22. Muscle Action and Glycogen Phosphorylysis

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It can no longer be doubted that phosphorylysis plays an important part in the breakdown of glycogen in liver and muscle. The amylolytic breakdown mechanism has in recent years steadily receded into the background [Parnas, 1937; Gill & Lehmann, 1939; Cori, 1940; Lee & Richter, 1940]. The primary importance of the phosphorylysing system is confirmed by the finding that its action is reversible, catalysing either the breakdown or the synthesis of glycogen. It seems unlikely that more than one system would be capable of this function.

To clarify further the part played by phosphorylysis *in vivo*, an investigation of the relationship between different types of muscle action and phosphorylysis was undertaken.

The magnitude of the phosphorylytic breakdown of glycogen by muscle brei and extract was determined by estimation of the decrease of inorganic phosphate in the presence of NaF. With fresh preparations the entire amount of glycogen phosphorylysed under these conditions is fixed as phosphoglyceric acid.

In the experiments described below, the following aspects of this subject are investigated: (1) phosphorylysis in muscle inactivated by denervation; (2) phosphorylysis in muscle inactivated by other means (tendotomy—by application of plaster cast); (3) phosphorylysis in embryo muscle; (4) phosphorylysis in different types of normal adult muscle (white, red, heart, and smooth muscle); (5) synthesis of glycogen by phosphorylase of different types of normal and inactivated muscle; (6) activity of phosphoglucomutase of different types of normal and inactivated muscle.

EXPERIMENTAL

Animal material. The animals used were bred in the laboratory and had long been maintained under observation.

Tissue and enzyme material. (a) Muscle brei. Muscle was removed from the bled animal, and reduced to a brei on an ice-cold plate. There was no difference in results between muscle brei from narcotized animals and from animals simply killed by a neck blow. Rapidity of operation in removing the muscle is essential.

(b) Muscle extract. To obtain muscle extract, 2 parts of muscle were extracted with 3 parts ice-cold water for 10–15 min.

(c) Eluate for synthesis of glycogen. This eluate was prepared by treating extract solution in the cold with $\text{Al}(\text{OH})_3$ for 15–20 min. The adsorbent was then sedimented and twice washed with cold water. Elution was carried out with 0.5 vol. of 0.25 *M* glycerophosphate for 1 hr. at room temperature. The adsorbents, $\text{Al}(\text{OH})_3$ C β and C γ , were prepared as described by Willstätter *et al.* [1925]. Our preparation of C β were less than a week old and did not contain appreciable amounts of the γ modification (general appearance, boiling test in conc. NH_3). The C γ preparations were at least 3 months old and failed to give a positive reaction when examined as indicated above for the presence of appreciable amounts of β modification.

Substrates. The glycogen used was free of N and P and prepared as described by Somogyi [1934]. Cori ester was used in the form of its dipotassium salt as prepared enzymically with potato juice from starch by the method of Hanes [1940]. Muscle adenylic acid was obtained from the firm of Laokoon, Lwow.

Buffer. The buffer solution contained 60 ml. *M*/10 phosphate buffer solution of pH 7.2, 100 ml. 0.9% NaCl, and 1 ml. 0.8% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

Analytical methods. Phosphate was determined according to Fiske & Subbarow [1925], glycogen according to Pflüger, glucose according to Somogyi [1937], and lactic acid according to Lieb & Zacherl [1932].

Test mixtures were incubated at 37° in a water thermostat and shaken during incubation. Synthesis of glycogen by eluate was carried out at room temperature.

(1) *Phosphorylysis by denervated muscle*

The sciatic nerve was cut under deep ether narcosis at its emergence from the pelvis and in part removed. The point of scission was confirmed during section. Denervation of muscle is known to affect excitability and contractility and evokes the degeneration reaction, fibrillation and finally contracture. Details as to these reactions as well as a review of previous knowledge on the chemical changes caused by denervation have been given by Tower [1939]. The known enzyme effects of denervation are surprisingly few. There is mention of a persistence of the enzymes responsible for glycogenolysis and lactic acid formation. In the respiratory system specific enzyme changes after denervation other than a rapid fall in extractable succinic dehydrogenase have not been observed. It is stated that in the second week after denervation succinic dehydrogenase decreases to 55% [Knowlton & Hines, 1934]. The variation in muscle water content due to denervation is negligible.

