ON THE INFLUENCE OF THE PANCREAS ON THE GLYCOLYTIC POWER OF MUSCLE

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Since Minkowski and von Mering published twenty years ago the results of their investigations on pancreatic diabetes and showed that some internal secretion of the pancreas played an important part in the mechanism of the metabolism of sugar in the body, many investigators have tried to discover the method by which the internal secretion worked.

Certain observations by O. Cohnheim¹ in a series of papers in 1903-5 seemed to throw light on this question. He found that while neither fresh muscle juice nor pancreatic extract had alone any marked tendency to the destruction of sugar *in vitro*, a mixture of the two possessed such an action in a marked degree; and he further appeared to have demonstrated that the relationship of the two factors was similar to Ehrlich's conception of ferment and amboceptor; the muscle moiety being thermolabile, insoluble in alcohol and readily destroyed by any adverse conditions; the pancreatic moiety, on the other hand, thermostable, soluble in alcohol, and when in excess having an inhibitory power.

Arnheim and Rosenbaum,² Hirsch,³ Dewitt,⁴ and Hall⁵ have published papers in confirmation of his observations, but these have been contested by Claus and Embden,⁶ McGuigan⁷ and others, and, unfortunately, the results obtained clinically by pancreatic feeding in diabetes have not been such as to confirm the hopes built on his observations.

As a preliminary to an intended investigation of this clinical failure I attempted to repeat Cohnheim's results, but found that in my hands no satisfactory results could be obtained.

PREPARATION OF EXTRACTS

At first slaughter-house muscle and pancreas obtained within half-an-hour to one hour after death were used, warm and not in rigor. The muscle was cut up into small pieces and well washed in cold tap-water, then pounded in a mortar with sand and expressed by a hand-press as completely as possible; usually water was added to the residue and it was then further expressed, and these washings added to the fluid obtained from the first expression. The pancreas was treated in the same way, but the expression was not carried far, as only a few cubic centimetres were required for each experiment. One hundred grammes of muscle was found to yield about 30 to 40 c.c. of juice by first expression; 5 to 7 c.c. of pancreatic extract is equal to 1 gramme of fresh pancreas.

The instruments and apparatus used both in extracting the juices and in the further course of the experiments were sterilised by boiling or by steam, except the pipettes and measuring flasks, which were thoroughly washed in water and then dried by alcohol. The juices were measured into flasks, roughly equivalent amounts of sterile solution of dextrose added to each; about 5 or 6 c.c. of 10 per cent. solution of phosphates (nine parts of di-sodium to one part of mono-sodium phosphate) was added to preserve neutrality, and 2 to 5 c.c. of toluol, the amounts in each experiment were then made up to constant volume with boiled cold water. The concentrations were, approximately, extract of 600 grammes muscle, extract of 20 grammes pancreas, 1.5 to 2 grammes dextrose, 10 to 20 grammes phosphate, 40 to 60 c.c. of toluol per litre.

Parts of the mixtures were diluted to the required proportion and estimated directly by Pavy's method; the rest incubated at 37° C. for twenty to twenty-four hours, and again estimated. The fluid in the incubated flasks was tested in most experiments as regards alkalinity, sweetness, presence of a surface layer of toluol, and sterility in aerobic organisms, at the end of the experiments. The first three conditions were present in all; a note of culture results is made in the experiment. The end-point of the estimation with Pavy was rather obscured by a marked biuret reaction in many experiments and rarely by blood pigment; fortunately as a rule the same errors came into parallel estimations and so cancel each other. After a time I came to look on the biuret reaction in slight degree as a rather favourable indicator of the end-point: there was a fairly definite point at which the last tinge of blue vanished and a pure pink colour only was left, and this point was determinable to 0.1 c.c. = 0.005 gramme error per 100 c.c. The point at which the biuret reaction

itself finally disappeared was very variable, sometimes a further 0.2 c.c., sometimes 5 c.c. of solution being required, and the amount further varied with the dilution, the rate of addition and the degree of ebullition to a marked and inconstant degree.

SERIES A.—Slaughter-house muscle and pancreas, obtained within one hour of death; expressed juices used; phosphate solution and toluol added with dextrose; sugar estimated before and after without removal of protein.

Experiment I.—PIG.

	MJ †	PJ †	Amount of solution		Sugar per litre		Loss of sugar			Cultures, colonies, and nature
					Before	After	Per litre	Per cent.	Per 40 c.c.	
<i>a</i>	25 c.c.	0 c.c.	in	40 c.c.	2.1604	1.6822	0.4782	21	0.019	—
<i>b</i>	25 c.c.	2 c.c.	in	40 c.c.	2.1874	1.0566	1.1308	51.5	0.045	—
<i>c</i>	0 c.c.	2 c.c.	in	40 c.c.	2.1552	1.8648	0.2904	13.5	0.016	—

Percentage excess of joint action (P+M) over sum of actions of its constituent actions + 17

† MJ = Muscle Juice. PJ = Pancreatic Juice.

Experiment II.—PIG.

	MJ	PJ	Amount of solution		Sugar per litre		Loss of sugar			Cultures, colonies, and nature
					Before	After	Per litre	Per cent.	Per 50 c.c.	
<i>a</i>	20 c.c.	0 c.c.	in	50 c.c.	2.3243	2.0270	0.2973	12.7	0.0148	0
<i>b</i>	20 c.c.	3.5 c.c.	in	50 c.c.	2.1583*	1.2362*	0.8321	38.6	0.0416	0
<i>c</i>	0 c.c.	3.5 c.c.	in	50 c.c.	2.3041	1.6529	0.6512	28.2	0.0325	1 mould

Percentage excess of joint action (P+M) over sum of actions of its constituent actions — 2.5

Experiment III.—SHEEP.

