THE RELATIONSHIP OF DIASTATIC EFFICIENCY TO AVERAGE GLYCOGEN CONTENT IN THE DIFFERENT TISSUES AND ORGANS

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The first demonstration of an enzyme capable of hydrolysing glycogen is associated with the names of von Wittich (1) and of Claude Bernard (2). The brilliant researches of the latter observer, which ultimately led up to the discovery of glycogen in 1855 (3), formed the basis on which the probability of the presence of such an enzyme rested, but the actual proof of its existence was first furnished by von Wittich; a short time afterwards independent evidence was advanced by Bernard, both observers having found the substance in the liver.

These discoveries, however, were not allowed to pass unchallenged, and to Pavy (4) belongs the credit of having settled beyond dispute the fact that the liver really contains a substance of the nature of an enzyme which is capable of converting glycogen and starch into sugar, and acts quite independently of the vitality of the tissue cells from which it is derived.

The presence of such an enzyme both in the animal and vegetable organism is now universally admitted, but its exact function is still in certain quarters a matter of controversy; the fact that glycogen occurs in the body tissues, and that the diastatic enzyme¹ possesses the power of transforming this substance into dextrose and intermediate products, suggests that the normal function is directly associated with the conversion of glycogen into the less complex substance—sugar.

Pavy (5), however, is unable to accept this view, and suggests that the enzyme is really a product of the dead or dying cell which is generated from an inactive pre-existent zymogen. On the other hand, analogy with other intracellular enzymes points to the probability that this diastatic enzyme actually exerts its influence during life in the same manner as

1. The term 'diastatic enzyme' is used in this paper to indicate the substance or substances by which glycogen is transformed into reducing bodies—maltose, dextrose, etc.; it is generally agreed that at least two distinct enzymes participate in the formation of *dextrose* from starch or glycogen.

its activity is evidenced *in vitro*—as a hydrolyser of glycogen—the difference being solely one of degree, and its activity being called forth in response to some condition not yet understood. That the conversion of glycogen is not regulated by the condition of the animal is suggested by the experiments of Kisch (6) carried out on adult muscle; his results seem to indicate that regulation of sugar formation from glycogen is apparently not brought about by the needs of the tissues for sugar.

If, then, the function of this ferment is to act on the glycogen present in the organs and change it into sugar in response to some unknown body stimulus, it might be expected that the amount of glycogen normally present in any particular organ would bear some general relationship to the richness of that organ in diastase. It has been known for many years that the chief seat of glycogen in the animal body is the liver; the muscles may also show a very considerable amount, while the other organs contain but traces. Consequently the literature of the subject abounds with data concerning the diastatic activity of the liver and muscles, but comparatively few observations dealing with the other organs are available; it has generally been recognised that many or all the tissues of the body contain traces of a glycogen hydrolysing ferment, but until quite recently no attempt has been made to investigate the relationship of average glycogen content to amylolytic efficiency.

A short time ago, however, an interesting series of observations have been undertaken by Mendel and Saiki (7); for their experiments muscle and liver of pig embryos of various sizes was utilised. In the case of the liver it was found that the diastatic activity increased markedly with the growth of the embryos, while in the case of muscle, which showed in general a well-marked initial activity, the increase was not nearly so pronounced. Hence it would seem that the diastatic activity of embryonic liver and muscle varies directly with the normal glycogen content, for it has been shown by Lockhead and Cramer (8) that the liver of foetal rabbits is at first very poor in glycogen, but that after twenty-five days a considerable amount is present; again the estimations of Mendel and Leavenworth (9) indicate that in the very young pig embryos no glycogen at all may be found. Thus, the foetal liver is at first very poor in glycogen, and apparently also in glycogen hydrolysing substances, while on the other hand the embryonic muscles may contain a fair amount of glycogen even at very early age, but tend to show only slightly increased amounts as age advances; in correlation with this slight rise in glycogen content the increase in diastatic power is correspondingly slight.

