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FERMENT ACTIONS OF THE PANCREAS IN DIFFERENT ANIMALS.

By VINCENT D. HARRIS, M.D., F.R.C.P., and WILLIAM J. GOW, M.D., M.R.C.P.

(*From the Laboratories of the Royal Colleges of Physicians and Surgeons.*)

In the research of which the present paper is a report, the authors desired to obtain information upon the following points, viz. :—

(1) Whether the ferments with the possession of which the pancreas is usually credited are present in the pancreases of animals of different classes, and if so whether there is any marked difference to be discovered in the activity (or amount) of each ferment in each class;

(2) Whether the activity or amount of the ferments bears any constant relation to the food of the animal;

(3) Whether the ferments of the human pancreas are markedly affected in activity or amount in morbid conditions of the body; and

(4) Whether in addition to the generally accepted pancreatic ferments the gland possesses any additional ferment action (*a*) in inverting cane sugar, or (*b*) in producing any chemical action upon dry or unboiled starch.

In carrying out the experiments connected with these problems we have employed material obtained from the ordinary laboratory animals, e.g. cats, dogs and the like; human pancreases obtained from the post-mortem room, and also, by the kindness of Mr Frank Beddard, F.R.S., the pancreases of many different animals dying in the Zoological Society's Gardens, Regent's Park. We would take the present opportunity of offering our cordial thanks to Mr Beddard for his courtesy in the matter.

Our knowledge of the functions of the pancreas dates from the investigations of Claude Bernard, which were carried out from the year 1846 to 1855. Up to this time the pancreas had always been considered to be identical in structure and function with the salivary

glands. It was in May, 1855, that Bernard delivered a series of lectures which embodied the results of his very important work. In these lectures he traced in detail the history of our knowledge of the anatomy and physiology of the gland up to the commencement of his research. He shewed that Wirsung in 1642 discovered the main duct of the pancreas in man, and Hoffman a few months previously had demonstrated the same in the turkey. From experiments made in the 17th and 18th centuries it was concluded either that the gland had no important function whatever, or that its function was identical with that of the salivary glands. In this century Majendie was one of the first to obtain a specimen of pure pancreatic secretion, and this he did by laying open the duodenum and collecting a small quantity as it oozed out of the orifice of the duct. He shewed that it was alkaline in reaction and coagulated on heating. Leuret and Lassaigne collected a considerable quantity of the secretion by introducing a cannula into the pancreatic duct of a horse, but their experiments with the fluid so collected led them to believe that its function was identical with that of saliva, and this idea was confirmed by the experiments of Tiedemann and Gmelin.

In 1846, Bernard, whilst investigating the phenomena of the digestion of fat in herbivorous and carnivorous animals, was struck by the fact that in rabbits no alteration in the fatty food took place until it had passed some distance from the pylorus, whilst in dogs it began to be altered much nearer the pylorus. The absorption of fat by the lacteals shewed the same difference, and he found that this difference coincided with a difference in the point of entry of the pancreatic duct into the duodenum. In dogs it enters very near the pylorus, whilst in rabbits it enters at a point about fourteen inches from the pylorus, and it was not until the fatty matters had passed this point that they began to be altered and absorbed. Bernard examined the pancreatic secretions of many animals and came to the conclusion that it was almost identical in all of them. He shewed too that the pancreatic juice shaken up with oil leads to the formation of an emulsion, and also that chemical changes ensued, from the splitting up of the oil into glycerin and fatty acids, this latter power being only possessed by the juice itself or by fresh pancreases pounded up in a mortar.

It is very clearly seen from the above short epitome that Bernard's researches were highly comprehensive and at once placed the physiology of the gland upon a sure foundation. Since his day several important additions have been made to our knowledge of the subject, but they

have been after all more with regard to details. Of these it is unnecessary to do more than mention the most important, viz. those of Heidenhain[1], Kühne[2], Langley[3], Lea[4], Bernstein[5] and Roberts[6], as they have been for the most part introduced into the ordinary text-books of physiology.