(a) *Experiments with muscle brei.* Muscle brei experiments were carried out at 37° in mixtures composed of 0.3 g. muscle brei, and 2 ml. phosphate buffer containing 1% glycogen and *M*/20 NaF. Experiments of series 1 were made on rabbits.

The effect of denervation on phosphorylation by preparations of gastrocnemius muscle is described below. In every case the innervated gastrocnemius muscle served as a control. In the first 10 days after denervation no certain changes in phosphorylytic ability occur. Atrophy of the denervated muscle is already well advanced by the end of this

Fig. 1. Phosphorylysis by 0.3 g. muscle brei. A, normal muscle; B, denervated muscle; 10–20 days after denervation.

period, and in the second week may amount to 25–30%. Later, 10–15 days after denervation, a definite inhibition of phosphorylysis is clearly apparent during brief incubation times, but may disappear almost completely after prolonged incubation. After 15–20 days, however, the inhibition is considerable even after long incubation times. Fig. 1, which summarizes the results obtained for the period between 10 and 20 days after

denervation, shows these relations clearly. The inhibition averaged 37 % after 15 min. incubation and 18 % after 60 min. incubation. The results for the period of 20–50 days after denervation (17 experiments) are summarized in Fig. 2. During this period phosphorylysis by brei of denervated muscle amounted to one-quarter, one-third and two-fifths of the normal value after 15, 30 and 60 min. respectively. No direct relationship was found between phosphorylytic activity and atrophy as measured by loss in muscle weight. Although extreme atrophy was always accompanied by a marked inhibition of phosphorylysis, an increase in atrophy was not constantly correlated with an increase in the degree of this inhibition, or *vice versa*.

Fig. 2. Phosphorylysis by 0.3 g. muscle brei. A, normal muscle; B, denervated muscle; 20–50 days after denervation.

Experiments with rats yielded results very similar to those described for rabbits. In all, 20 rats were tested. Inhibition of phosphorylysis in these animals was found to become appreciable within 6–12 days of the scission of the nerve. After 11 days, a marked inhibition, which persisted even after long incubation, was found. After 20 days, as Table 1 shows, the inhibition amounted to 50 % even after an incubation of 60 min.

Table 1

No. of exp.	Days after operation	Wt. of gastrocnemius (mg.)		Decrease in P_2O_6 in mg. with 0.3 g. muscle brei after 60 min.	
		Normal	Denervated	Normal	Denervated
10	22–68	1.24	0.55	3.13	1.41

(b) *Experiments with muscle extract.* In addition to tests with muscle brei, comparisons were conducted between phosphorylysis by extracts of normal and denervated gastrocnemius muscles. The tests were carried out in mixtures composed of 0.6 ml. extract, 2 ml. phosphate buffer, 1 % glycogen and $M/20$ sodium fluoride. Fig. 3 shows the average results for the period between 16 and 40 days after denervation of six experiments, all of which showed the same trend. The phosphorylysing effect of denervated muscle extract is frequently so slight as to approach the limit of experimental error.

(c) *Presence of inhibitors or deficiency of activators of phosphorylysis in denervated muscle?* An attempt has been made to solve this question by experiments on muscle extract. The possibility that extract of denervated muscle is deficient in activators was tested by adding known activators, adenylic acid (0.004 M) and KCN ($N/10$), to the usual incubation mixtures. No activation due to the addition of these substances could be

demonstrated. The question of the presence in denervated muscle of inhibitors was tested as follows: to 0.5 ml. extract of normal muscle were added 0.5 ml. extract of denervated and strongly inhibited muscle extract. In control mixtures 0.5 ml. water was added to 0.5 ml. of normal extract. No inhibition of phosphorylysis by addition of denervated muscle to normal muscle extract could be demonstrated.

Fig. 3. Phosphorylysis by 0.6 ml. muscle extract. A, normal muscle; B, denervated muscle.

(d) *Inhibition of glycogen decrease in denervated muscle.* Concurrently with the inhibition of phosphorylysis there is an inhibition in denervated muscle of glycogen decrease (Table 2). The experiments show that for breis of denervated and normal muscles the relationship glycogen decrease/phosphate decrease is roughly the same. It follows that the same fixation product accumulates in both these cases.