So far, therefore, as the subject has been investigated there is, in the case of embryonic tissues, undoubted evidence pointing to a relationship between diastatic efficiency and glycogen content, but whether varying diastatic activity is directly dependent on the relative glycogen content, or whether the increase of diastatic power constitutes a factor which directly influences the amount of glycogen, is still obscure. An answer to this question might help us in attributing its proper function to the diastatic enzyme, for it is not impossible that its chief sphere of action during life is synthetical rather than destructive. If the view entertained by several authorities with regard to glycogen is correct—that under normal circumstances it does not pass on to any marked extent to form dextrose, but is utilised in other ways—then it might perhaps be expected that the measurement of post-mortem amylolytic activity in an organ would afford some clue to its glycogenetic power during life. It will be shown, however, that the amount of diastatic enzyme actually found in different adult tissues bears no apparent relationship to the glycogen content of the same tissues; it is thus obvious that amylolytic efficiency and glycogen storage do not necessarily go together. It might be argued that the comparative absence of glycogen in an organ did not of itself imply the inability of that organ to form glycogen, for the latter substance might be formed and carried away in the blood; or again it might be immediately utilised at the seat of formation by the tissue cells. That the blood does not carry the glycogen as such from an organ is suggested by the very low glycogen content of this fluid, and the conception of a simultaneous process of formation and destruction of glycogen by which this substance might be kept at a minimum, can hardly be entertained if we accept the theory that it is as dextrose that carbohydrate is utilised by the tissues; such a procedure would be quite out of harmony with the ordinary methods of nature, for in that case formation of glycogen would be but a useless step; only on the theory that glycogen is utilised as such by the tissue cells could the latter possibility be entertained.

This apparent lack of correlation between glycogen content and glycogen hydrolysing capacity makes it much more difficult to understand what the amylolytic activity as evidenced post-mortem really means in connection with the vital processes. It may well be that these enzymes exert their destructive action chiefly as the result of some interference which threatens the life of the organ.

It is well known that the kidney, for instance, contains normally

but a very small amount of glycogen, and yet it is often found to be much richer in diastatic enzyme, per unit weight, than any organ in the body. On the other hand, muscle which generally contains a fair amount of glycogen is often very weak in diastatic effect. The following figures, taken from an experiment recorded by Pick (10), are of interest with regard to this point; they indicate the results of some comparative estimations of amylolytic activity of different adult tissues.

It was found that

100 grms. kidney digested 2.37 grms. glycogen in 3 hours.

100 grms. liver digested 0.69 grms. glycogen in 3 hours.

100 grms. blood digested 0.31 grms. glycogen in 3 hours.

Here it is seen that kidney possessed between three and four times the diastatic power of liver and about eight times that of blood. Experiments made by the writer gave in general very similar results, and showed that almost invariably the kidney is the organ which possesses, weight for weight, the greatest amylolytic efficiency. Often other organs were also found to have quite a marked effect. It is interesting to note that in the case of another endoenzyme—*erepsin*—it has been shown by Vernon that the kidney possesses far and away a greater ereptic action than any other tissue, with the exception of small intestine. The richness of the kidney in these and perhaps other enzymes is not easily explained in the light of our present knowledge of vital processes.

In order to demonstrate the comparative efficiency of the different organs the following procedure was adopted.

ESTIMATION OF AMYLOLYTIC ACTIVITY

The majority of observers conducting research on amylolytic enzymes have chosen as an index of activity the amount of reducing substances formed in a given time. Either the dried tissue or the expressed tissue juice was incubated with a starch or glycogen solution of known strength, and subsequently the extent to which reducing substances had been formed estimated by Fehling's or some similar alkaline copper solution.

This method supplies information as to the activity of the enzyme in terms of its power of forming products which reduce copper solutions, but affords no information as to the relative amounts of these substances actually present—dextrose, maltose, etc. For general comparative work, however, such detailed information is not necessary, and this method is quite suitable.

