CVII. THE PRODUCTION OF LACTIC ACID IN FROG'S MUSCLE *IN VIVO*.

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ALMOST all the work which has been done upon the production of lactic acid in muscular activity (Fletcher and Hopkins, Meyerhof, etc.) has been carried out upon muscles removed from the animal body and consequently deprived of what must certainly be a potent factor in the recovery process *in vivo*, viz. the circulating blood. The experiments here described were carried out in order to see to what extent lactic acid produced by the activity of the living animal would accumulate in the muscles.

The work was begun in 1922 and as there is no prospect of our being able to complete the investigation we have considered it worth while to publish it in its present stage.

If a frog be placed in a large vessel of water at 35–40° it swims round with great violence and after a period of about 1 to 2 minutes it exhibits a jerky spasmodic movement of its legs as though the muscles were unable any longer to respond to the nervous stimuli being sent into them. Shortly after this stage all movement ceases and the frog becomes stiff and unconscious. But if, now, the animal be removed from the hot bath it will recover consciousness in a few minutes and gradually reacquire the power of movement in its limbs, though frequently the muscles do not appear to have recovered completely for at least half an hour. The various stages of this process were used for investigating the production and removal of lactic acid.

The following are typical observations made upon frogs under these conditions.

At 33°. Frog became weaker but could still swim about after being in the water up to 5 minutes.

At 35°. Frogs came to rest after 3 minutes and when taken out they seemed quite limp and toneless (cf. at 38°) until breathing and movements returned and then the tone seemed to return to the muscles all at once.

At 37°. Came to rest in 1 minute. Taken out at once. Eyes closed. Breathing started after 1 minute. Eyes slowly opened and breathing quickened. Legs not nearly so fixed as at 38° but still tend to return to a semi-extended position. Complete recovery after 2 minutes.

At 38°. (i) Frog swims about much more violently than at the lower temperatures tried. Stiff after 1 minute. Taken out at once. Legs stopped in a semi-extended position and returned to this point whether forcibly flexed or extended. Could first move legs very weakly after 2 minutes. Could first hop effectively after 4 minutes. Complete recovery in about 15 minutes.

(ii) Stiff after 1 minute. Left in bath 30 seconds longer. Breathing recommenced after about 1½ minutes. Reflexes in hind legs began to appear after 3½ minutes but could not be repeated at short intervals. Front legs remained stiff. Remained very sluggish for upward of 20 minutes. Breathing as in the normal frog during this time. Could not jump properly. Could not execute many movements properly. At end of 2 hours recovery in all limbs was complete and frog appeared normal in every way.

The observations on the lactic acid content of the muscles have been made only at 38°, the frog being left in the bath in each case until stiff, *i.e.* about 1 minute. The lactic acid was estimated by the micro-method of Meyerhof [1920], the muscle being removed as rapidly as possible from the hind legs of the frog used and one estimation made on each leg.

Procedure	g. lactic acid per 100 g. muscle	
"Resting minimum" of normal frog...	0.031	0.031
Muscle removed from frog and kept at 40° for 1 hour. ("Lactic acid maximum")	0.406	—
Muscle removed from frog and kept at 40° for 1 hour. ("Lactic acid maximum")	0.443	—
Pithed frog in bath at 38° for 1 min. 30 secs.	0.042	0.044
Active frog left in bath at 38° until stiff	0.112	0.125
" " "	0.125	—
" " "	0.112	0.113
" " "	—	0.176
" " "	—	0.125
Frog in bath as above, after recovery at room temperature for 18 minutes	0.107	0.111
" " " " 20 "	0.091	0.096
" " " " 32 "	—	0.070
" " " " 30 "	0.039	0.045
" " " " 60 "	0.037	0.040

It will be seen that at the time when the animal is unable any longer to bring about the contraction of its muscles, the latter contain a high percentage (average 0.125 %) of lactic acid. Meyerhof [1920] showed that the gastrocnemius of the frog, removed from the body and stimulated indirectly by means of a tetanising current until it could no longer respond, attained a concentration of lactic acid of about 0.19 %. Hence it is not improbable that the movements of the frog are arrested by the lactic acid accumulating under the conditions of our experiments more rapidly than it can be removed.

If the frog is pithed and left in the hot bath for a corresponding period, there is very little more lactic acid present than the normal resting minimum (0.043 % as against 0.031 %). The lactic acid is, clearly, the result of muscular activity.

The figures also show that the removal of this accumulated lactic acid is very slow. It is often not completely removed in half an hour, though after the lapse of an hour the resting minimum value has been practically regained.

The large lactic acid production and the slowness of the recovery process under these conditions should make this material very suitable for studies on lactic acid in the living organism.

SUMMARY.

1. When placed in a bath at 38° the frog is at first violently active, but becomes stiff and motionless in about 1 minute.
2. In this state the muscles of the hind legs show an average content of lactic acid of 0.125 %.
3. The muscles of a pithed frog under similar conditions contain little more lactic acid (0.043 %) than the normal resting minimum (0.03 %).
4. On removal to room temperature the frog recovers in a few minutes, but the lactic acid content of the muscles does not regain the normal level until half to one hour later.

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REFERENCES.

- Meyerhof (1920). *Pflüger's Arch.* **182**, 232.