Table 2

	Decrease in P_2O_5 (mg.)		Decrease in glycogen (mg.)	
	After 30 min.	After 60 min.	After 30 min.	After 60 min.
Normal	3.84	4.20	8.00	9.00
20 days after denervation	1.96	2.52	4.00	4.00
Normal	5.10		7.13	
17 days after denervation	2.88		4.00	

The glycogen decrease is slightly greater than suggested by calculation from the phosphate decrease (assuming phosphoglyceric acid to be the sole product). A small fraction of glycogen may be decomposed by a breakdown path other than phosphorylysis, i.e. by amylolysis. The existence of this by-path is further confirmed by experiments in phosphate-free medium as described below.

(e) *Formation of lactic acid by denervated muscle.* The marked drop of phosphorylysis in denervated muscle suggested the advisability of experiments to establish whether glycolysis too is affected under these conditions.

In the first 14 days after denervation, no definite decrease in glycolysis was demonstrable. Later, when the effect on phosphorylysis becomes most pronounced, a corresponding decrease is also noted in glycolytic ability. The experiments summarized in Table 3 were carried out as follows: 0.3 g. muscle minced to a fine brei with scissors over ice, was suspended in 2 ml. of 0.04 M phosphate buffer ($pH=7.2$) containing 1% glycogen. The mixtures were then placed at 37° in an atmosphere of N_2 . The values shown in Table 3 were obtained.

Table 3

No. of exp.	Days after operation	Incubation time (min.)	P ₂ O ₅ decrease in mg.		Lactic acid formed in mg.	
			Normal	Operated	Normal	Operated
4	16-40	30	3.92	1.44	2.78	1.34
		16	4.19	2.07	3.50	2.05

(2) *Phosphorylysis and inactivity atrophy*

The question arises whether the observed decrease in phosphorylytic ability after denervation is a direct effect of denervation as such, or a general effect of inactivity atrophy. In the latter alternative it is to be expected that a similar decrease in phosphorylytic ability would also accompany all other inactivity conditions.

(a) *Inactivity atrophy of the limb due to fixation in a plaster cast.* Single limbs of rabbits were maintained in a plaster cast. After an interval of between 18 and 49 days, the atrophy induced was measured and the phosphorylysis curves of the gastrocnemius muscle of bound and normal limbs compared. The results of four parallel experiments are seen below (Table 4). Despite a loss of 46 % in the weight of the bound gastrocnemius muscle, no deviation from normal was noted in phosphorylytic power.

Table 4

Muscle wt. in g.		Decrease in mg. P ₂ O ₅ with 0.3 g. muscle brei after incubation.					
		15 min.		25 min.		60 min.	
Normal	Bound	Normal	Bound	Normal	Bound	Normal	Bound
9.2	5.0	3.36	3.70	4.12	4.37	4.68	4.75

(b) *Inactivity atrophy induced by tendotomy.* The Achilles tendon of single limbs of five rabbits was severed and the atrophy and the phosphorylytic curves of the inactivated gastrocnemius muscles determined after 28-45 days. In a second experimental series, three guinea-pigs and three rats were used. The results are shown in Table 5.

Table 5

Animal	Decrease in mg. P ₂ O ₅ with 0.3 g. muscle brei after incubation							
	Muscle wt. in g.		15 min		30 min.		60 min.	
	Normal	Tendotomy	Normal	Tendotomy	Normal	Tendotomy	Normal	Tendotomy
Rabbits	7.06	3.28	3.48	3.05	3.99	3.96	4.45	4.38
Guinea-pigs	1.60	0.82	2.24	2.52	3.08	3.00	3.48	3.32
Rats	1.17	0.61	—	—	2.66	2.33	3.23	2.70

Although tendotomy caused a loss of gastrocnemius weight in both guinea-pigs and rabbits of the order of 50 %, no deviation from normal in phosphorylytic ability became apparent. In rats 50 % loss in gastrocnemius muscle weight as a result of tendotomy was found to be accompanied by a 15 % decrease in phosphorylytic ability. This inhibition is very small compared to that produced by denervation.

