CXXXI. PANCREATIC EXTRACTS IN RELATION TO LACTIC ACID FORMATION IN MUSCLE.

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THE pancreatic factor of Winfield and Hopkins [1915] which inhibits the formation of lactic acid in muscle and muscle extracts, has been shown [McCullagh, 1928] to owe its effect to suppression of esterification of phosphates. The first portion of the work herein reported was carried out, using a new technique to be described, with a view to discovering the properties of the pancreatic factor and its distribution throughout various body tissues. Results were soon obtained, however, which led us to suspect the conclusions of former workers in regard to the possible identity of this factor with other tissue constituents. It seems to have been established that neither trypsin nor insulin is responsible for the inhibition; and Downes [1927], Reay [1927] and Ronzoni [1927] state that in their opinion the inhibitory effect of pancreas and pancreatic extracts is not due to amylase. Our preliminary results, however, indicated the necessity of further work in this connection.

PART I. PROPERTIES AND DISTRIBUTION OF THE FACTOR.

Methods. Preparation of the muscle extract was at first carried out as described in the previous paper. However, it was speedily found that such elaborate precautions in regard to temperature were superfluous; the extracts obtained by the following procedure are, in general, if anything more active. Immediately after amytal anaesthesia the rabbit is bled from the heart, the hind quarters are at once stripped and the leg muscles removed. Mincing is followed by extraction with three times the weight of ice-cold distilled water for half an hour, the extracting vessel being kept immersed in a freezing mixture. After filtering through muslin the filtrate is kept cold in the same way, and apart from this no cooling measures are necessary.

Technique employed for the study of the pancreatic factor.

In the presence of fluoride, hexosephosphate is not hydrolysed by muscle enzymes and free phosphate therefore rapidly disappears owing to its removal by esterification. As formerly shown the free phosphate does not disappear

in the presence of the pancreatic extract. The new method of studying the pancreatic inhibitor is based on the measurement of the suppression of esterification of phosphate in the presence of fluoride.

The following substances are placed in a test-tube:

(*a*) 1 cc. extract containing the pancreatic factor (or distilled water for control);

(*b*) 2 cc. 2 % soluble starch;

(*c*) 2 cc. fluoride buffer (as described in former work);

(*d*) 5 cc. muscle extract.

The contents of the tubes are thoroughly mixed and a 3 cc. sample is immediately withdrawn and delivered into a test-tube containing 10 cc. 6 % trichloroacetic acid. The mixture is then incubated for 1 hour at 30° and again sampled in the same manner. The samples are thoroughly mixed with the acid, allowed to stand 5 minutes and filtered. Free phosphate is estimated in 3 cc. of the filtrate by the Briggs modification of the Bell-Doisy method.

In studying the distribution of the factor in various tissues, the extract was prepared in each case by triturating the tissue with sand plus ten times its weight of water, allowing it to stand in the ice-chest for 1 hour previous to centrifuging, and using the supernatant fluid.

Some properties of the inhibiting factor.

Thermostability. The statements in the literature concerning the thermostability of the inhibiting factor vary somewhat. 5 cc. of pancreatic extract were placed in a thin glass test-tube which was immersed in a boiling water-bath for 2 minutes. This caused a complete destruction of the inhibiting factor as shown by Table I.

Table I.

	mg. lactic acid in 15 cc. sample.		
	Before incubation	After incubation	Change
1. Water control	9·7	25·8	16·1
2. Pancreas	9·7	10·3	0·6
3. Boiled pancreas	9·7	25·7	16·0

In this experiment the technique of the former paper was followed, viz. each flask contained 25 cc. muscle extract, 10 cc. 2 % starch, 10 cc. phosphate buffer as previously described, and 5 cc. pancreatic extract or water. The production of lactic acid during an hour's incubation was used as a criterion of activity. The slight increase in lactic acid in the flask containing unboiled pancreas was probably derived from preformed hexosephosphate.

Dialysability. As a result of several attempts we have found that the inhibiting factor will not pass through a collodion membrane. The formation of hexosephosphates was in no case suppressed by the addition of the outer dialysing fluid to the incubation mixture, whereas the inner dialysing fluid was at the same time very active. Table II gives typical results, dialysis in this case being continued for a week.

