3,100, which can be considered a reasonable value, being in fact identical with the turnover number of crystalline lactic dehydrogenase from muscle⁴, though this is no doubt a coincidence, as the two enzymes belong to different groups. The maximum turnover number of cytochrome c is 3850^5 . The calculated purity of our final enzyme solutions depends on the assumed molecular weight, but is probably of the order of 10-20 per cent. The Q_{MB} of the pure enzyme would be 30,000-100,000, according to the molecular weight assumed. A calculation from the activity of the Lebedew juice shows that I kgm. of yeast contains only a few mgm. of the pure enzyme.

The idea that a cytochrome, or indeed any other hæmatin compound, may act as a dehydrogenase is a new one and may throw light on the nature of other dehydrogenases of the third group.

The work will be reported in greater detail elsewhere.

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- ¹ Bernheim, F., Biochem. J., 22, 1178 (28).
- ⁹Dixon, M., and Zerfas, L. G., NATURE, 143, 557 (1939).

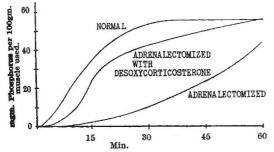
Bach, S. J., Dixon, M., and Keilin, D., NATURE, 149, 19 (1942).
Straub, F. B., *Biochem. J.*, 34, 483 (1940).
Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.*, B, 129, 227 (1940).

Decrease in Glycogen Phosphorylation in Muscles in vitro after Adrenalectomy and Restoration with Desoxycorticosterone

IT has been supposed that a diminution of phosphorylation is the main disturbance after adrenalectomy, and that this is proved by a study of the direct phosphorylation of glycogen by muscle.

If 0.5 gm. of leg muscle of a normal rat is minced and placed in 2 c.c. of a solution of 1 per cent sodium bicarbonate with 1.75 per cent sodium fluoride, then inorganic phosphate is dissolved from the muscle. If 0.25 per cent of glycogen is then added to such a solution, the inorganic phosphoric acid content of the solution decreases and the glycogen is phos-phorylated (Bodnár and Tanko, Lohmann, Parnas). The muscles of adrenalectomized rats lose this capacity of phosphorylating glycogen in vitro. This was first shown by Schumann (1940), but simultaneously Helve (1940) did not find this difference.

We have studied the time relations of this reaction with the muscles of normal and adrenalectomized rats. Normal rat muscle uses about 54 mgm. phosphorus per 100 gm. of muscle. At 20° C. the reaction ends in 30-60 minutes, and at 37°C. in



PROSPHORYLATION OF GLYCOGEN BY RAT MUSCLES in vitro AT 20° C.

10-20 minutes. With muscles of adrenalectomized rats, the reaction is much slower and within the physiological time limit it is strikingly decreased. In thirty-seven adrenalectomized rats only about 11 mgm. phosphorus was used up after thirty minutes. The reaction then slowly continues and may, after three hours, give values equal to those found in normal muscle after thirty minutes at 20° C. or after twenty minutes at 37° C. This explains why Helve, who kept the muscle at 37° C. for 31 hours, did not observe a decrease in phosphorylation.

The accompanying figure shows the time relations of the reaction based on mean values of experiments on normal and adrenalectomized rats' muscle at 20° C. The decrease in phosphorylation in the latter is obvious. It is already found in animals which are still only slightly adynamic. Only animals which were still in good condition were used for these analyses. Generally speaking the more time had elapsed after the adrenalectomy, the less phosphorylation occurred. Table 1 gives some values at 20° C. after sixty minutes and at 37°C. after twenty minutes for comparison.

Table 1.

		M	GM. P	HOSPHORUS	PER	100 GM.	MUSC	LE USED.
Noi	mal	rat	muscl	e (21 anima	ls)			45-64 (mean 54)
On	4th	day	after	adrenalecto	my			26, 22
**	5th	,,	,,	,,				36, 36, 22, 40, 22
,,	6th	"	,,	,,				28, 26, 20, 26
,,	7th	,,	,,	55				8, 4, 39, 15, 8
,,	8th	,,	,,	"				6, 20, 16, 18
					(Each figu	re ref	ers to a separate rat)

We then tried to restore phosphorylation by adding desoxycorticosterone acetate to these muscles in vitro. A 1 per cent solution in 66 per cent propandiol-water was made and 0.1 c.c. added to the system described of 0.5 gm. muscle with glycogen. Desoxycorticosterone restored the decreased rate of phosphorylation with muscle of adrenalectomized rats to the normal rate in all cases (muscles of twelve animals at 20° C., and four at 37° C.); it did not increase the rate of glycogen phosphorylation of normal rat muscle where it was already optimal (three experiments). As a control the action of other steroid hormones, cestradiol and similarly of testosterone, was compared. Neither of these hormones. had any action whatever on the rate of phosphorylation, with normal or adrenalectomized rats' muscle.

Table 2. MGM. PHOSPHORUS PER 100 GM. MUSCLE USED. Desovveorticosterone

							DUSUAYCOLUCUSUCIONC	
							(Without)	(With)
On	4th	day	after	adrenalectomy			20	36
,,	,,	,,	,,	**		•••	42	54
,,	5th	,,	,,	""			36	64
,,		.,	"	**			48	68
,,	6th		,,	,,			26	40
,,	,,	,,	,,	"			28	52
,,	7th	,,	,,	**			39	62
,,	,,	,,	,,	,,			8	30
,,	8th	,,	**	,,			10	19
,,	,,	,,	,,	**	•••		18	30

These experiments seem to give a proof that phosphorylation disturbances are the main factor in adreno-cortical insufficiency. It must be emphasized that the restoration took place not in the animal but in vitro. A detailed report will be given in Helvetica Chimica Acta.

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