In other cases the amount of glycogen present before and after incubation was calculated, and the difference taken as an index of amylolytic action. This again merely indicates the power of the enzyme to transform glycogen into substances soluble in alcohol, but gives no indication as to the extent to which this change has been carried on towards the formation of the final products. As exact estimation of glycogen, especially where many experiments have to be undertaken, is a tedious process, a good indication can be obtained by substituting starch for glycogen and testing with iodine in order to ascertain when the starch ceases to give its characteristic iodine reaction. This method has been used and recommended by Wohlgemuth (11), but in my experiments on dried tissues it was found occasionally to give somewhat indefinite results; only a few experiments, however, were carried out, and so far as they went the results were in general similar to those obtained by the reduction method. On the whole the most satisfactory method was found to be the estimation of the reducing power, and dried tissue was mostly employed; in a few experiments moist fresh tissue was also used.

METHOD

The method utilised is in general the same as that made use of by many observers. The various organs were obtained as soon as possible after death, freed from adherent fat, and quickly cut up into small pieces; these were washed in normal saline solution in order to free them from blood, dried by being pressed gently in a cloth, and finally passed through a mincing machine. The finely divided substance was placed in a large volume of alcohol and left there for 48 hours. The alcohol was then filtered off and the tissue dried in vacuo over H_2SO_4 , and ground to a fine powder by a coffee mill. To 50 c.c. of a 1-2% solution of soluble starch or glycogen, 1 gm. of the dried powder was added, and the mixture placed in the thermostat at 37° and left there for 18 to 20 hours. Small flasks of about 100 c.c. capacity were found to be most convenient, and the utmost precautions were adopted in order to ensure the sterility of all vessels used; toluol, alone or with sodium fluoride, was always added to the fluid. The flasks were thoroughly shaken up from time to time, and next day the contents were boiled in order to coagulate the albumin; after filtration a measured volume (10-30 c.c.) was taken and gradually added to excess of boiling Fehling's solution. After boiling for about 5 minutes, the cuprous oxide was

collected on a weighed Gooch crucible containing asbestos; after thorough washing of the precipitate with distilled water, the crucible was strongly heated in order to oxidise the lower oxide, and the precipitate then weighed as cupric oxide. Parallel experiments with boiled controls were also made. In all cases the ordinary Fehling's solution was employed, diluted with an equal volume of water, and in every comparative experiment the same amount of Fehling's solution was used; this precaution was necessary in order to obtain accurate results, and likely depends on the power of caustic alkali to dissolve small amounts of cuprous oxide.

At first some difficulty was occasionally experienced in filtering a too finely divided cuprous oxide precipitate; it was found, however, that this could be overcome in all cases by boiling both the sugar-containing fluid and the alkali before bringing them together, or by adding the sugar solution very slowly to the boiling Fehling's fluid; by adopting this small precaution a well defined red precipitate was easily obtained in every case, and no difficulty arose in filtering.

Some preliminary experiments showed that this method, when carried out carefully as above described, gave practically identical results in parallel experiments with the same tissue. Wohlgemuth, however, raises the objection to results based on reduction methods, that they do not necessarily indicate the diastatic activity, since there may be present glycolytic enzymes which destroy part of the sugar as it is formed. The evidence for the presence to any appreciable extent of active glycolytic enzymes in the tissues is by no means very convincing, as may be seen by consulting the literature of the Conheim controversy relating to the alleged glycolytic action of muscle juice combined with pancreatic extract. In my experiments in which it is sought to establish the different diastatic powers of the various tissues, it is obvious that glycolytic effect is of most importance from a comparative point of view in cases where low results were obtained. Some experiments made on this point showed that mixtures of sugar and tissue, treated exactly as in the diastatic experiments, did not tend to decrease in strength to any appreciable amount; the results obtained indicate that this factor is of little importance in determining the results of diastatic efficiency calculated in terms of reducing substance. In one experiment the following figures were obtained:—

10 c.c. sugar sol. = 0.1126 grm. CuO.