(c) *Phosphorylysis by gastrocnemius muscle after scission of the spinal cord.* The spinal cord of rats was cut at the level of the sixth breast segment. After 1½ months the gastrocnemius muscle of the calf was removed and tested as to phosphorylytic ability. The results are shown in Table 6. No difference from normal in phosphorylytic power was found.

Table 6

No. of animals	mg. P ₂ O ₅ decrease after incubation with 0.3 g. muscle brei		
	10 min.	30 min.	60 min.
3	2.6	3.5	3.9

(d) *Exercise and phosphorylysis.* To supplement the experiments comparing the effect on phosphorylysis of denervation and other modes of inactivation, the question was examined whether exercise or artificial stimulation affects phosphorylytic ability.

In the first place a comparison was carried out between breast muscle from free-living and caged pigeons. Birds in the latter category had been maintained in narrow cages for 2 months. No difference of phosphorylytic ability between the two groups was found.

In a further experiment normal gastrocnemius muscle of rat was stimulated during 10 days by massage, or by electrical treatment (induction current) 4 times daily for intervals of 2 min. Similar treatment was applied to denervated gastrocnemius muscle. In both cases stimulation failed to affect the phosphorylytic ability.

Exhaustion produced by prolonged cramps also failed to affect the phosphorylytic ability. The experiments on this point were conducted as follows: the sciatic nerve on one side of the experimental animal was severed. On the following day 2-4 doses of strychnine sulph. (0.15 mg./100 g. body wt.) were injected subcutaneously, producing cramps which lasted 30-60 min. The effect of this treatment on phosphorylysis by gastrocnemius muscle is shown in the results of four experiments given below (Table 7).

Table 7

mg. P_2O_5 decrease after incubation					
15 min.		30 min.		60 min.	
Strychnine	Control	Strychnine	Control	Strychnine	Control
2.47	2.41	3.08	3.31	4.00	3.96

Tetanic rigor fails likewise to affect phosphorylytic ability. Experiments testing this point were conducted as follows: rats were given 2-3 injections of half M.L.D. (as determined for guinea-pigs) of tetanus toxin into the thigh muscle of the hind limb. Contracture of the treated limb was always induced by this treatment. After 1-2 weeks a comparison was carried out between the phosphorylytic ability of the contracted and the normal muscle. The figures (Table 8), showed no difference in phosphorylytic ability between normal and tetanus rigid muscle.

Table 8

mg. P_2O_5 decrease after incubation					
10 min.		30 min.		60 min.	
Normal	Tetanus rigor	Normal	Tetanus rigor	Normal	Tetanus rigor
2.04	1.97	2.98	2.98	3.43	3.43

(3) *Phosphorylysis by embryo muscle and by muscle of the newly born*

In excitability, chronaxie and contractility, embryo muscle resembles denervated and smooth muscle more closely than striped adult muscle. This resemblance is still manifest in the newly born animal and only disappears gradually as the muscle assumes its normal active function.

The phosphorylytic ability of muscle preparations from embryo of rats and rabbits a few days before birth has been found to be extremely low and remains so up to 3 days after birth. With increasing age the phosphorylytic power gradually increases, but in rats was still below the normal of adult animals even after 25 days (Table 9). Heart and liver preparations from the embryo and suckling showed the same phosphorylytic power as the corresponding tissue from grown animals. In this connection it may be recalled that, unlike muscle, heart and liver of embryo and suckling animals are in full function.

Table 9

Animals	Age in days	mg. P_2O_5 decrease during incubation				Remarks
		15 min.	30 min.	60 min.	120 min.	
Rat	Embryo	—	0.34	0.56	—	
Rat	Embryo	—	—	0.95	—	
Rabbit	Embryo	—	0.32	0.70	0.86	
Rat	3	—	0.40	0.64	0.80	
Rat	12	0.95	1.26	1.82	—	
Rat	25	1.6	2.16	2.94	—	
Rabbit	Embryo	—	1.10	1.48	1.90	Heart muscle (3 exp.)
Rat	3	—	—	0.98	1.20	Liver

(4) *Comparison of phosphorylytic abilities of different types of muscle*
(white and red, heart and smooth muscle)

Two main groups of striped muscle have long been distinguished: the red muscle, which reacts and fatigues comparatively slowly, and the less pigmented so-called white muscle which reacts and fatigues rapidly. White muscle yields steep action curves; red muscle, which has its most extremely developed representative in the smooth muscle system, yields flat action curves. In general the red-muscle group tends to resemble embryo and denervated muscle in mode of behaviour. Heart muscle is intermediate in position between red-striped muscle and smooth muscle. The differences between the various categories manifest themselves not only in chronaxie and in the contraction curves but also in chemical composition [cf., e.g., Embden & Adler, 1921]. It seemed of interest therefore to ascertain whether these functional groups differ also in phosphorylytic ability.