	MJ	PJ	Amount of solution		Sugar per litre		Loss of sugar			Cultures, colonies, and nature
					Before	After	Per litre	Per cent.	Per 60 c.c.	
<i>a</i>	30 c.c.	0 c.c.	in	60 c.c.	1.695	1.61	0.085	5	0.005	0
<i>b</i>	30 c.c.	5 c.c.	in	60 c.c.	1.43*	1.575	—0.14	—10	—0.008	0
<i>c</i>	0 c.c.	5 c.c.	in	50 c.c.	1.94	1.88	0.06	3	0.004	0

Percentage excess of joint action (P+M) over sum of actions of its constituent actions — 18

Experiment IV.—SHEEP.

	MJ	PJ	Amount of solution		Sugar per litre		Loss of sugar			Cultures, colonies, and nature
					Before	After	Per litre	Per cent.	Per 60 c.c.	
<i>a</i>	15 c.c.	0 c.c.	in	60 c.c.	1.92	1.44	0.480	25	0.027	0
<i>b</i>	15 c.c.	2 c.c.	in	60 c.c.	2.00	1.47	0.530	26	0.035	0
<i>c</i>	0 c.c.	5 c.c.	in	50 c.c.	2.24	2.03	0.110	5	0.007	1 culture

Percentage excess of joint action (P+M) over sum of actions of its constituent actions — 1
(P* = loss by 2 c.c. PJ).

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The following experiment is included in this series for comparison; human muscle and pancreas; P.M. eighteen hours after death.

Experiment V.

	MJ	PJ	Amount of solution		Sugar per litre		Loss of sugar			Cultures, colonies, and nature
					Before	After	Per litre	Per cent.	Per 50 c.c.	
a	22.5 c.c.	0 c.c.	in	55 c.c.	1.733*	1.684*	0.085	5	0.0042	—
b	22.5 c.c.	3.5 c.c.	in	55 c.c.	1.852*	1.129*	0.724	39	0.0362	—
c	0 c.c.	3.5 c.c.	in	50 c.c.	1.753	1.125	0.629	35	0.0314	—

Percentage excess of joint action (P+M) over sum of actions of its constituent actions - 1

* Indicates cases in which excessive haemoglobin or biuret reaction rendered estimation difficult.

Comments on Series A

In all this series the destruction of sugar by the action of pancreas and muscle together gave the highest actual and percentage value, with the exception of Experiment III; but on further analysis it is seen that the sum of the separate actions of muscle and pancreas alone is in only one instance (Experiment I) less than the amount of the action of pancreas and muscle together. This difference is shown below each experiment.

The pancreas alone in Experiments I, II and III showed a very high glycolytic figure contrary to the results obtained by previous observers, and for this reason, in spite of the negative results of the cultures used in Experiment B, an attempt was made to maintain a higher degree of asepsis in the work. In the next two experiments, C and D, a considerably lower figure was obtained for the pancreatic glycolysis, but also a lower one for the parallel observations in the presence of muscle.

In Experiment III an actual increase in sugar value, as measured by the reduction power, was obtained for the first time by me; this was at first attributed to some error in manipulation or possibly to an error in estimation due to the strong biuret reaction of the control; but as the same effect occurred in later experiments of mine and also in practically all the other recorded series, this was probably no error, and its reason will be discussed later.

This series, then, excepting Experiment I, gave no confirmation of Cohnheim's results, and so the use of alcoholic extracts of boiled pancreas was begun in the hope that by that a higher degree of glycolysis might be obtained, as stated by Hall and by Cohnheim, and also that a lessened chance of error from protein and bacterial contamination would exist, since on each occasion only the muscle would need expression, the sterile pancreatic extract being prepared beforehand.

The extract was made from the pancreas used in Experiments I, II, III and IV, Series A. The residue of pancreas and of expressed juice,

after a sufficient quantity for the experiments had been obtained, was again thoroughly pounded and ground with sand and water and evaporated nearly to dryness on a water bath; the residue was collected and extracted with successive portions of 95 per cent. alcohol; four to six lots of alcohol were used, each for one or more days; the successive portions of alcohol were mixed together, and, where necessary, reduced by heating on a water bath until of such a strength that 1 c.c. = 0.2 to 0.4 gramme pancreas.

In Series B, Experiments VI, VII and VIII, the extracts used were those of Series A, Experiment I and Experiment II, which are called respectively P.E. (i) and P.E. (ii). After that the four extracts made were mixed together and used for the remainder of the series (as P.E. (iii)). The muscle juice, toluol and sugar, and general course of the observations was as in the first series.

SERIES B.—Fresh muscle juice and alcoholic extract of pancreas from laboratory animals used: dextrose, toluol, and phosphate solution.

Experiment VI.—SHEEP'S MUSCLE. EXTRACT OF PIG'S PANCREAS.

				Sugar per litre		Difference in Sugar		Per ex- peri- ment	Cultures Aerobic	End reaction to litmus
	MJ	PE (i)	Volume	Before	After 24 hrs.	Per litre, grms.	Per cent.			
<i>a</i>	30 c.c.	0 c.c.	in 60 c.c.	1.695	1.610	-0.085	- 5	-0.005	Sterile	Alkaline
<i>b</i>	30 c.c.	+ 2 c.c.	in 60 c.c.	1.538*	1.850	+0.312	+20.3	+0.019	Sterile	Alkaline
<i>c</i>	0 c.c.	3.5 c.c.	in 60 c.c.	1.560	1.370	-0.190	-12	-0.011	Sterile	Alkaline

Percentage excess of joint action over separate actions - 32.