From these observations, however, it has been clearly established firstly, that very definite ferments exist in pancreatic juice, and may be extracted from the gland by means of glycerin and other vehicles; secondly, that these ferments have special relations to the different classes of food stuffs; and thirdly, that during secretion the ferments which had been previously deposited within the pancreatic cells in granular and undeveloped form are discharged into the lumen of the tubes. Although many different animals appear from incidental mention to have been used for the purposes of experiment by different workers, yet we are unaware of any systematic endeavour to investigate the question whether the ferments, four in number, which the pancreas is said to possess, appear in the gland in all animals, and it was with the idea of testing this question in a number of different kinds that this research was done.

Section I.

The experiments under the first head, viz.: to ascertain whether the usual ferments are present in the pancreases of such mammals as we were able to obtain, may be treated of in two divisions, (*a*) qualitative experiments and (*b*) quantitative experiments, the former having to do with four different ferment actions and the second with two only.

(*a*) *Qualitative Experiments.*

Experiments more or less complete have been made with the pancreases of the following animals to discover whether they contain ferments, (i) converting starch into sugar, *i.e.* diastasic or amylolytic; (ii) converting proteids into peptones, *i.e.* proteolytic; (iii) splitting fats into their fatty acids and glycerin, *i.e.* lipolytic, and (iv) curdling milk, *i.e.* rennettic.

[1] *Pflüger's Archiv*, x. 557, and xiv. 437 et seq.
[2] *Arch. f. Path. Anat.* xxxix. 130, and elsewhere.
[3] This *Journal*, iii.
[4] *Verhand. d. Heidelberg naturhist. med. Vereins V. e.* 1 Heft. v.
[5] *Sitzimge b. d. Akad. d. Wiss.* 36.
[6] *Digestion and Diet.*

English Name.	Scientific Name.	Class.	Food.	Cause of Death.
1. Lion	Felis leo	Carnivora felidæ	Meat	Paralysis, destroyed
2. Serval	Felis serval	Carnivora felidæ	Meat	?
3. Brown Bear	Ursus arctos	Carnivora ursidæ	Biscuits, bread	Pneumonia
4. Otter	Lutra vulgaris	Carnivora mustelidæ	Fish	?
5. Seal	Phoca vitulina	Carnivora	Fish	?
6. Wallaby	Halmaturus bennetti	Marsupial	Cabbage, corn bread	?
7. Axis deer	Cervus axis	Ruminant	Hay, corn	Died during parturition
8. Dingo dog	Canis dingo	Carnivora canidæ	Meat	Destroyed
9. Paradoxure	Paradoxusus typus	Carnivora viverridæ	Meat	?
10. Armadillo	Tatusia peba	Edentata	Meat, vegetables	?
11. Leopard	Felis uncia	Carnivora felidæ	Meat	?
12. Glutton	Gulo luscus	Carnivora mustelidæ	Meat	Congestion of lungs
13. Ocelot	Felis pardalis	Carnivora felidæ	Meat	?
14. Moufflon	Ovis musimon	Ruminant	Hay, corn	Intestinal obstruction
15. Drill Baboon	Gynocephalus leucophæus	Quadrumana	Fruits, bread, rice	Bronchitis
16. Horse	Equus Caballus	Pachyderm	Hay, corn	Killed

In addition to the Pig, Cat, Dog, etc. and several birds, *e.g.* Bonelli's Eagle, Sea Eagle, Rhea Americana, as well as one or two Snakes.

Extracts of the glands made with the following different vehicles have been employed:

(*a*) Distilled water.
(*b*) Glycerin.
(*c*) Chloroform water.
(*d*) Saline solutions of different strengths.
(*e*) Dilute and slightly acidulated spirit,

and experiments have been also made with the pounded gland itself, without any admixture of fluid. The extracts have invariably been made after the following method: The pancreas either fresh, or after twenty-four hours' exposure to the air, or after immersion for a variable time in methylated spirit, has been carefully freed from extraneous tissues and has then been either passed several times through a sausage-making machine until quite pulpy or pounded up in a mortar until in that condition; the pulp has then been weighed and the vehicle to be used for the purpose of dissolving the ferments has been added in the amount to make a solution of a definite and known strength: as an example of the method the following instance may be given:

Sept. 1890. Human pancreas deprived of fat and connective tissue as far as possible by careful dissection, then placed in methylated spirit, and afterwards dried under an air-pump over sulphuric acid, weighed 51 grms.: then passed several times through sausage-making machine and any connective tissue picked out and the pulp again weighed, = 46 grms. The pulp used to make three 20 % extracts—(1) with diluted methylated spirit (1 in 10) rendered slightly acid with acetic acid (1 in 50), (2) with 10 % solution of ammonium chloride, and (3) with brine solution, made by saturating distilled water with common salt.

The comparison of the activity of the pancreatic ferments in different animals, weight for weight of the gland, we may say at once would be quite misleading, as the differences in the amount of the connective tissue present in different pancreases is most marked. For example in the above instance of the human pancreas about $\frac{1}{8}$ of the weight consisted of obvious masses of connective tissue, in other animals *e.g.* in that of the dingo dog, the proportion was even greater, whilst in others, *e.g.* the fox, there was hardly any loss of weight by connective tissue, and in the pancreases of some birds and snakes (experiments with which are not for the most part included in this report) the whole gland was capable of being powdered up almost like dried liver. Again, without committing ourselves to a more definite statement, comparison of different parts of the same pancreas, weight for weight, would in many instances be quite unreliable, since the amount of connective tissue obviously varies in different parts of the same gland.

The use of chloroform water as a solvent for digestive ferments is open to objection in cases where the amount of amylolytic ferment present in the pancreas is small, because of the property possessed by chloroform of reducing Fehling's solution. Watery solutions of chloroform, half the strength of the Aqua Chloroformi of the *British Pharmacopeia*, readily reduce Fehling's solution, so that for qualitative testing in the case of pancreases possessing only feeble amylolytic power or none at all, brine or spirit solutions are better.

The brine extracts were found on the whole to be the most satisfactory. They were made by pounding up the pancreatic tissue freed from connective tissue, in a mortar with salt. The presence of the salt made it possible to reduce the pancreatic tissue to a powdery consistence and this powder, consisting of salt and gland tissue, was then mixed with a known quantity of distilled water.

We made experiments also with slightly acidulated spirit extracts as recommended by Sir W. Roberts, but found that in some cases after

filtering the solution became quite inactive, both with starch and proteids.

Extracts made with 10% solution of ammonium chloride did not appear to possess any advantages over the brine (sodium chloride) extracts.

The qualitative experiments were very numerous, with varying amounts of the extracts, and were done in flasks in the cases of the amylolytic, tryptic and rennet ferments.

(*a*) *Amylolytic Experiments.*

In these experiments we directed our attention to:

(i) The amount of standard solution of the pancreas required to produce any change in the starch.

(ii) The length of time required to produce any change; as well as the length of time at which all ferment action appeared to cease.

(iii) The resulting product or products.

(iv) The influence of temperature.

Example I. Of negative action. Lion's pancreas—15% extract of the lion's pancreas made with saturated salt solution. The solution was not coagulated or rendered opalescent on boiling, but gave two of the proteid reactions fairly, viz. the xanthoproteic and the biuret reactions, but only a white precipitate not rendered pink on boiling with Millon's reagent.

Sept. 30, 1890. Fresh starch mucilage solution (about 3%) with 1% sodii bicarbonate. 100 c.c. of the solution were placed in 3 flasks: *A*, with 1 c.c. of above-mentioned brine extract, *B* with 2 c.c. of the same extract, and *C* with 4 c.c. of the same extract.

Exposed at the temperature of the room about 20° C. for 24 hours.

Oct. 1. No change whatever in the starch solution.

Flasks *A*, *B* and *C* put in incubator at 40° C.

And an additional flask *D*, containing 50 c.c. of 1% solution of starch mucilage and sodii bicarb. to the extent of 1% and 2·5 c.c. of the above described brine extract.

In incubator for 22½ hours.

No substance in solution in any of the flasks was capable of reducing copper sulphate solution on boiling.