50 c.c. of this sugar solution digested with 1 gm. of following organs for 20 hours; other 50 c.c. + tissue boiled and used as control:--

	Digest CuO (10 c.c. used)		Control CuO (10 c.c. used)		Difference between original sugar solution and digest
Liver	0.1130 gm.	...	0.1109 gm.	...	+ 0.0004 gm.
Heart	0.1121 ..	...	0.1082 ..	...	- 0.0005 ..
Lung	0.1046 ..	...	0.1090 ..	...	- 0.0080 ..
Muscle	0.1102 ..	...	0.1113 ..	...	- 0.0024 ..

While not excluding the possibility of some slight glycolytic effect, the differences obtained are probably within the bounds of experimental error; at any rate, any glycolytic action that may exist is much too small and indefinite to be of any appreciable account in determining diastatic activity, especially from a general comparative point of view.

RELATIVE DIASTATIC EFFICIENCY OF DIFFERENT ORGANS IN TERMS OF CuO

Experiments were carried out with tissues obtained from different animals, and it was found that great variations existed not only as regards animals of different species, but in different animals of the same species. The result of an average experiment in each species of animal examined is given below. In almost all cases where sufficient material was available, two sets of parallel experiments were carried out; by this means the possibility of accidental results due to contamination by micro-organisms was excluded. In a few cases in which the organ was too small to yield 1 gm. dried tissue, $\frac{1}{2}$ gm. was taken and the result calculated for 1 gm.

Experiment I.—Cat.

1 gm. dried tissue + 50 c.c. 2 per cent. starch solution + toluol + NaF digested for 18 hours.

Amount of digest used = 20 c.c.		Result in grms. CuO
Kidney	=	0.3708
Lungs	=	0.3008
Liver	=	0.2720
Heart	=	0.2566
Stomach	=	0.2210
Bladder	=	0.1984
Muscle	=	0.1448

Here it is seen that kidney shows the greatest diastatic action, while lung comes second and liver third; muscle is apparently weaker than any of the tissues examined. Almost identical comparative results were found in another cat examined, except that the lung was less active.

Experiment II.—Rabbit

1 gm. dried tissue + 50 c.c. 2 per cent. starch solution + toluol. Digested 19 hours.
20 c.c. digest used

		Result in grms. CuO
Kidney	=	0.2694
Liver	=	0.2028
Stomach	=	0.1572
Lung	=	0.1546
Bladder	=	0.1348
Heart	=	0.1128
Muscle	=	0.0621

In the case of another rabbit, experiments were made with the fresh tissue in order to test the relative effects immediately after death. The animal was killed by bleeding, and the organs removed as quickly as possible and cut up into small pieces. They were then thoroughly minced up and 1 gm. of each quickly weighed and then digested with the above starch solution + toluol. Only kidney, liver and muscle were used for this experiment.

		Time digested	Amount of digest	Result in grms. CuO
Kidney	5 minutes after death	19 hours	10 c.c.	0.1046
Liver	10 " "	"	"	0.0516
Muscle	12 " "	"	"	0.0294

In order to ascertain whether there was any difference if the organ was not used quite fresh, it was tested again three hours after death, exactly as mentioned above.

Result—Kidney	=	0.1016 grms. CuO
Liver	=	0.0584 "
Muscle	=	0.0318 "

In another rabbit the following figures were obtained; same starch solution as above + 1 gm. tissue were used, and digestion went on for 20 hours 30 minutes:—

20 c.c. digest used.			
Kidney	10 minutes after death	=	0.1872 CuO
	4 hours	=	0.2000 "
Liver	5 minutes after death	=	0.0640 "
	4 hours	=	0.0599 "
Lung	14 minutes after death	=	0.0576 "
	4 hours	=	0.0521 "

From these results it appears that tissue does not lose any of its activity on standing, at least for a few hours; other experiments show that no change is apparent after standing for a very much longer time.