In a first series of experiments rabbits were used. As representatives of the white motor muscles, gastrocnemius muscle, biceps femoris muscle and the great thigh extensors were selected. They were found to possess roughly the same phosphorylytic ability. As representatives of red muscle we used *M. semitendinosus*, adductor longus, *M. vastus* and the diaphragm muscle. (Terminology as in *Anatomie des Kaninchens* by W. Krause, 1868.)

Fig. 4. Phosphorylysis by 0.3 g. muscle brei. A, white muscle; B, red muscle; C, heart muscle.

Fig. 4, showing the differences in phosphorylytic ability between white, red, and heart muscle, summarizes the result obtained. The slower the reactivity of a muscle in these groups, the smaller is its phosphorylytic ability. It will be seen that the difference in phosphorylytic activity is particularly pronounced when red and white muscles are compared; the activity of heart muscle approaches that of red muscle.

In a second series of experiments a comparison was carried out between different functional muscle types of birds. From the hen, white breast muscle, red leg muscle, heart muscle and smooth stomach muscle were taken. The phosphorylytic ability was again found to be related to the reactivity, declining in the following series: white muscle > red muscle > heart muscle > smooth muscle.

A comparative experiment, using muscle of pigeon, yielded a similar result. The red pigmented breast muscle of the pigeon was found to possess a lower phosphorylytic activity, than the white breast muscle of the hen. This was as expected from the different function in flight of the breast muscles of these species (Table 10).

Table 10

Bird	No. of exp.	Muscle	Phosphorylysis with 0.3 g. muscle brei after				
			15 min.	30 min.	60 min.	120 min.	180 min.
Hen	4	Breast	2.97	4.29	4.49	4.40	—
		Leg	1.49	2.37	2.87	3.16	—
		Heart	—	1.24	1.45	2.20	2.88
		Stomach	—	0.42	0.84	1.23	1.56
Pigeon	6	Breast	2.00	2.56	3.36	3.37	—
		Leg	1.12	1.75	2.30	3.06	—
		Heart	1.67	1.67	2.66	3.10	—
		Stomach	—	0.20	0.33	0.65	—

(5) *Diastatic activity*

Towards the conclusion of the experiments on phosphorylysis, parallel measurements on the diastatic activity were undertaken. In general, these were carried out with 0.5 g. muscle brei in 2 ml. bicarbonate-Krebs-Ringer containing 0.5% added glycogen. The mixtures were incubated at 37° and shaken during incubation. Diastase activity was assumed to correspond to the decrease in glycogen content over and above that calculated as due to phosphorylysis.

In denervated gastrocnemius muscle of rabbits 30 days after scission of the sciatic nerve, no loss of diastatic activity due to denervation was found, though the atrophy induced was of the order established in the previous experiments (see Table 11). A corroboration of this result was provided by corresponding experiments in which diastatic activity was estimated from the increase in reducing power.

Table 11. *Decrease of glycogen in mg.*

No. of exp.	1 hr.		2 hr.	
	Normal	Denervated	Normal	Denervated
3	1.27	1.13	2.55	2.13

A comparative experiment on the diastatic activities of white, red and heart muscle is summarized in Table 12.

Table 12. *Average of glycogen hydrolysis in mg. after incubation for 2 hr.*

No. of exp.	White muscle	Red muscle	Heart muscle
5	3.07	2.19	3.49

Diastatic activity is smaller in red muscle and somewhat larger in heart muscle than in white muscle. The relative distribution of diastase seems very different from that of the phosphorylysing system.