Experiment VII.—SHEEP'S MUSCLE. EXTRACT OF PIG'S PANCREAS *a*.

				Sugar per litre		Difference in Sugar		Per ex- peri- ment	Cultures Aerobic	End reaction to litmus
	MJ	PE (i)	Volume	Before	After 24 hrs.	Per litre, grms.	Per cent.			
<i>a</i>	15 c.c.	0 c.c.	in 60 c.c.	1.92	1.44	-0.48	-25	-0.027	Sterile	Neutral
<i>b</i>	15 c.c.	2 c.c.	in 60 c.c.	1.90	1.70	-0.20	-10.5	-0.012	Sterile	Alkaline
<i>c</i>	0 c.c.	2 c.c.	in 50 c.c.	1.515	1.44	-0.07	-4.6	-0.004	Sterile	Alkaline

Percentage excess of joint action over separate actions - 20.

Experiment VIII.—SHEEP'S MUSCLE. EXTRACT OF PIG'S PANCREAS *a*.

				Sugar per litre		Difference in Sugar		Per ex- peri- ment	Cultures Aerobic	End reaction to litmus
	MJ	PE (ii)	Volume	Before	After 24 hrs.	Per litre, grms.	Per cent.			
<i>a</i>	15 c.c.	+0 c.c.	in 60 c.c.	1.92	1.44	-0.48	-25	-0.027	Sterile	Neutral
<i>b</i>	15 c.c.	2 c.c.	in 60 c.c.	1.92	1.49	-0.43	-22.4	-0.026	Sterile	Alkaline
<i>c</i>	0 c.c.	3 c.c.	in 50 c.c.	1.63	1.47	-0.16	- 9.8	-0.010	Sterile	Alkaline

Percentage excess of joint action over separate actions - 12.4

NOTE.—In each of above experiments separate estimations of each sub-division (*a*, *b* and *c*) were made before and after incubation, as in Series A. In the remainder of this series this procedure was not followed, as the pancreatic extracts had little reducing power, and so the addition caused little error. See estimations *a* and *b* in each of above.

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Experiment IX.—**PIG'S MUSCLE.** Juice expressed as in previous experiments; sugar solution, phosphate solution, and toluol added, and the whole diluted with sterile water and well shaken. Strength such that every 60 c.c. contained the juice of 10 grammes of muscle. Estimated reducing power of 60 c.c. was equal to 0.119 gramme sugar (the same mean value being given by the estimation of two separate portions diluted to four times the volume), that is 1.98 grammes per litre. Separate portions of 60 c.c. were measured into sterile flasks, and the desired amount of pancreatic extract added to each. This extract contained the extract of about 3 grammes of pancreas in 10 c.c., and had a glycolytic power of 0.002 to 0.003 gramme of sugar per cubic centimetre.

Experiment IX.—

MJ	PE (iii)	Amount of sugar corrected for dilution by pancreas				Difference in sugar in grms.				
		Per experiment		Corrected to per litre original		Per Ex- periment	Per cent.	Per litre	Per kilo. muscle	
		Before	After 24 hrs.	Before	After 24 hrs.					
<i>a</i>	Juice of 10 grammes of muscle in 60 c.c. of alkaline sugar solution	+0 c.c.	0.119	0.124	1.98	2.08	+0.005	+ 5	+0.10	+0.6 gm.
<i>b</i>		0.5 c.c.	0.119	0.112	1.98	1.87	-0.007	- 6	-0.11	-0.7 gm.
<i>c</i>		1 c.c.	0.119	0.117	1.98	1.95	-0.002	- 2	-0.03	-0.2 gm.
<i>d</i>		2 c.c.	0.119	0.107	1.98	1.78	-0.012	-10	-0.20	-1.2 gm.
<i>e</i>		3 c.c.	0.119	0.135	1.98	2.27	+0.016	+13	+0.29	+1.7 gm.
<i>f</i>		4 c.c.	0.119	0.143	1.98	2.37	+0.024	+19	+0.39	+2.4 gm.
<i>g</i>		6 c.c.	0.119	0.147	1.98	2.46	+0.028	+22	+0.48	+2.9 gm.
<i>h</i>		8 c.c.	0.119	0.136	1.98	2.26	+0.017	+14	+0.28	+1.7 gm.
<i>k</i>		10 c.c.	0.119	0.141	1.98	2.35	+0.022	+18	+0.37	+2.2 gm.

Reactions all alkaline after twenty-four hours, had excess of toluol on surface, and smelt quite sweet. No cultures taken.

Experiment X.—As Experiment IX, but incubated for forty-eight hours.

MJ		PE (iii)	Amount of sugar corrected for dilution by pancreas				Difference in sugar in grms.			
			Per experiment		Corrected to per litre original		Per Ex-periment	Per cent.	Per litre	Per kilo. muscle
			Before	After	Before	After				
a	Juice of 10 grammes of muscle in 60 c.c. of alkaline sugar solution, containing toluol in excess	0 c.c.	0.119	0.130	1.98	2.17	+0.011	+10	+0.19	+1.14
b		0.5 c.c.	0.119	0.101	1.98	1.68	-0.018	+15	-0.30	-1.80
c		1 c.c.	0.119	0.139	1.98	2.31	+0.020	+16	+0.33	+1.98
d		2 c.c.	0.119	0.103	1.98	1.72	-0.016	-13	-0.26	-1.56
e		3 c.c.	0.119	0.127	1.98	2.12	+0.008	+ 7	+0.14	+0.84
f		4 c.c.	0.119	0.028	1.98	0.47	-0.091	-76	-1.41	-8.46
g		6 c.c.	0.119	0.076	1.98	1.27	-0.043	-36	-0.71	-4.26
h		8 c.c.	0.119	0.087	1.98	1.45	-0.032	-28	-0.53	-3.18
k		10 c.c.	0.119	0.022	1.98	0.37	-0.097	-81	-0.61	-3.66

Reactions all alkaline excepting f, which was markedly acid. All smelt quite sweet, had toluol on surface and no signs of organic scum (moulds, etc.). Aerobic cultures of f yielded no growth.