Here, again, it is seen that kidney possesses the most marked amylo-lytic efficiency, while liver has a very much less marked effect and muscle comparatively little. In one rabbit, however, it will be noticed that liver tissue was twice as active as in the next case, while muscle was about equal in both. In the last case, lung is as effective as liver, though lung at most contains only traces of glycogen under normal circumstances. In another case it was found that muscle was somewhat richer in diastatic ferment than liver.

Here, when treated exactly as other dried tissues for 18 hours, it was found that 20 c.c. digest gave the following figures:—

Kidney	=	0.2264	grms. CuO
Liver	=	0.1136	„
Muscle	=	0.1200	„
Lung	=	0.0716	„

These results indicate sufficiently well the marked fluctuations that may exist in the corresponding tissues of the same species of animal.

Experiment III

Pig

In this case only one experiment was made, and the lung gave higher results than any of the other tissues examined. Liver was also somewhat more active than kidney, while skeletal muscle was lowest of all. In this experiment the tissues were used fresh in the moist state, and glycogen taken instead of starch.

2 grms. fresh tissue + 50 c.c. 2 per cent. glycogen + toluol + NaF. Digested 16 hours.

20 c.c. digest used.		Result in grms. CuO
Lung	=	0.3128
Liver	=	0.2296
Heart	=	0.2246
Kidney	=	0.2190
Muscle	=	0.1628

In many experiments carried out on animals of other species, it was often found that lung had a relatively high value.

Experiment IV

Dog

The figures obtained from two dogs examined showed that kidney was again most effective, but the difference was not so marked as in some other animals. It is likely that an increase in the number of experiments would have shown greater variations than are here indicated.

1 grm. dried tissue + 50 c.c. 2 per cent. starch solution + toluol. Digested 20 hours.

10 c.c. digest used.		Result in grms. CuO
Kidney	=	0.1612
Liver	=	0.1322
Lungs	=	0.1288
Stomach	=	0.1051
Bladder	=	0.1214
Spleen	=	0.1333
Muscle	=	0.1051
Small intestine	=	0.1850 ¹

1. In all cases examined small intestine gave a high figure, but as this result is obviously complicated by the effect of the probable presence of ordinary pancreatic juice, this tissue has not been included in the other tables.

Experiment V

SHEEP

A very considerable number of experiments were conducted with tissues derived from this animal, and, as in other cases, marked variations were observed, both in the activity of corresponding organs of different animals and in the relative efficiency of the different tissues derived from the same animal. Sometimes kidney was found to be very much more active than liver, or any other organ; in other cases liver and kidney displayed practically an equal effect. In a few cases the heart muscle exerted but very slight diastatic action. Some of the variations obtained are indicated by the following figures from two different animals:—

1 gm. dried tissue + 50 c.c. 1½ per cent. starch solution + toluol + NaF. Digested 20 hours.
20 c.c. digest used. Result in grms. CuO

	(1)	
Liver	=	0.1876
Kidney	=	0.1796
Muscle	=	0.1414
Heart	=	0.1384
Lungs	=	0.1204
	(2)	
Kidney	=	0.2576
Liver	=	0.1846
Lungs	=	0.1642
Stomach	=	0.1401
Muscle	=	0.0920
Heart	=	0.0741

A few investigations were carried out with sheep's tissues in order to ascertain whether there was any change in diastatic activity after the fresh moist tissue had stood for a considerable time at room temperature or in a cold chamber.

2 grms. liver were taken at different times after death and digested with starch solution in the usual way, under exactly similar conditions. The results indicate that liver does not lose its full diastatic activity for a very considerable time:—

	Result in CuO given by 10 c.c. digest
½ hour after death	= 0.0852
24 hours „	= 0.0754
86 „ „	= 0.0835

In another case liver was left for several days in alcohol, part being taken out at different times and dried in the usual way. No difference could be detected between liver treated for 24 hours with alcohol and the same liver treated for four days; the figures given are merely comparative, the same amount of dried tissue being used in each case:—

	Result in grms. CuO
24 hours in alcohol	= 0.0876
48 " "	= 0.0851
96 " "	= 0.0836

Samples of powdered tissue kept in a dry state did not seem to have changed in the slightest degree after several months. No indication of any difference in the action of the liver when used as soon as possible after death, and a very considerable time afterwards, was observed, though it is possible that this may not obtain for the glycogen *actually deposited in the liver itself* when acted upon by the cells *immediately* after the death of the animal.

