenodemedullated animals, this is the rule. 2) Total liver glycogen store of the rat is relatively small, compared to the capacity of glucose disposal mechanisms. Changes in rates of the major glucose disposal reactions can completely mask the anticipated effect of hepatic glycogenolysis on blood glucose concentration. Such changes may be induced by fluctuations of endogenous insulin secretion.

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