Numerous subsequent experiments confirmed the deduction that the pancreas of the lion, extracted by brine or by dilute acid spirit or by water, contained no diastasic ferment. The same extract dissolved fibrin with great readiness.

Example II. Of complete action. Pig's pancreas.

The pig's pancreas is so active that 1 or 2 c.c. of any extract is able to convert a certain amount of starch into sugar in 2 or 3 minutes. The following experiment shews what appears to be a complete action :—

Oct. 9. Two flasks—*A* and *B*.

A contained 50 c.c. of a 1 % solution of starch mucilage = 0·5 grms. of dry starch, sodii bicarb. to extent of 1 % and 5 c.c. of a brine extract of pig's pancreas.

B with similar contents but with perchloride of mercury to the extent of 1 in 1000, i.e. $\frac{1}{20}$ grm. placed in the incubator at 40° C.

Oct. 10. In flask *A* there was no reaction with iodine but a good reaction of reducing sugar, probably maltose.

The amount of sugar estimated by Pavy's method gave 0·416 % of sugar, or, in other words, 0·28 grms. of sugar were obtained from 0·5 grms. of dry starch.

Flask *B* gave the same amount of sugar in the solution.

This was the largest amount of sugar we were able to obtain in any case from the same amount of starch.

In a similar experiment with 4 c.c. of a brine solution of a human pancreas we obtained our nearest approach to this, viz. 0·1 grms. of sugar from 0·5 grms. of starch.

Both the amounts appear to be somewhat small.

Example III. Of delayed action. Dingo Dog. The pancreas was noted as being long and broad but very loose, with a great amount of connective tissue and large lobules—a 10 % brine extract was used.

April 2, 1891. The flask contained 40 c.c. of a 1 % starch solution and 5 c.c. of the extract. Hardly any effect upon the starch was observed during the first 24 hours.

On *April* 4, a slight amount of sugar was present, both erythrodextrin and starch being also present in the solution.

The solution remained in the incubator at 40° C. for several more days but there was never complete conversion of the starch into sugar; dextrin was always present.

The three examples will explain the terms active, inactive, and slight or delayed, to be used in the following table :

The observations made of flask digestion of starch appeared to suggest : I. That a certain definite amount of ferment must be present before any change occurs, as below a certain minimum no diastasic action could be demonstrated; this fact we noticed and tested over and over again.

II. That a certain definite amount of the pancreatic extract could

TABLE I.

Diastasic action on Starch Mucilage at a temperature of 40° C.

A. Active.	B. Slight or delayed[1].	C. Inactive[2].
Pig. Far the most powerful action of any animal (1 grm. of starch entirely converted, leaving no starchy or erythrodextrin residue by 5 c.c. of extract in between 5 and 10 mins.)	Ocelot (almost nil)	Lion[3]
Glutton	Otter (much delayed, 70 hours)	Rhea
Ox	Serval (slight, after 24 hours)	Leopard
Brown Bear	Wallaby (70 hours)	
Seal	Horse (slight & delayed)	
Dog	Dingo dog (slight and delayed)	
Cat	Puma (very incomplete)	
Fox	Moufflon (incomplete)	
Sea Eagle		

do a definite amount of work and no more. The amount of ferment work done in different cases varied as shewn in the above table, in some cases being small and in other cases large. The action of the ferment then is obviously limited and the removal of the product of the action, *i.e.* the dextrin and sugar formed, is not enough to prolong the change indefinitely. This confirms the observation of Sir William Roberts[4]: "The notion that the energy of diastase is not consumed in action seems, on *a priori* ground, to be quite untenable—such a notion contravenes a general principle in physics that energy in performing work is expended and finally exhausted."

III. That the power of conversion of starch into dextrin may be very active whilst the further power of changing dextrin into sugar may be only feebly present, this will be again referred to in a future section.

IV. That a moderately high temperature, viz. 40° C. (to 45° C.) appeared to be the optimum for the diastasic action.

[1] Larger amounts of extracts used than in A.

[2] Larger amounts of extracts used than in B.

[3] The pancreases of two lions gave same result.

[4] *Digestion and Diet*, p. 31.