Table II.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change
1. Water control	0·81	0·32	−0·49
2. Inner fluid	0·80	0·80	0·00
3. Outer fluid	0·80	0·32	−0·48

Adsorbability. Reay and Downes made several attempts to adsorb the factor with but little success; in view of other work to be reported it seemed unnecessary to confirm these results. We have, however, carried out experiments in regard to the precipitability of the factor by means of heavy metals, etc., but have obtained only negative results.

Distribution of the inhibiting factor.

Pancreas preparations were always found to be 50 to 500 times as efficacious as any other preparations except those from tryptic juice, saliva, and salivary glands. Table III gives figures showing the effect of various tissues on the formation of hexosephosphate.

Table III.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change	% inhibition	
1. Water control	0·84	0·16	−0·68	0	
2. Pancreas (ox, no. 1)	0·86	0·84	−0·02	97	
3. Pancreas (ox, no. 2)	0·85	0·36	−0·49	28	
4. Parotid (rabbit)	0·89	0·89	0·00	100	
5. Kidney (rabbit)	0·85	0·31	−0·54	21	
6. Liver (rabbit)	0·86	0·48	−0·38	44	
7. Suprarenals (rabbit)	0·86	0·32	−0·54	21	
8. Spleen (rabbit)	0·87	0·46	−0·41	40	
9. Brain (rabbit)	0·84	0·23	−0·61	10	
10. Bladder (rabbit)	0·85	0·21	−0·64	6	
11. Leg muscle (rabbit)	0·86	0·16	−0·70	3 %	acceleration
12. Ovary (rabbit)	0·85	0·18	−0·67	1	
13. Lung (rabbit)	0·85	0·23	−0·62	9	
14. Kidney (ox)	0·84	0·34	−0·50	26	
15. Liver (ox)	0·85	0·56	−0·29	57	
16. Spleen (ox)	0·84	0·45	−0·39	43	
17. Brain (ox)	0·85	0·26	−0·59	13	
18. Bladder (ox)	0·85	0·21	−0·64	6	
19. Lung (ox)	0·84	0·28	−0·56	18	

All the above preparations are aqueous extracts. With the exception of the pancreas all are prepared so that 1 g. of tissue is equivalent to 10 cc. of extract. In the case of pancreas (ox, no. 1) 1 g. represents 25 cc., while in the case of pancreas (ox, no. 2) 1 g. represents 1·25 l.

All tissues except parotid show an inhibition of the same order as the very dilute pancreas preparation. Unreported experiments show inhibitions between 15 and 80 % to be in almost direct proportion to the amount of inhibitor present.

Table IV is the protocol of an experiment to show the relative concentrations of inhibitor in saliva, tryptic juice, and pancreas.

Table IV.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change	% inhibition
1. Water control	0·84	0·38	−0·46	0
2. Saliva 1 : 25	0·86	0·81	−0·05	89
3. Saliva 1 : 100	0·86	0·67	−0·19	59
4. Saliva 1 : 500	0·86	0·49	−0·37	20
5. Tryptic juice 1 : 50	0·85	0·84	−0·01	98
6. Tryptic juice 1 : 100	0·85	0·77	−0·08	82
7. Tryptic juice 1 : 500	0·85	0·60	−0·15	67
8. Tryptic juice 1 : 1000	0·85	0·48	−0·37	20
9. Pancreas 1 : 250	0·85	0·74	−0·11	76
10. Pancreas 1 : 500	0·85	0·60	−0·25	56
11. Pancreas 1 : 1000	0·86	0·43	−0·43	7

From this it is evident that these tissues and fluids all possess approximately the same power to inhibit the formation of hexosephosphate, 1 cc. of a 1 in 500 dilution of saliva being slightly less potent than the 1 in 500 tryptic juice and the 1 in 500 pancreas preparation.

From the results of the foregoing experiments concerning the properties and distribution of the inhibitor it was noted that:

(*a*) it is not more thermostable than amylase;

(*b*) like amylase it will not pass through a dialysing membrane;

(*c*) it resembles amylase in being difficult to adsorb;

(*d*) the distribution corresponds to that of amylase.

This led us to suspect that the phenomenon was nothing more than a manifestation of amylolytic activity. Part II of this paper reports work which confirms this view.

Part II.