(6) *Phosphorylase and phosphoglucomutase in different muscles*

Decrease of inorganic phosphate as measured in the previous experiments is the resultant of the joint activity of at least two enzymes—phosphorylase and phosphoglucomutase. Experiments have shown that denervated muscle does not contain an inhibitor of phosphorylysis. It seemed likely that the loss in phosphorylytic power after denervation is due to a decrease in the amounts of enzyme present. To clear up this point, a differentiation of the activities of the several enzymes concerned is necessary. In the following, data are reported which show the separate activities of phosphorylase and phosphoglucomutase.

(a) *Phosphoglucomutase*. Glucomutase activity was estimated from the speed of conversion of Cori ester into Robison ester in mixtures containing 0.3 g. muscle brei, and 2 ml. bicarbonate buffer pH 7.2 containing 20 mg. dipotassium salt of Cori ester and $M/20$ NaF. Incubation was at 37° and the reaction was interrupted by addition of 0.5 ml. 20 % trichloroacetic acid. Cori ester was calculated from the increase in inorganic phosphate on 10 min. hydrolysis at 100° in N HCl (Table 13).

Table 13. *Decrease of Cori ester in mg. P_2O_5 after incubation*

Muscle	15 min.	30 min.	45 min.
White, normal	2.73	3.30	3.01
White, denervated (34 days after denervation)	0.86	1.58	2.06
Red muscle	1.11	1.49	2.20
Heart	0.78	1.20	1.05
Muscle of 2-day old rabbits	—	2.08	—
Hen stomach muscle*	0.25	—	0.80

* In this material phosphatase assays in the absence of NaF were also carried out. The findings were negative.

In general, muscle types which phosphorylyse less actively than normal white muscle are seen to contain subnormal quantities of phosphoglucomutase. Shortly after denervation, however, denervated muscle occasionally manifested a phosphoglucomutase activity equal to that of normal white muscle, as shown in Table 14.

Table 14. *Decrease of Cori ester in mg. P_2O_5*

Muscle	5 min.	15 min.	45 min.
Normal	1.60	1.40	1.31
Denervated (23 days after denervation)	1.45	1.70	1.65

(b) *Phosphorylase*. In brei or extract the activity of the phosphorylase cannot be separately measured. For this measurement phosphorylase preparations, which were freed of glucomutase as described by Cori *et al.* [1939], were used. Phosphorylase activity in this preparation is difficult to measure from the decrease in glycogen since equilibrium in the reaction $\text{Cori ester} \rightleftharpoons \text{glycogen}$ is strongly shifted to the left (10 % Cori ester, 90 % glycogen). The total phosphate changes, however, are small and rapidly ended. Cleavage-time curves are therefore difficult to establish. In the following experiments, phosphorylase activity was therefore estimated from the effect of the preparations on the velocity of the reaction to the right, i.e. on glycogen synthesis (Table 15). Under the conditions of the tests as Table 15 shows, white, red and heart muscles yield eluates of equal activity. These muscles appear to contain equal concentrations of phosphorylase. A similar rate of activity is found in muscle of new-born animals. Eluate of denervated muscle, on the other hand, shows approximately half the normal activity. Smooth muscle eluate manifests only a slight activity.

A surprising observation with heart muscle may be noted. With $C\gamma$ $\text{Al}(\text{OH})_3$ as the adsorbent, prepared according to Willstätter *et al.* [1925] and applied as described by Cori, eluates obtained from heart are practically inactive. The same adsorbent in white and

red muscle extracts yields eluate of good activity. With $C\beta$ $Al(OH)_3$, on the other hand, heart and other muscles yielded eluates of good activity.

Table 15. *Glycogen synthesis from Cori ester by eluate of different muscle*

Mixtures: 1 vol. eluate, and 0.5 vol. of addition mixture. Of the latter 1 ml. contained 10 mg. dipotassium salt of Cori ester, 1 mM muscle adenylic acid, and 0.5% glycogen. The selected concentrations of Cori ester adenylic acid, and glycogen are the optimal concentrations for synthesis as established by Cori & Cori [1940]. The final pH was 7.0, and the adsorbent was Cy in all experiments.