(*b*) *Proteolytic or Tryptic Experiments.*

These were also flask experiments. The method of experiment was as follows:

Fibrin obtained from ox's blood was thoroughly washed free of colouring matter, was torn into small shreds, and used unboiled for the tryptic experiments. The solution used was sodii bicarb. (1%) and the extracts of the glands were as in the amylolytic experiments. The flasks were plugged with cotton-wool and placed in the incubator at 40° C.

Example of method. Brine solution of lion's pancreas. Three flasks used, *A*, *B* and *C*.

In *A* Flask—Fibrin 2 grms., 50 c.c. 1% solution of sodii bicarb. and 2 c.c. of pancreatic extract.

B Flask—Fibrin 2 grms., 50 c.c. 1% solution of sodii bicarb., but with 3 c.c. of extract.

C Flask—Fibrin 2 grms., 50 c.c. 1% solution of sodii bicarb., but with 5 c.c. of extract, put in the incubator at 12 noon on Oct. 2, 1890.

TABLE II.

Tryptic action of the Pancreatic Extracts on Fibrin.

Active.	Slightly Active.	Inactive.
Human, very active	Gazelle	None
Lion ,,	Leopard ?	
Pig ,,	Badger	
Glutton ,,	Serval	
Ocelot, less than Glutton	Wallaby	
Otter, very active		
Puma ,,		
Dog ,,		
Cat ,,		
Fox ,,		
Dingo Dog ,,		
Seal		
Axis Deer		
Ox		
Brown Bear		
Sea Eagle, very active		
Rhea		

On *Oct.* 3, at 11 a.m.

Fibrin in *A* Flask about half digested.
do. *B* do. three-quarters digested.
do. *C* Flask practically wholly dissolved.

The undigested solids from *A* flask separated and dried—weighed 1 grm. The solid material &c. weighed being yellow and jelly-like, and not easily soluble in dilute hydrochloric acid, was probably swollen fibrin.

Oct. 3. In a fourth flask *D*, was placed 2 grms. fibrin, 50 c.c. 1 % solution of sodii bicarb., 2 c.c. of the brine extract of lion's pancreas, and carbolic acid added in the proportion of 1 in 100.

Oct. 4. The fibrin was partially dissolved and the undigested residue was dried and weighed less than 1 grm. So that in each case with 2 c.c. of the extract, nearly the same amount of fibrin had gone into solution and the presence of carbolic acid in flask *D* had not the effect of diminishing the tryptic action of the ferment.

(c) *Fat-splitting or Lipolytic Action.*

In no single case tried were we able to demonstrate the presence of any fat-splitting ferment in extracts of pancreases which had been kept in spirit. In some instances, however, we have had satisfactory evidence of the presence of a ferment in fresh pancreases, acting upon fats. This action was far the most pronounced in the case of the dog's pancreas, less so in that of the pig, practically absent from the human pancreas and from the cat's pancreas.

Effect of Pancreatic Extract on Oil.

The fresh pancreas of a dog was pounded up in a mortar with a little distilled water, the reaction being very faintly alkaline.

Neutral Lucca Oil was used. 5 c.c. of Lucca oil and 5 c.c. of aqueous solution of pounded pancreas unfiltered were placed in a test-tube and thoroughly shaken up and then placed in water at 32° C. In ten minutes the reaction was distinctly acid, and at the end of half-an-hour this acid reaction was very marked. The fluid shewed only a very slight tendency to separate into two layers.

Experiments were then made with a view to determining whether the development of the acid reaction was prevented or interfered with by the presence of carbolic acid, and whether emulsification was dependent either on the action of a ferment or on the presence of the acid.

Example I. Four test-tubes were taken.

Number 1.

2 c.c. of Lucca Oil.
2 c.c. of freshly pounded pancreas.
4 c.c. of Solution of Carbolic Acid (1—20).

Carbolic acid was therefore present in the mixture in the proportion of 1 in 40. The mixture was neutralized.

Number 2.

2 c.c. of Lucca Oil.
2 c.c. of freshly pounded pancreas.
4 c.c. of water.

Number 3.