The first point which presented itself was whether or not the inhibition might arise from the muscle enzyme system being destroyed or inactivated by the pancreas. This point was settled by the following experiment, the results of which are given in Table V. In tubes (1) and (2) muscle extract and pancreas were incubated together for 15 minutes at 30°, while in tubes (3), (4) and (5) the muscle extract was simultaneously incubated alone. At the end of this primary incubation the remainder of the substances (starch, etc.) was added to all the tubes and the initial samples were withdrawn. Incubation was then carried on as usual for 1 hour at 30°. The pancreas preparation used was an aqueous extract of ox pancreas, 250 cc. of which represented 1 g. of pancreas, 1 cc. being used in each tube as usual. A preliminary experiment had shown that this extract would bring about only a partial inhibition with respect to the muscle enzyme used.

Table V.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change	% inhibition
1. Pancreas	0·87	0·71	−0·16	71
2. Pancreas	0·86	0·70	−0·16	71
3. Pancreas	0·84	0·67	−0·17	70
4. Pancreas	0·83	0·67	−0·16	71
5. Water control	0·84	0·33	−0·51	0

The incubation of numbers 1 and 2 with pancreas has made no appreciable difference in the amount of inhibition. It is probable that the slight excess of phosphate present in tubes 1 and 2 was derived from hexosephosphates during the first incubation. During this period the resynthesis of hexosephosphate from glycogen and free phosphate would not take place extensively owing to the presence of pancreas. In tubes 3 and 4 there would be some synthesis of hexosephosphate during the primary incubation. Tube 5 was the water control. This demonstrates that the muscle enzyme is not being destroyed by the pancreatic extract.

The extent of inhibition, if due to an amylolytic effect on the starch, should decrease if the percentage of starch in the incubation mixture is increased. After preliminary experiments to ascertain the amount of pancreas necessary to give a partial inhibition with the muscle enzyme preparation in use, the following experiments were performed to obtain information on this point.

Table VI.

	mg. P as free phosphate in 3 cc. sample. Before incubation	After incubation	Change	% inhibition
1. H_2O and 2 % starch	0·68	0·12	−0·56	0
2. Pancreas and 2 % starch	0·68	0·66	−0·02	96
3. H_2O and 4 % starch	0·68	0·12	−0·56	0
4. Pancreas and 4 % starch	0·68	0·32	−0·36	36
5. H_2O and 6 % starch	0·68	0·12	−0·56	0
6. Pancreas and 6 % starch	0·68	0·22	−0·46	18

From this it appears that an increase in starch concentration within the above limits does not affect the rate of formation of hexosephosphate by the muscle enzyme system. The increasing amounts of substrate, however, do cause a marked decrease in potency of the pancreatic inhibitor. This indicates that the inhibition must be due, at least in part, to amylase.

Wohl and Glimm [1910] have shown that the activity of amylase is suppressed in the presence of large amounts of maltose, whereas sucrose, galactose, and fructose have no effect on the amylolytic activity. We find that in the presence of large amounts of sugar the muscle enzyme system is less active. The pancreas, however, still causes its usual inhibition except in the presence of maltose. Table VII is the protocol of an experiment to demonstrate this.

Table VII.

	mg. P as free phosphate in 3 cc. sample. Before incubation	After incubation	Change	% inhibition due to pancreas
1. Water control	0·66	0·05	−0·61	0
2. Pancreas	0·67	0·68	+0·01	101
3. H_2O and 2 g. galactose	0·60	0·28	−0·32	0
4. Pancreas and 2 g. galactose	0·60	0·60	0·00	100
5. H_2O and 2 g. sucrose	0·60	0·05	−0·55	0
6. Pancreas and 2 g. sucrose	0·61	0·60	−0·01	99
7. H_2O and 2 g. fructose	0·59	0·25	−0·34	0
8. Pancreas and 2 g. fructose	0·59	0·60	+0·01	101
9. H_2O and 1·5 g. maltose	0·62	0·21	−0·41	0
10. Pancreas and 1·5 g. maltose	0·62	0·44	−0·18	56

Tube no. 1 is a water control without any pancreas or sugar. Another water control was incubated with each of the sugars used, as the different sugars varied in the degree in which they themselves inhibited the muscle enzyme system. In each case much more pancreas was used than was necessary to cause complete inhibition. The sugars were added in crystalline form to the incubation mixtures.

Percentage inhibitions by pancreas in the above table are calculated, in each case, by considering the corresponding tube containing sugar alone to show no inhibition. In the presence of galactose, sucrose, or fructose, the pancreas causes complete inhibition. In the case of maltose, however, in spite of the large amount of pancreas present there was only a partial inhibition.

The experiment on maltose was repeated using a pancreatic preparation which, by means of previous experiments, we knew to be sufficiently dilute to cause only a partial inhibition in the absence of maltose. Table VIII gives the results.