Comments on Series B

Experiments VI, VII, and VIII. Pancreatic extract has a glycolytic power of its own of 6 to 10 milligrammes dextrose per gramme of pancreas in twenty-four hours; fresh muscle juice a glycolytic power equivalent to from 8 to 20 milligrammes of dextrose per gramme of muscle in twenty-four hours; that is to say a fractionally larger power.

In mixtures of the two in the proportion of 0.6 gramme pancreas to 25 to 50 grammes of muscle, less glycolytic power than the sum of that of the constituents was generally apparent; whether from some antibody, or from the inhibitory action of excess of the glycolytic factor of the pancreas, or from veiling of the glycolysis by the formation of some breakdown product with power of reducing Pavy's solution.

Experiments IX and X. In these experiments it is seen that autolysis of muscle alone can lead to the formation of reducing substance; that the presence of pancreatic extract in large amount leads to increased formation of reducing substance, at any rate in the first twenty-four hours. Smaller amounts of pancreatic extract (0.15 to 0.6 gramme per 10 grammes muscle) led at first to a glycolytic action which reached as a maximum 1.2 grammes of sugar destroyed in twenty-four hours per kilogramme of muscle with 60 grammes pancreas, or corrected for the autolytic increase of reducing contents to 1.8 grammes sugar per kilogramme muscle in twenty-four hours.

Since the glycolysis was greater with 60 grammes pancreas per kilogramme muscle than it was with 15 grammes per kilogramme, it would appear that the small value of this factor was not due to any inhibitory action of excess of pancreatic extract. Also the total glycolysis is not nearly high enough to be comparable to the amount of sugar metabolised in the body during life.

On longer incubation (Experiment X), a great deal of variation is seen, the autolytic production of reducing bodies in the presence of large amounts of pancreatic extract is replaced by a large glycolytic action, while with smaller amounts the results become variable. In spite of the absence of any signs of bacterial or organism action, I cannot help feeling that some such action was possibly present.

This increase of reducing power first seen in Series A, Experiment III, and Series B, VI, and so very manifest in Series B, IX, X, led me to read the literature of the subject carefully once more, looking for this effect, and I found that the complication had occurred to previous observers.

Cohnheim did not obtain it in the series first communicated, in which he obtained his extract by freezing the muscle and then pulverising it, and in which he frequently mixed his pancreas and muscle from the start. He obtained it very frequently when he used normal saline solution, or other solutions, as oxalate and Ringer's solutions, for his extracts, or where he used such solutions to extract muscle, and also where he carried

on the extraction at temperatures above 0° C. It also appeared on the addition of pancreas in excess, blood serum and pancreas to slightly glycolytic muscle, or small amounts of pancreas to highly active muscle.

Claus and Embden, using saline solution to obtain constant concentration in their experiments, obtained a similar increase in several experiments.

Hall obtained an increase in two experiments only when using the juice of the fresh pancreas alone; never with muscle or muscle and pancreas, though working with large amounts of pancreas in many experiments.

Thinking that the presence of glycogen in the muscle might be a reason for the appearance of this complication, it was attempted at a later stage to estimate the amount of glycogen present in the muscle juice used by comparing its reducing power before and after boiling with hydrochloric acid. In none of the cases in which this was tried were any signs of glycogen breakdown observed, and it can therefore be assumed that the glycogen in the muscle changes rapidly and completely into sugar in the process of extraction, and so does not add to the sugar contents of the fluid during the later incubation. The experiments in which I had thus eliminated the glycogen factor showed, on incubation, only some very slight increase in glycolytic power (maximum 0.2 gramme per kilogramme muscle), and so are not very conclusive. I incline, however, to the belief that the increase is not due to the formation of dextrose from glycogen, but is an apparent one due to the formation of protein breakdown derivatives with reducing powers, on account of the fact that it generally appears in the presence of a large amount of pancreatic extract or juice, even though an alcoholic extract of boiled pancreas has not as a rule a high tryptic power.

In support of this compare Experiments IX and X, Series B, where the muscle alone yielded an apparent increase of 1.14 grammes dextrose per kilogramme after forty-eight hours, while in the presence of pancreatic extract there were increases equivalent to 2.9 and 2.4 grammes of dextrose per kilogramme muscle in twenty-four hours.

Other observers do not seem to have attempted to find any explanation for this factor, which greatly adds to the difficulties of the experiment, and which, when glycolysis is obtained, must be considered in comparing the observed power of glycolysis with the amount of glycolysis required for the metabolism of sugar in the body.

In answering Claus' and Embden's criticisms, Cohnheim alludes to the fact that in some of the experiments where he used saline, the

addition of pancreatic fluids seemed to reduce the inherent power of glycolysis of the muscle, and ascribes this to some inhibitory influence of the sodium chloride on the activating power of the pancreas, the nature of which he makes no attempt to explain.