2 c.c. of Lucca Oil.
2 c.c. of freshly pounded pancreas (boiled).
4 c.c. of water.

Number 4.

2 c.c. of Lucca Oil.
4 c.c. of water.

The contents of all the test-tubes were thoroughly shaken up and the tubes were then placed in a beaker of warm water at a temperature of 32° C. At the end of 10 minutes they were all examined. In Number 4, there was almost complete separation into two layers. The fluid below the oil was slightly milky. The three other tubes all shew some degree of separation into two layers and all to about an equal extent. The lower layer of fluid in these cases is buff-coloured. The contents of tubes 1 and 2 have a distinctly acid reaction. Tubes 3 and 4 still remain neutral. The presence of carbolic acid in the proportion of 1 in 40 does not interfere with the development of this acid reaction. This would suggest that the formation of the acid is not due to organized ferments. It will be observed that the power of producing the fatty acid is destroyed by boiling.

The tubes were examined again at the end of twenty-four hours. Increased separation into two layers was noticed in tubes 1, 2 and 3, but in all of them the degree of separation was as nearly as possible equal. Emulsification does not therefore seem to depend on the presence of any ferment, nor does it seem to be increased by the presence of an acid, as in tube 3 the pancreas had been boiled before it was mixed with the oil and this tube remained neutral to the last. Emulsification depends entirely on the thickness and consistency of the fluid which is mixed with the oil.

Brine extracts of pancreas mixed with oil failed to lead to the development of any acid.

Example 2.

Pig's pancreas. In this case the experiment was repeated, but pig's fresh pancreas was used instead of dog's.

There was rapid and complete emulsion, and considerable, but less rapidly developed, acid reaction.

Example 3.

Cat's pancreas. A perfectly fresh pancreas from a healthy cat was taken. Half to 1 hour after removal from the body it was pounded in a mortar with distilled water. The extract was perfectly neutral in action. Four test-tubes with an equal quantity of oil (pure and neutral) in each; in (i) an equal quantity of the pancreatic extract, in (ii) boiled pancreatic extract, in (iii) pancreatic extract and carbolic acid to the extent of 1 in 40, in (iv) oil and water only.

All of the tubes were exposed to a temperature of 40° C. and examined at intervals. In no case was there any evidence of fat-splitting up to two days.

An extract of the gland made twenty-four hours after removal from the body gave similar and negative results.

A second cat's pancreas was used for a series of similar experiments, but neither in this could we obtain evidence of a fat-splitting ferment being present.

It is a remarkable fact that the fat-splitting ferment should be so difficult to prove in pancreatic extracts since in pancreatic juice, according to Claude Bernard, it is always present. We have been able in a large number of experiments to confirm his statement that the ferment or ferments acting upon fats do not occur in pancreases, except when fresh.

(*d*) *Rennettic Experiments.*

There can in our opinion be no possible doubt as to the very frequent presence of a rennet ferment in pancreatic extracts. Of course this does not necessarily imply its presence in natural pancreatic juice. We have made no experiments to shew its presence in the secretion of the gland. In the latest English text-books on Physiology, little stress has been laid upon the presence of a rennet ferment in the pancreatic juice or in pancreatic extracts by Foster (1889), Mc Kendrick (1889), Halliburton (1890), and Waller (1891). The latter however makes the following observation on the subject: "for the sake of completeness, rather than because we have any reason to believe that the action is normally exercised, it may be mentioned that pancreatic juice or extract possesses the rennet property of coagulating milk; we have, however, no ground for supposing that milk can ever escape coagulation in the

stomach either by rennet ferment or by acid and be coagulated by pancreatic juice." We feel quite disposed to agree with these statements, but at the same time are unable to understand why such a very marked ferment action can be without some use. For, as has been pointed out by Roberts and others, the action is certainly remarkable. Thus, for example, 1 c.c. of an ordinary brine extract of pig's pancreas (and the ferment is so active in the pigs' pancreases we have tried, that we may generalize) will completely clot 15 c.c. of milk at 37° C. in less than a minute.