Table VIII.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change	% inhibition due to pancreas
1. Water control	0·78	0·05	−0·73	0
2. Pancreas	0·78	0·63	−0·15	79
3. H_2O and 1·5 g. maltose	0·71	0·51	−0·20	0
4. Pancreas and 1·5 g. maltose	0·70	0·50	−0·20	0

It is evident that with these more dilute solutions of pancreas the maltose is capable of completely preventing activity of the inhibitor.

In both the above experiments there is an apparent discrepancy between the figures given for phosphates previous to incubation. This is caused by the changes in volume produced by the addition of the solid sugars.

If the inhibiting substance is amylase, different amylolytic preparations should have the same relative diastatic power as they have relative power of inhibition. On April 30th, 1928, we had the following preparations in the ice-chest, where they had been stored: (1) an alcoholic preparation of desiccated pancreas prepared March 2nd, 1928; (2) a similar preparation of March 29th, 1928; (3) a preparation which had been subjected to tryptic digestion at 37° for 3 days, previous to removing trypsin and protein with alcohol in the usual manner; (4) an alcoholic preparation of April 26th, 1928; (5) an alcoholic preparation of saliva made on April 26th, 1928.

The inhibiting power of these preparations was now tested. In each case a series of tubes containing the preparation in various dilutions was incubated for 1 hour in the ordinary manner at 30°, with the results shown in Table IX. Only those dilutions which are of interest in demonstrating the relative inhibiting power are given in the table.

Preparations 2 and 4 are the most potent and about equal in inhibiting power. Preparation 5 is next in strength, whereas 1 and 3 are both less effective.

Table IX.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change	% inhibition
1. Water control	0·72	0·10	−0·62	0
2. Pancreas prep. 1 (undiluted)	0·72	0·55	−0·17	77
3. Pancreas prep. 2 dil. 1 : 10	0·73	0·52	−0·21	66
4. Pancreas prep. 3 (undiluted)	0·73	0·54	−0·19	69
5. Pancreas prep. 4 dil. 1 : 10	0·74	0·55	−0·19	69
6. Saliva prep. dil. 1 : 5	0·74	0·42	−0·32	48

The relative diastatic powers of these same preparations were measured by mixing 1 cc. of each preparation with 5 cc. 1 % soluble starch and 3 cc. of chloride-containing buffer, and then incubating at 30°. In each case the time was noted at which a two-drop sample showed with iodine a change from the pure starch blue colour.

The results are given in the following table:

Table X.

Preparation	1	2	3	4	5
Time in mins.	30	5	40	5	20

Once more preparations 2 and 4 proved to be the strongest, followed by 5, with 1 and 3 the least potent. That is, they have the same relative diastatic activity as they have power of inhibiting esterification.

It seemed desirable to add confirmation by measurement of the power to inhibit the actual production of lactic acid. To this end flasks were set up as follows, and lactic acid determinations carried out before and after incubation. The technique was the same as in the former paper except that the method of Friedemann, Cotonio and Shaffer [1927] was used for the determination of lactic acid. Each flask contained 25 cc. muscle extract, 10 cc. soluble starch, and 10 cc. phosphate buffer. Besides this, flask no. 1 contained 5 cc. water; no. 2, 5 cc. of a 1 in 2 dilution of pancreas preparation 1; no. 3, 5 cc. of a 1 in 20 dilution of preparation 2; no. 4, 5 cc. of a 1 in 2 dilution of preparation 3; no. 5, 5 cc. of a 1 in 20 dilution of preparation 4; no. 6, 5 cc. of a 1 in 10 dilution of saliva preparation no. 5. These dilutions were all calculated to give a partial inhibition. The results of the lactic acid determinations are given in Table XI.

Table XI.

mg. lactic acid in 15 cc. sample.

	Before incubation	After incubation	Change	% inhibition
1.	6·4	23·7	17·3	0
2.	7·0	15·4	8·4	51
3.	6·4	12·2	5·8	66
4.	6·6	14·0	7·4	57
5.	5·8	11·8	6·0	65
6.	5·4	12·2	7·8	55

When the dilutions of the various preparations are considered it is seen that again the distribution of inhibiting power is the same as in the two

previous experiments; preparations 2 and 4 being more potent than 5, which is half as strong, and 1 and 3 which are about one-tenth as strong.