Muscle	% Cori ester converted into glycogen					
	5 min.	10 min.	15 min.	30 min.	60 min.	75 min.
White, normal	50	62	—	63	—	78
Denervated (3 weeks after operation)	25	36	—	56	—	71
Red	49	65	—	71	—	70
White, normal	28	—	61	63	—	—
Heart	22	—	44	59	—	—
Muscle of 2 day old rabbits	24	39	—	72	80	—
Pigeon stomach	—	—	6	10	13	—
Hen stomach	—	1	8	12	—	—

DISCUSSION

It has been shown that the phosphorylytic activity towards glycogen of denervated muscle is abnormally low. Low phosphorylytic activity is also shown by embryo muscle and by muscle of newly born animals. In the adult animal, the phosphorylytic activity of different kinds of muscle decreases in accordance with the following sequence: white muscle > red muscle > heart muscle > smooth muscle. It is known that denervation is followed by a loss in both contractility and excitability. These functions grow in intensity from a minimum in the embryo to a normal maximum in the adult. Moreover, the contractility and excitability of different muscles are known to follow a sequence which is identical with that noted above for the phosphorylytic activity. In every case lowered excitability and contractility is seen to be accompanied by a lowering in phosphorylysis. The existence of a relationship between these functions would thus appear most probable.

It seemed of importance to determine the biochemical cause of the observed difference in activity. Its explanation as an effect of the presence of inhibitors, or of the absence of coenzymes has been eliminated. There remains the alternative that the difference is one in enzyme concentration. Since glycogen phosphorylysis in muscle is the resultant of the joint action of phosphorylase and of the phosphoglucomutase of Cori, a comparison of the separate activities of these enzymes in the various types of muscle investigated was desirable. In every case of a marked lowering in the rate of phosphorylytic breakdown a pronounced deficiency in glucomutase was encountered. The concentration of phosphorylase, on the other hand, except in the case of denervated and smooth muscle where both phosphorylase and glucomutase were lowered, remained roughly constant. The primary limiting factor of phosphorylytic glycogen breakdown in muscle was always in these experiments the phosphoglucomutase. It seems probable therefore that this factor is also limiting in respect of synthesis.

Changed activity to glycogen in denervated muscle occurs only after denervation has caused atrophy. However, other types of inactivity atrophy such as those following tendotomy or application of plaster, failed to affect the enzyme activity. Scission of the spinal cord was likewise without effect on the phosphorylytic activity. Phosphorylysis appears to depend rather on the intact maintenance of the mechanism of innervation from the anterior horn cells. The changes in phosphorylytic ability go hand in hand with a diminution of the concentration in muscle of the birefringent substance. The latter is decreased after denervation atrophy but, as in the case of phosphorylytic ability, is

affected neither by tendotomy, nor by scission of the spinal cord [Fischer, 1941]. It seems likely that the phosphorylytic breakdown enzymes are contained in the birefringent complex.

All the conditions for which a lowered phosphorylytic ability has been shown above to exist are stated in the literature to be accompanied by lowering in phosphagen content. No explanation of this correspondence can be offered as yet. Experiments *in vitro* have failed so far to reveal any direct relationship between phosphagen and phosphorylytic ability.

SUMMARY

Brei and extract of denervated muscle have subnormal phosphorylytic activities towards glycogen. The phosphorylytic activity begins to fall off about 10 days after denervation. Other forms of severe atrophy, such as that induced by tendotomy or application of plaster cast, do not entail any loss of phosphorylytic activity towards glycogen.

In the third week after denervation some decrease is also manifest in glycolytic ability.

Electrical stimulation of muscle failed to affect the phosphorylytic ability. Strychnine convulsions were likewise without effect.

Embryo muscle and muscle of the newly born animal manifest only minimal phosphorylytic activity. As the muscles come into full function, the phosphorylytic activity increases and gradually approaches the normal.

The phosphorylytic activity of different muscle types decreases as follows: white muscle > red muscle > heart > smooth muscle. Except for denervated and smooth muscle, the phosphorylase content of different muscle types is fairly uniform. Denervated muscle shows half the phosphorylase activity of normal muscle. Lowered phosphorylytic activity was found, however, always to entail a marked diminution of the phosphoglucomutase concentration. In muscle the primary limiting factor of phosphorylytic glycogen breakdown and glycogen synthesis is the glucomutase.

The significance of phosphorylytic glycogen breakdown for muscle function has been discussed.

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