Method of Experiment. Our experiments relating to the rennet ferment were done in the following way: Fresh skimmed milk was boiled and then cooled down to 38° C. A varying amount of the extract of pancreas to be tested was added with or without the addition of sodii bicarb. (which is by no means necessary for the action), and the mixture was watched and the time in which coagulation took place noted. If 1 c.c. was unable to curdle 10, 15 or 20 c.c. of milk, 2 c.c., 4 c.c. or more, of the extract were added and the experiment was repeated as often as necessary. Control experiments were done with the same extracts of pancreas previously boiled.

TABLE III.

The curdling action of the different extracts on milk.

1.	Horse	50 c.c.	milk	+ 3	c.c. extr.	Firm clot in 3 minutes.
2.	Seal	15	„	+ 3	„	Firm clot.
3.	Otter	15	„	+ 5	„	Firm clot in 10 minutes.
4.	Wallaby	15	„	+ 5	„	Imperfect slow coagulation.
5.	Serval	15	„	+ 5	„	Imperfect slow coagulation.
6.	Brown Bear	15	„	+ 5	„	No coagulation.
7.	Ocelot	15	„	+ 5	„	No coagulation.
8.	Glutton	15	„	+ 5	„	Firm coagulation.
9.	Moufflon	15	„	+ 5	„	Slow coagulation.
10.	Lion	15	„	+ 5	„	No coagulation.

The pancreatic extracts of Man, Pig, Dog, and Cat were all very active in their power of rapidly curdling milk. Also those of several varieties of Eagle, but that of the Rhea was inactive.

A 25 % extract was used in all cases.

There seems to be no relation between the activity of the diastasic ferment and that of the rennet ferment. The pancreases of the Otter and Wallaby possessed nearly the same diastasic power, but clotting

occurred in 10 minutes with extract of the Otter's pancreas and only imperfectly, and after several hours with that of the Wallaby.

The pancreas of the Horse produced firm and rapid clotting, but its diastasic activity was extremely slight.

There seems also to be no very definite relation between the tryptic power of pancreas as estimated by the metacasein reaction and the power of curdling milk. In the Horse $T^1 = 62{\cdot}5$, and rapid clotting is produced in milk, but in the Seal where T is less than 1, firm and rapid clotting is also noticed. In the case of the Wallaby $T = 6$, and milk is clotted only slowly and imperfectly.

In some of the above experiments a brine extract was used. According to the recent researches of J. S. Edkins[2] the presence of sodium chloride seems to aid the action of the rennet ferment.

The different results shewn in the above table cannot however be attributed to this factor. Brine extracts of the pancreases of the Lion, Bear, Wallaby, Seal and Horse were used, and it will be seen that while the pancreatic extracts of the Lion and Bear were incapable of coagulating milk, that of the horse was exceedingly active and that of the Wallaby only moderately so.

Differences in the curdling activity appear therefore to exist in the pancreatic extracts of different animals.

(*b*) *Quantitative Experiments.*

Diastasic power. A series of experiments were made to determine the relative activity of the diastasic ferment in the pancreases of the following animals, which on the removal from the body had been preserved in methylated spirit.

1. Brown Bear.
2. Drill Baboon.
3. Otter.
4. Axis Deer.
5. Paradoxure.
6. Dingo Dog.
7. Armadillo.
8. Man.
9. Pig.
10. Seal.
11. Wallaby.
12. Serval.
13. Horse.

In all cases except the Wallaby and Serval a spirit extract was used, the extracts of the pancreases of these two animals were made with brine.

Each pancreas was pounded up in a mortar and mixed with four times its weight of dilute alcohol containing 25 % of rectified spirit.

[1] For explanation of T see p. 487.

[2] This *Journal*, Vol. XII.

This mixture was digested at a temperature of 40° C. for several days and then filtered, a very small quantity of acetic acid having been added before filtration.

A 1 % starch solution was employed.

A slight modification of a method suggested by Sir W. Roberts was employed. His method described in his own words was the following:— "In principle the method consists in ascertaining the quantity of starch mucilage of known strength which can be transformed, by a unit measure of a diastasic solution, to the point at which it ceases to give a colour reaction with Iodine, in a unit of time and at a fixed temperature."