This incidentally demonstrates the admissibility of results obtained by the phosphate technique, as did other lactic acid measurements made at intervals throughout the work.

It seemed possible that, although a part of the inhibition was due to amylase, there might be some other factor present in pancreas which would inhibit esterification. If such a factor be present it is unlikely that it would be destroyed by heat at exactly the same rate as the amylase.

10 cc. samples of a preparation made from desiccated pancreas (as described in the former paper) were placed in test-tubes and immersed in a boiling water-bath for 18 and 21 seconds respectively. The diastatic action of these preparations was tested by the same method as that described above. The times before a change in colour was observed are as follows:

	Original preparation	Sample no. 1	Sample no. 2
Time (mins.)	4	8·25	12

From the above the original preparation is seen to be practically twice as powerfully diastatic as sample no. 1, and three times as strong as no. 2.

These amylolytic solutions were now tested for inhibiting power by setting up tubes in various dilutions. The results obtained with the tubes which contained suitable dilutions of the various solutions are given in Table XII.

Table XII.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change	% inhibition
1. Water control	0·72	0·13	−0·59	0
2. Original pancreas dil. 1 : 6	0·72	0·48	−0·24	59
3. Sample no. 1 dil. 1 : 3	0·72	0·45	−0·27	54
4. Sample no. 2 dil. 1 : 2	0·72	0·45	−0·27	54

This demonstrates that in respect to inhibiting power the original preparation is twice as active as sample no. 1, and three times as active as sample no. 2, which indicates that the only substance in these preparations capable of inhibiting esterification is destroyed by heat at the same rate as amylase.

Meyerhof [1927] has recently pointed out that the muscle enzyme system is not only capable of producing lactic acid from starch, glycogen, and hexosans, but can also produce it from glucose if that sugar be activated. He describes methods of preparing activator from muscle or from yeast. If the inhibiting effect of pancreas on the lactic acid production is a function of amylase, one should not expect inhibition to occur when lactic acid is produced from glucose, even in the presence of pancreas. The activator in the following experiments was prepared from yeast by the method described by Meyerhof. In this experiment six flasks were set up as follows:

1. 25 cc. muscle extract + 10 cc. phosphate buffer + 10 cc. 2 % starch + 5 cc. H_2O.

2. 25 cc. muscle extract + 10 cc. phosphate buffer + 10 cc. 2 % starch + 5 cc. pancreas.
3. 25 cc. muscle extract + 10 cc. phosphate buffer + 15 cc. H_2O.
4. 25 cc. muscle extract + 10 cc. phosphate buffer + 5 cc. 4 % glucose + 10 cc. H_2O.
5. 25 cc. muscle extract + 10 cc. phosphate buffer + 5 cc. 4 % glucose + 5 cc. H_2O + 5 cc. activator.
6. 25 cc. muscle extract + 10 cc. phosphate buffer + 5 cc. 4 % glucose + 5 cc. pancreas + 5 cc. activator.

Lactic acid estimations were carried out before and after incubation and Table XIII gives the results.

Table XIII.

	mg. lactic acid in 15 cc. sample. Before incubation	After incubation	Change
1.	10·9	19·3	8·4
2.	11·9	14·9	3·0
3.	10·8	12·8	2·0
4.	10·4	12·8	2·4
5.	10·8	27·2	16·4
6.	13·1	32·0	18·9

Flasks 1 and 2 show that both the muscle enzyme preparation and the pancreas are quite active. The lactic acid formation from glycogen and hexose-phosphate in the muscle extract is small as demonstrated by flask 3. Flask 4 shows that the addition of glucose causes no increase in the amount of lactic acid formed, unless, as in flask 5, activator be added. Flask 6 makes it quite evident that the formation of lactic acid from activated glucose is not affected by the presence of pancreas.

It has been shown above that animal amylases have the power of inhibiting the formation of hexosephosphates, and *ipso facto*, that of lactic acid from starch. If this is a purely amylolytic phenomenon, plant diastases would be expected to have the same effect. We therefore tested takadiastase and malt diastase, with the results given in Table XIV.

Table XIV.

	mg. P as free phosphate in 3 cc. sample. Before incubation	After incubation	Change	% inhibition
1. 1 cc. H_2O	0·78	0·08	−0·70	0
1 cc. takadiastase	1·52	1·51	−0·01	99
2. 1 cc. H_2O	0·66	0·05	−0·61	0
1 cc. malt diastase	0·68	0·50	−0·18	70

Controls with boiled enzymes showed no inhibition. The high values for takadiastase are due to the phosphate content of the commercial preparation.