It occurred to me that the work of Cole,⁸ Schutz,⁹ and others on ferments possibly held out an explanation of this fact. They showed that the nature of the ions in the solutions of ferments had a very considerable influence on their activity, and sodium was especially seen to have large inhibitory powers. Cole found that in some cases where sodium salts had an inhibitory effect ammonium salts had an accelerating effect, and this was tried in one case (compare Series C, Experiments XVII and XVIII, Nos. c and d), but without any result. I did not proceed further on those lines, since I was by then convinced that the production of reducing bodies, which undoubtedly occurs, might be favoured by such methods of extraction and so would quite account for the lessening of apparent glycolysis.

Very slight variations in the conditions of experiments on autolysis or protein digestion may lead to very divergent lines of breakdown, and while this factor cannot be eliminated, it must cause great difficulty in any work on the glycolytic influence of the pancreas by methods such as we have been using; and I feel that some such factor has to be reckoned with in such cases as that recorded by Cohnheim in his Experiment XX, Paper No. 2:—

<i>a</i>	Muscle alone gave glycolysis	=	0 sugar
<i>b</i>	Muscle + pancreas 8 c.c.	=	-107 mg. sugar
<i>c</i>	Muscle + pancreas 8 c.c. + serum 7 c.c.	=	- 75 mg. sugar
<i>d</i>	Muscle + Serum 7 c.c.	=	- 50 mg. sugar
<i>c</i>	Muscle + 4 c.c. pancreas + 3 c.c. serum	=	-100 mg. sugar

Cohnheim suggests that the serum contains the same activator as the pancreas for the glycolytic ferment of the muscle; that 8 c.c. pancreas contains the optimum amount of activator for the amount of muscle used (100 grammes); that 7 c.c. of serum contains sufficient activating body to partially inhibit the action; and, finally, that 3 c.c. serum and 4 c.c. pancreas again contain the optimum amount of activator.

Surely, if this be so, 3 c.c. of serum are equal to 4 c.c. of pancreas for the purposes of the experiment, and if that is right the activating effect obtained by 7 c.c. of serum should be almost of an optimum value; on the contrary, either it is below maximum, since it only causes half the maximal glycolysis, or else it is far over the optimum (6 c.c. should, strictly speaking, be optimum and equivalent to 8 c.c. pancreas), and has

a marked inhibitory effect. But this latter cannot be true, since 7 c.c. serum in excess reduces the maximum glycolysis by only 25 per cent., so 1 c.c. would scarcely reduce it by 50 per cent.

Moreover, Cohnheim states that 8 c.c. pancreas means the alcoholic extract of 0.8 gramme of fresh pancreas; it is scarcely conceivable that 10 c.c. of blood serum should contain the same amount of activating body as 1 gramme of the gland that secretes it.

A further point that invites criticism in the above experiment is the fact that the muscle alone gave no glycolysis at all. Now it is scarcely possible even in an animal killed by bleeding that the tissues should be so freed of blood and serum that 100 grammes of muscle (amount said to be used in these experiments) should not contain, say, 2 c.c. of serum. If so, and if 7 c.c. is the optimum value, then surely the muscle itself should show some considerable glycolytic power if the serum used was, as seems probable, taken from the same cat.

In the next experiments I attempted to imitate exactly the method followed by Cohnheim. The muscle was obtained from rabbits and cats killed by bleeding, by as rapid and aseptic a dissection as was possible; it was passed, after weighing, through a mincing machine into ice cold water; sugar, about 1 gramme per 100 grammes muscle, 10 per cent. toluol and 2 per cent. magnesium carbonate (to preserve neutrality, instead of the solution of mixed phosphates previously used) added, and the whole made up to nearly the required volume with water and ice. After standing for six to twenty-four hours at 0° C., the remaining ice was removed, and the volume of the fluid again adjusted. The mass was then well shaken and filtered through sterilised cloth and separated into equal portions. The minced pancreas of the same animal was used in some cases, in the others the mixed pancreatic extract previously prepared. Determinations were made in some cases by estimating with Pavy's solution in the presence of protein; in other cases after removal of the protein. Average dilution to five or ten times the volume. After the control determination the flasks were sealed with cotton wool and incubated twenty hours and upwards at 37° C., and then re-estimated.

To remove the protein 40 c.c. of the fluid was measured, neutralised by acetic acid, and then a slight excess of acetic acid and about 10 grammes of magnesium sulphate added; the whole diluted to 100 c.c. and heated for twenty minutes on a water bath. The precipitate was broken up finely by a glass rod, and the whole removed by washing to a measuring cylinder and made up to 40 c.c. or other required volume and allowed to stand twenty-four hours, then filtered and used in the estimation. In

earlier experiments I had found that even prolonged washing did not extract all the sugar from the protein precipitate, but in this way the results obtained gave only a slighter lower value than direct estimations with Pavy before freeing from protein.

SERIES C.—Fresh muscle minced and extracted with ice cold water in the presence of dextrose, magnesium carbonate, and toluol.

Experiment XI.—Cat's muscle; extracted 6 hours at 0°, filtered. 200 c.c. = 100 grms. muscle. Minced fresh pancreas of same cat. Estimated by Pavy direct at dilution of 10 volumes.

	Reducing power per portion in grms. dextrose		Difference in reducing power as grms. sugar		
	Before	After 24 hrs.	Per ex- periment	Per cent.	Per kilo. muscle
<i>a</i> 200 c.c. (100 grms. muscle)	0.980	0.971	-0.009	-1	-0.09
<i>b</i> 200 c.c. + 1 grm. pancreas	0.980	0.909	-0.071	-7	-0.71
<i>c</i> 200 c.c. + 0.3 grm. pancreas	0.980	0.961	-0.019	-2	-0.19

Reactions alkaline after incubation toluol abundant. Smell sweet. Cultures gave no growth.

