blood supply. Packing CO_2 snow around the muscle, starting at the insertion and proceeding to the origin, has given the lowest values for lactic acid.

Fletcher[1] found 59 mg. per cent in rabbit muscle, but excised the muscle before freezing it in liquid air. We have obtained similar results from animals which died (from the anesthetic) during the dissection, even though the muscles were frozen *in situ* immediately and without stimulation. It seems likely that mammalian muscle forms lactic acid with such rapidity when oxygenation is stopped that it is possible to obtain a value approximating the normal only when it is frozen with the circulation still intact.

We have used ice cold trichloracetic acid (5% aqueous solution) as a protein precipitant. After freezing, the muscles were excised and the unfrozen portion at the origin discarded. They were then sliced, while frozen, into sections of about 0.1 mm. thickness and immediately dropped into the trichloracetic acid. Lactic acid was determined by the Friedemann, Cotonio and Shaffer[2] procedure adapted to quantities between 0.02 and 0.2 mg.

[1] Fletcher, W. M., *J. Physiol.*, 1914, xlvii, 361.

[2] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, lxxiii, 335.

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Inhibition of Lactic Acid Formation in Muscle by Extract of Desiccated Pancreas.

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Hopkins and Winfield[1] showed that extracts of pancreas could inhibit the formation of lactic acid in chopped muscle. This work did not exclude the possibility that the inhibitory factor was trypsin. Foster and Woodrow[2] working in Hopkins' laboratory made further investigation of this substance. They found that it was neither trypsin, insulin nor anti-gloxylase, and that heating it for 10 minutes at 85° C. greatly reduced its activity but did not completely destroy it. They reported an inhibition between 40 and 60% of the total lactic acid which was formed in control samples of muscles, a fact which they interpreted as evidence of two mechanisms for lactic

acid production in muscle, one of which was inhibited by the pancreatic factor.

In an attempt to determine the mechanism of the action of this substance, we confirm the observations of Foster and Woodrow,[2] obtaining an inhibition of between 40 and 60% of the total lactic acid production in muscle hash in concentrations similar to theirs (extract of 5 mg. of desiccated pancreas acting on between 3 and 4 gm. of muscle in 10 cc. of phosphate buffer). Increasing the concentration produces no greater inhibition. This action is not due to trypsin as Foster has demonstrated. That the inhibiting factor, as prepared, usually contains no trypsin is shown by the fact that filtrates after HgC12 precipitation of the proteins give no Biuret test. The non-protein nitrogen content of muscle extracts treated for 3 hours with the inhibiting factor usually shows no increase. In a few cases, there occurs a rise from 1 to 2 mg. percent, an increase which does not affect the inhibitory action of the extract upon lactic acid formation.

A change in pH or in salt concentration is not the cause of the inhibition. Certain inactive extracts having the same salt concentration and pH as active extracts have had no inhibiting effect. In all experiments which are reported here the pH has been carefully controlled by regulating the pancreatic extract to the pH of the buffer before adding it to the reaction mixture. It has been shown that the inhibition is not due to direct action of the extract upon the carbohydrate itself. That the inhibiting factor as prepared is rich in amylase is shown by its action on glycogen solutions. In a period of 2 hours, this extract acting on a 0.5% glycogen solution, in a concentration similar to that used in the experiments, converts from 50 to 60% of the glycogen into a free reducing substance. However, some pancreatic preparations showing a marked amylolytic activity have been inactive as inhibitors of lactic acid formation. In hashed muscle, the inhibiting factor not only prevents the formation of lactic acid but also interferes with the disappearance of carbohydrates. The inorganic phosphates, on the other hand, are increased to the same extent as when the inhibiting factor is not employed.

If the increase in free phosphate may be taken as a measure of the breakdown of hexose phosphate and if the presence of hexose mono- and diphosphate may be assumed, the changes in phosphate will account for the lactic acid which is formed. In muscle, therefore, it appears that the formation of lactic acid from preformed hexose phosphate is not inhibited by the pancreatic extract.

TABLE I.

The influence of the inhibiting factor on changes occurring in hashed muscle. Expressed as mg. per 100 gm. of muscle.

Source of muscle	Lactic Acid Increase		Phosphate Increase expressed as glucose from hexose monophosphate		Total Carbohydrate Loss expressed as glucose	
	Control	Inhibitor	Control	Inhibitor	Control	Inhibitor
Rabbit	842	420	480	496	430	20
Rabbit	647	342	378	393	315	13
Rabbit	728	389	420	430	340	00
Chicken	467	238	252	268	220	16

Upon the muscle extract of Meyerhof[3] the inhibiting substance has a similar but more striking effect. It almost completely prevents the formation of lactic acid. It allows of no loss in the total carbohydrate. Its effect upon free phosphate concentration, however, differs from that observed in hashed muscle. In Table II it will be seen that there is no change in free phosphate concentration in the experiments where the inhibitor has been added.

TABLE II.

Influence of the inhibiting factor on changes occurring in muscle extract. Expressed as mg. per 100 cc. of muscle extract.

No.	Lactic Acid Increase		Phosphate Changes expressed as glucose from hexose monophosphate		Total Carbohydrate Changes expressed as glucose	
	Control	Inhibitor	Control	Inhibitor	Control	Inhibitor
1	+ 93.6	+2.3	—13.6	+2.60	—100.0	±0.0
2	+106.0	+0.6	—12.9	+1.8	—113.0	—0.3
3	+101.0	+0.9	—15.2	+1.2	—110.0	—1.1
4	+ 68.0	±0.0	—23.2	±0.0	— 95.0	—0.4

The inhibiting factor prevents the loss of phosphate in muscle extract. Since the same extract shows no loss in carbohydrate, we must assume that the formation of hexose phosphate has been inhibited and that the point of action of the inhibiting substance is on the formation of hexose phosphate. Since there is no preformed hexose phosphate in muscle extract and its formation can be inhibited by the pancreatic factor, complete checking of carbohydrate loss and of lactic acid formation is possible.

[1] Hopkins, F. G., and Winfield, G., *J. Physiol.*, 1915, l, 5.

[2] Foster, F. L., and Woodrow, C. E., *Biochem. J.*, 1924, xviii, 562.

[3] Meyerhof, Otto, *Biochem. Z.*, 1927, clxxxiii, 176.