As expected, both these preparations are quite active as inhibitors. The malt diastase did not cause complete inhibition, but was not a highly active diastatic preparation.

As starch has been used as a substrate in nearly all the work herein recorded, it seemed advisable to show that the inhibition also occurs when the substrate is glycogen. Using a 2 % solution of glycogen instead of the usual 2 % soluble starch the figures given in Table XV were obtained.

Table XV.

mg. P as free phosphate in 3 cc. sample.

	Before incubation	After incubation	Change	% inhibition
1. Water control	0·84	0·29	0·55	0
2. Pancreas	0·84	0·84	0·00	100

The inhibition therefore occurs whether starch or glycogen is used.

In many of the above experiments the mixture was tested with iodine immediately before incubation. Starch was found to be present, even in tubes where inhibition proved to be complete. This eliminates the possibility that starch has been removed by enzymic hydrolysis before it becomes accessible to the muscle enzymes.

In all the experimental work reported above, guarded pipettes were always employed, in order to avoid the possibility of salivary contamination.

Discussion and Summary.

The first part of the paper demonstrates that the properties and distribution of the factor are the same as those of amylase. It is shown in the second part of the paper that the muscle enzyme system is not itself affected by the inhibitor. As different concentrations of substrate cause variation in the inhibiting power of pancreas, the action would appear to be on the starch. Amylase is inhibited in its activity by maltose but not by certain other sugars; this is also the case with the pancreatic factor. The amylolytic power and the ability to inhibit esterification and lactic fermentation have been shown to correspond in the case of different preparations, and to be destroyed to the same extent by heat. The production of lactic acid from glucose is not in the least inhibited by the pancreas. Both animal and vegetable amylases act as inhibitors when the substrate is starch or glycogen.

These results all indicate that the so-called pancreatic factor which inhibits lactic acid production by muscle enzymes is merely amylase. Statements in the literature to the effect that preparations diastatically active cause no inhibition are probably explained by the fact that the workers in question employed muscle hash, in which partial inhibitions are much more difficult to measure. The same fact undoubtedly accounts for their failure to obtain complete inhibitions. Not only does the question of permeability come into play, but the presence of an activating mechanism in muscle hash would probably result in the production of considerable amounts of lactic acid from glucose, even in the presence of pancreas. Using the muscle enzyme system of Meyerhof, we have never succeeded in obtaining either a preparation containing the inhibiting factor which was not diastatically active or *vice versa*.

Dakin and Dudley [1913] have demonstrated the presence of an antiglyoxalase in pancreas. Ariyama [1928] has shown the production under certain circumstances of a glyoxal-like compound from hexosephosphate by muscle enzymes. After the destruction of glyoxalase by heat or by means of antiglyoxalase, the best yield of this substance obtained was only 11 % of the theoretical amount. Thus antiglyoxalase might conceivably cause a partial inhibition. It is our opinion that other than antiglyoxalase and amylase there is no substance in pancreas which inhibits the formation of lactic acid in muscle.

The observations and conclusions recorded above render slight the possibility that some specific pancreatic factor controls lactic acid production in muscle. Apart from any physiological significance, however, the results are of great interest from a purely biochemical standpoint. It is possible that this work may open up a new path whereby a study may be made of the carbohydrate precursor of hexosephosphates in muscle. Moreover, in view of the relatively small amounts of pancreas which are capable of bringing about complete and instantaneous inhibition, it may be that some molecular union between amylase and its substrate, prior to enzymic hydrolysis, can be demonstrated.

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REFERENCES.

Ariyama (1928). *J. Biol. Chem.* **77**, 395.
Dakin and Dudley (1913). *J. Biol. Chem.* **15**, 463.
Downes (1927). Thesis (Cambridge).
Friedemann, Cotonio and Shaffer (1927). *J. Biol. Chem.* **73**, 335.
McCullagh (1928). *Biochem. J.* **22**, 402.
Meyerhof (1927). *Biochem. Z.* **183**, 176.
Reay (1927). Thesis (Cambridge).
Ronzoni (1927). *Proc. Soc. Exp. Biol. Med.* **25**, 178.
Winfield and Hopkins (1915). *J. Physiol.* **50**, *Proc.* v.
Wohl and Glimm (1910). *Biochem. Z.* **27**, 349.