Experiment XII.—Cat's muscle: extracted 6 hours at 0°, filtered. 200 c.c. = 100 grms. muscle. Minced fresh pancreas of same cat. Estimated after removal of protein.

	Reducing power per portion in grms. dextrose		Difference in reducing power as grms. sugar		
	Before	After 24 hrs.	Per ex- periment	Per cent.	Per kilo. muscle
<i>a</i> 200 c.c.	0.962	0.962	0.000	0	0.00
<i>b</i> 200 c.c. + 1 grm. pancreas	0.926	0.909	-0.017	-2	-0.17
<i>c</i> 200 c.c. + 3 grms. pancreas	0.961	0.971	-0.014	-1.5	-0.14

Experiment XIII.—Fresh minced Cat's muscle extracted as above. Mixed alcoholic pancreatic extract used. Estimated by Pavy in presence of protein. Incubation 20 hours.

	Reducing power per Experiment in grms. dextrose		Difference in grms. Dextrose		
	Before	After 20 hrs.	Per ex- periment	Per cent.	Per kilo. muscle
<i>a</i> 200 c.c. = 100 grms. muscle ...	0.980	0.971	-0.009	-1	-0.09
<i>b</i> 200 c.c. + 20 c.c. PE(iii) ...	0.980	1.026	+0.047	+5	+0.47
<i>c</i> 200 c.c. + 10 c.c. PE(iii) ...	0.980	0.931	-0.049	-5	-0.49
<i>d</i> 200 c.c. + 1 c.c. PE(iii) ...	0.980	0.956	-0.024	-2.5	-0.24

All alkaline and sweet after 24 hours; excess of toluol.

Experiment XIV.—Fresh cat's muscle extracted six hours at 0°. Mixed pancreatic extract. Estimated after removal of protein. Incubation 24 hours.

	Reducing power per Experiment in grms. dextrose		Difference in grms. dextrose		
	Before	After	Per ex- periment	Per cent.	Per kilo. muscle
<i>a</i> 200 c.c. = 100 grms. muscle ...	0.962	0.962	0	0	0
<i>b</i> 200 c.c. + 20 c.c. PE (iii) ...	0.981	0.982	+0.001	0	+0.01
<i>c</i> 200 c.c. + 10 c.c. PE (iii) ...	0.982	0.932	-0.050	-6	-0.5
<i>d</i> 200 c.c. + 1 c.c. PE (iii) ...	0.990	0.971	-0.019	-2	-0.2

All alkaline and sweet after 24 hours; excess of toluol.

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Experiment XV.—Fresh cat's muscle extracted at 0° for six hours. Fresh cat's pancreas. Incubated 48 hours. Pavy in presence of protein.

				Reducing power per Experiment in grms. dextrose		Difference in grms. dextrose		
				Before	After	Per ex- periment	Per cent.	Per kilo. muscle
<i>a</i>	200 c.c. = 100 grms. muscle	...	...	0.980	0.980	0	0	0
<i>b</i>	200 c.c. + 1 gm. pancreas	...	...	0.980	0.918	−0.062	−7.3	−0.72
<i>c</i>	200 c.c. + 0.3 gm. pancreas	...	...	0.980	0.971	−0.009	−1	−0.09

All alkaline and sweet after incubation; excess of toluol present.

Experiment XVI.—As Experiment XV, but pancreatic extract used. Incubation 48 hours.

				Reducing power per Experiment in grms. dextrose		Difference in grms. dextrose		
				Before	After	Per ex- periment	Per cent.	Per kilo. muscle
<i>a</i>	200 c.c. = 100 grms. muscle	...	...	0.980	0.980	0	0	0
<i>b</i>	200 c.c. + 20 c.c. PE(iii)	...	...	0.980	1.009	+0.029	+3	+0.29
<i>c</i>	200 c.c. + 10 c.c. PE(iii)	...	...	0.980	0.972	−0.008	−1	−0.08
<i>d</i>	200 c.c. + 1 c.c. PE(iii)	...	...	0.980	0.976	−0.004	−0.5	−0.04

All alkaline and sweet after incubation; excess of toluol present.

Experiments XVII and XVIII.—Cat's muscle extracted as above for six hours with ice cold water, and filtered. Residue again extracted for 24 hours with ice cold water with sugar, etc., and filtered. Extract such that 150 c.c. = 100 grms. muscle. Alcoholic pancreatic extract used. Incubation 24 hours.

Experiment XVII.—Estimations in presence of protein.

Extract				Reducing power per experiment in grms. dextrose		Difference in reducing power in grms. dextrose		
				Before	After	Per ex- periment	Per cent.	Per kilo. muscle
<i>a</i>	95 c.c. in 100 c.c.	...	...	0.143	0.150	+0.007	+5	+0.11
<i>b</i>	90 c.c. + 2 c.c. PE (iii) in 100 c.c.	...	...	0.135	0.130	−0.005	−3	−0.09
<i>c</i>	95 c.c. + 1 c.c. PE (iii) in 100 c.c.	...	...	0.143	0.143	+0.0	+0	0
<i>d</i>	95 c.c. + 1 c.c. PE (iii) + AmCarb in 100 c.c.	...	...	0.143	0.148	+0.005	+3	+0.08
<i>e</i>	90 c.c. + 5 c.c. PE (iii) in 100 c.c.	...	...	0.135	0.148	+0.013	+9	+0.22
<i>f</i>	45 c.c. + 30 c.c. PE (iii) in 100 c.c.	...	...	0.068	0.076	+0.008	+2	+0.26
<i>g</i>	95 c.c. + 5 drops PE (iii) in 100 c.c.	...	...	0.143	0.139	−0.004	−3	−0.06

All alkaline and sweet after 24 hours; excess of toluol present.