That diastatic enzymes in general are exceedingly stable bodies, especially when protected from moisture, is proved by many researches. Thus Sehrt found active diastatic ferments in the tissue of mummies several thousand years old, and lately it has been shown by White (12) that the seeds of such cereals as wheat, maize and barley contain diastatic enzymes, which, if stored dry, retain their activity for twenty years or more; that these substances are necessarily comparatively stable bodies is obvious from the observation that barley diastase remains unaffected in efficiency after being subjected to temperatures varying from -200°C . to $+138^{\circ}\text{C}$.

These facts indicate that observations on human tissues which can generally be obtained not sooner than 18 to 24 hours after death, probably give quite a fair picture of what would be produced by fresh tissues. In the cases of normal organs examined, the results were generally of the same order as those obtained for other animal tissues. Kidney was generally much more active than any other part, liver being second and lung third. Muscle was again about the bottom of the list; great variations were, however, in evidence, and only a few cases were investigated.

Tissues were also obtained from two patients who had died from diabetes; it has been shown by Bainbridge and Beddard and by others that diastatic activity is present in diabetes, but the relative power of the different organs has not been investigated. In these two cases, however, no appreciable variation from the normal was observed; in No. 1 the marked effect of the kidney over all the other organs is well brought out,

Experiment VI

DIABETIC TISSUES

1 gm. tissue + 50 c.c. 1 per cent. starch + toluol + NaF. Digested 20 hours.

30 c.c. digest used.

No. 1

Result in grms. CuO

Liver	==	0.1191
Kidney	==	0.2642
Muscle	==	0.0959
Heart	==	0.0820
Lungs	==	0.2214

No. 2

Kidney	==	0.1498
Liver	==	0.1089
Lungs	==	0.1186
Muscle	==	0.0707

In these two cases, at any rate, it would seem as if the diabetic condition had at least no tendency to cause diminished diastatic activity; obviously many more cases would be required before any definite statement could be made.

GENERAL SUMMARY

Just as the present investigation was almost finished, several papers by Wohlgemuth (13) on tissue diastases appeared. One contribution dealing with the relative diastatic power of certain organs of the rabbit—liver, kidney, muscle—states that kidney was most powerful, followed by muscle and liver in the order mentioned. The figures given show fairly marked variations, and in some cases liver and muscle appeared to be about equal; sometimes muscle was more powerful than liver.

From these investigations, and those given in this paper, it is quite obvious that there are considerable variations in the relative diastatic activity displayed by different animals; at the same time there is no very definite relationship between the results obtained for the different tissues of two animals of the same species. The experiments given above are intended to indicate chiefly the comparative results obtained with animals of the same species, for sometimes different samples of soluble starch were made use of. At first it was intended to make comparative observations on many different species, but the extent to which variations manifested themselves in animals of the same species indicated that such an investigation would lead to no definite results unless a very great number of animals were used.

The results obtained, however, give sufficient data for an answer to the question of the existence of correlation of diastatic efficiency to glycogen content.

Just as the liver normally contains the most glycogen, and the muscles a considerable quantity, while such organs as the kidney, bladder and lungs contain but traces, it might be expected that these same organs would show diastatic power in the order of their glycogen storing power. This, however, is not the case, and sometimes an organ containing at most but a mere trace of glycogen—e.g. lung—shows more marked amylolytic power than liver; again, muscle may contain less ferment than any other organ.

It is obvious from these results that *there exists no definite correlation between glycogen content and diastatic efficiency in the case of adult tissues.*

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