Experiment XVIII.—Estimations after removal of protein.

					Reducing power per experiment in grms. dextrose		Difference in reducing power in grms. dextrose		
					Before	After	Per experiment	Per cent.	Per kilo. muscle
<i>a</i>	95 c.c. in 100 c.c.	...	...	...	0.140	0.148	+0.008	+5	+0.12
<i>b</i>	90 c.c. + 2 c.c. PE (iii) in 100 c.c.	...	...	...	0.133	0.136	+0.003	+2	+0.05
<i>c</i>	95 c.c. + 1 c.c. PE (iii) in 100 c.c.	...	...	...	0.140	0.140	0	0	0
<i>d</i>	95 c.c. + 1 c.c. PE (iii) + AmCl ₃ in 100 c.c.	...	...	...	0.140	0.137	-0.003	-2	-0.05
<i>e</i>	90 c.c. + 5 c.c. PE (iii) in 100 c.c.	...	...	...	0.133	0.139	+0.006	+4	+0.10
<i>f</i>	45 c.c. + 30 c.c. PE (iii) in 100 c.c.	...	...	...	0.066	0.075	+0.009	+6	+0.30 gm.
<i>g</i>	95 c.c. + 5 drops PE (iii) in 100 c.c.	...	...	...	0.140	0.137	-0.003	-2	-0.05 gm.

All alkaline and sweet after 24 hours; excess of toluol present.

Experiment XIX.—Minced fresh rabbit's muscle, extracted 24 hours at 0° C. in presence of sugar, mag.-carb. and thymol. Filtered through cloth and incubated 24 hours at 37° C. 200 c.c. = 100 grms. muscle.

					Reducing power in grms. dextrose		Difference in reducing power as grms. sugar		
					Before	After	Per experiment	Per cent.	Per kilo. muscle
<i>a</i>	40 c.c. extract of muscle	...	...	...	0.746	0.726	-0.02	2.7	-1 gm.
<i>b</i>	40 c.c. muscle extract + 20 c.c. PE (iii)	...	...	...	0.746	0.737	-0.01	1.3	-0.5 gm.

Apparently quite sweet. Reaction remained alkaline.

Experiment XX.—As above, but extracted 72 hours. Incubated 24 hours.

					Reducing power in grms. dextrose		Difference in reducing power as grms. sugar		
					Before	After	Per experiment	Per cent.	Per kilo. muscle
<i>a</i>	30 c.c. extract	...	...	...	0.054	0.008	-0.046	-90	-3.1 gm.
<i>b</i>	30 c.c. extract + 3 c.c. PE (iii)	...	...	...	0.054	0.057	+0.003	+6	+0.2 gm.
<i>c</i>	30 c.c. extract + 6 c.c. PE (iii)	...	...	...	0.054	0.053	-0.001	-2	-0.06 gm.

a Contained mould and bacilli in abundance. Reaction acid.

b and *c* Apparently sweet. Reactions alkaline.

Experiment XXI.—As Experiment XIX, extracted 24 hours; incubated 48 hours.

					Reducing power in grms. dextrose		Difference in reducing power as grms. sugar		
					Before	After	Per experiment	Per cent.	Per kilo. muscle
<i>a</i>	40 c.c. extract	...	...	...	0.727	0.708	-0.019	-2.6	-0.95 gm.
<i>b</i>	40 c.c. extract + 20 c.c. PE (iii)	...	...	...	0.727	0.729	+0.002	+0.3	+0.1 gm.

Reactions alkaline, apparently quite sweet.

Comments on Series C

Experiments XI to XVI. In these experiments the muscle extract alone had practically no power of glycolysis whatever. In the presence of fresh pancreas a slight glycolysis was seen, never amounting to more than 0.72 gramme per kilogramme muscle, that is to 10 grammes of sugar or 7 per cent. of the total sugar used; an amount, which, considering the elaborate technique and the numerous chances of error from evaporation of toluol from large dilution, etc., seems to approach too nearly to the limits of experimental error to be of any value.

Experiments XVII and XVIII form two exactly parallel series; the production of reducing substance by large amounts of pancreatic extract is again seen, but small amounts led to extremely little glycolysis, never in amount which could possibly be sufficient to account for the mechanism of the metabolism of carbohydrate in the body. Experiments XIX to XXI are again negative.

On further consideration of Cohnheim's articles, the fact again suggested itself that of all his experiments he obtained the most regular and striking results in those cases where the muscle and pancreas of the animal were mixed intimately from the first by being minced together, and in line with this was the fact that in the first series of experiments, where somewhat similar conditions obtained, my results had approached his most nearly.

Now if the muscle contains some substance, ferment or complement, which can, when influenced by the activator or amboceptor contained in the pancreas, cause glycolysis, this body must be extremely liable to be destroyed by slight changes in its environment, possibly susceptible to the autolytic ferments of muscle itself. If so, it is possible that just as other ferments are protected from destruction by the presence of the substances on which they exert their action, so this muscle ferment might be protected from destruction and obtained in greater amount and of higher activity if protected from the outset by the presence not of sugar (as obtained in Series C), for it does not on analogy combine directly with that substance, but of its pancreatic complement, and possibly even better protected by the presence of sugar also. This would explain the greater regularity of results obtained as described above by Cohnheim in his first contribution.

I had hoped that by some modification of the technique of the workers on protection and immunity that I might further be able to isolate this muscle ferment either alone or combined with its amboceptor

from the muscle proteids, and so get a more striking demonstration of its existence, but their work as yet holds out no hopes of such success.

However, fresh muscle was extracted in ice cold water for twenty-four and seventy-two hours in the presence of pancreatic extract in varying amounts, sugar, magnesium carbonate and thymol (toluol not being, unfortunately, at the time obtainable). Unfortunately the thymol did not prove as successful an antiseptic as toluol had done, and a large proportion of the flasks were found after incubation to be very foul and to contain mould and numerous other organisms. Those in which this was obviously present were not estimated, nor those in which the smell was other than sweet or in which the contents of the flask had not the usual appearance of a clear slightly reddish fluid, alkaline in reaction, with a loose flocculent deposit.

SERIES D.—Minced fresh rabbits' muscle extracted with ice cold water in the presence of pancreatic extract, sugar, and mag.-carb. Thymol as disinfectant in great excess (most flasks smelt strongly of thymol). Filtered through cloth after extraction. Fluids estimated before and after incubation with Pavy in the presence of protein. 300 c.c. extract equivalent to 100 grms. muscle.

Experiment XXII.—Controls. 24 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per ex- periment	Per cent.	Per kilo. muscle
200 c.c. muscle extract without pancreas = 66 grms. muscle	0.363	0.354	0.007	—2	0.105
			Alkaline, sweet.		

N.B.—72-hour control obviously contaminated.

Experiment XXIII.—24 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per ex- periment	Per cent.	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 4 c.c. PE (iii)	0.368	0.338	0.030	— 8	0.450

Though alkaline and apparently sweet, mould and bacillus (? Hay bacillus) present in considerable amount.

Experiment XXIV.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per ex- periment	Per cent.	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 10 c.c. PE (iii)	0.391	0.095	0.296	— 75	4.44

Mould and bacillus found in scanty amount microscopically. Reaction alkaline, smell not quite sweet, cultures lost.

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Experiment XXV.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per ex- periment	Per cent.	Per kilo. muscle
200 c.c. muscle extract (= 166 grms. muscle + 10 c.c. PE (iii)) and 16 c.c. PE (iii) added just before incubation ...	0.389	0.341	0.048	-15	0.72
Apparently sweet. Residue after 72 hours obviously foul.					

Experiment XXVI.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per ex- periment	Per cent.	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 4 c.c. PE (iii)	0.363	0.320	0.043	-14	0.65
Apparently sweet. Residue in 72 hours still sweet.					

Experiment XXVII.—72 hours.

	Reducing power in grms. sugar per experiment		Loss of sugar		
	Before	After	Per ex- periment	Per cent.	Per kilo. muscle
200 c.c. muscle extract = 66 grms. muscle + 6 c.c. PE (iii) 	0.376	0.372	0.004	- 1	0.06
Alkaline, remained sweet after 72 hours.					

Comments on Series D

Here Experiments XXVII, and possibly XXVI, alone could be relied on, and these are negative or small; but the series is put in at length as showing the varied action of the bacteria and the way in which their growth is favoured by pancreatic digestion of the medium.

CONCLUSIONS

1. That the glycolysis obtained by the combined action of pancreatic and muscle juices is not as a rule greater than the sum of the actions of its constituents.

2. That slight errors in technique tend to cause the appearance of such an increase, owing to bacterial growth being favoured by pancreatic digestion.

3. That such bacterial action may be masked at first when large amounts of pancreatic juice or extract are used by the appearance of protein breakdown products with reducing powers.

4. That the difficulty of maintaining asepsis in such a series of observations is almost insuperable without special apparatus, and that while antiseptics such as toluol retard, they do not prevent absolutely the action of micro-organisms in the solutions.

5. That in such a series of experiments the extreme difficulty of estimating sugar in the presence of protein leads to a high degree of experimental error; that removal of the protein before estimating only overcomes this by introducing a further series of errors, and the figures are quite unreliable for small changes in concentration in solutions originally containing from 2 to 5 grammes of dextrose per litre.

6. That by incubation of fresh muscle juice or extract, especially in the presence of pancreatic juice or extract, large amounts of reducing substance are often formed, possibly from glycogen in some degree, but more largely from autolytic or tryptic digestion of the muscle plasma, and that the nature of these bodies might well repay detailed investigation.

7. That, in face of these facts, it is useless to attempt to find any explanation, along the lines of the present research, for the failure of pancreatic extracts to benefit cases of diabetes.

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BIBLIOGRAPHY

1. Otto Cohnheim, *Zeitschrift für physiol. Chem.*, Bd. XXXIV, p. 336, 1903; Bd. XLII, p. 401, 1904; Bd. XLIII, p. 547, 1905; Bd. XLVII, p. 253, 1905.
2. Arnheim and Rosenbaum, *ibid.*, Bd. XL, p. 220, 1904.
3. Hirsch, *Beiträge z. chem. Physiol. u. Pathol.* V, p. 214, 1905.
4. Dewitt, *Journ. Exp. Medicine*, VIII, p. 193, 1906.
5. Hall, *Amer. Journ. of Physiol.*, XVIII, p. 283, 1907.
6. Claus and Embden, *Beiträge z. chem. Physiol. u. Pathol.* (two papers), VI, p. 214, 1905.
7. McGuigan, *Amer. Journ. of Physiol.*, XIX.
8. Cole, *Journ. Physiol.*, XXX, p. 202, p. 281, 1903.
9. Schutz, *Beiträge z. chem. Physiol. u. Pathol.*, Bd. V, p. 406, 1904.