The iodine solution used was made by diluting 1 vol. of the liquor iodi of the *British Pharmacopeia* with 200 volumes of water.

The temperature of the mixture during digestion was maintained at 40° C.

The experiments were conducted in the following manner:—A series of test-tubes each containing 1 c.c. of the iodine solution were first prepared.

A known volume of 1 % starch mucilage was taken and diluted with water containing 1 % of sodii bicarb. to ten times its original volume and was then raised to, and afterwards maintained at, a temperature of 40° C. A known quantity of pancreatic extract was then added to the diluted starch mucilage and the time at which this was done was noted. At intervals of one minute, 1 c.c. of the mixture was withdrawn and added to an equal bulk of the iodine solution contained in the test-tubes, and the point of time at which any colour ceased to appear when the two fluids were mixed was recorded. This is spoken of as the achromic point. Sir W. Roberts determined the achromic point by placing a drop of the enzymosing liquid on a white plate with a drop of iodine solution, but the authors found (after using this method for some time) that it was more convenient and more accurate to mix the liquids in a test-tube as above described.

Mode of Calculating and Expressing the Diastasic Value.

"In reducing this principle to a definite formula it was necessary to choose arbitrarily a unit of measure of the diastasic solution and a unit of time. The unit of measure fixed on was 1 c.c., and the unit of time five minutes. These selections seemed on the whole the best adapted for furnishing a convenient scale. On these bases the formula took the following form: the diastasic value of any solution—or *D*—is expressed

by the number of cubic centimeters of the standard starch mucilage which can be transformed to the achromic point by 1 c.c. of the solution to be tested in a period of five minutes at a given temperature. In the process of testing the quantity of the standard mucilage was made constant, namely 10 c.c., and the quantity of pancreatic extract and the time were made variable. In order to get the value of D the results must be so transformed as to make the quantity of extract and the time constant and the quantity of standard mucilage variable. This is accomplished by increasing or reducing a quantity of pancreatic extract employed to 1 c.c. and increasing or diminishing the standard mucilage in the same proportion. The product thus obtained is again increased or reduced in the same proportion as is requisite to increase or reduce the time to five minutes. The value of D is obtained by the following formula: Let p signify the quantity of pancreatic extract employed, and m the number of minutes found requisite to reach the achromic point, then :— $\frac{10}{p} \times \frac{5}{m} = D$.

The value of D as already explained signifies the number of cubic centimeters of the standard starch mucilage which can be changed to the achromic point by 1 c.c. of the diastasic solution in five minutes at a given temperature. As the standard mucilage contains 1 p.c. of dry starch, the value of D divided by 100 gives us the same value in terms of dry starch" (Roberts).

The following experiment may be taken as an example :

10 c.c. Standard Starch mucilage + 90 c.c. of water + 2 c.c. of pancreatic extract of Bear at 40° C.

Achromic point reached in 4 minutes.

$$\tfrac{10}{2} \times \tfrac{5}{4} = D = 6{\cdot}25,$$

or in terms of dry starch $D = {\cdot}0625$ grains.

The following table shews the value of D in the different cases :—

TABLE IV.

Diastasic value of the pancreatic extracts.

Brown Bear	D	=	·0625	grains	of dry	starch
Drill Baboon	D	=	·0555	,,	,,	,,
Otter	D	=	·1666	,,	,,	,,
Axis Deer	D	=	·0833	,,	,,	,,
Paradoxure	D	=	·025	,,	,,	,,
Dingo dog	D	=	·1666	,,	,,	,,

Armadillo	D = less than	·0083	grains	of	dry	starch
Seal	D =	·0625	,,	,,	,,	
Wallaby	D =	·1388	,,	,,	,,	
Serval	D =	·125?	,,	,,	,,	
Horse	D = less than	·004	,,	,,	,,	
Man	D =	·033	,,	,,	,,	
Pig	D =	·5	,,	,,	,,	
Rhea	D =	·01	,,	,,	,,	
Bonelli's Eagle	D =	·25	,,	,,	,,	

From our experiments we are led to believe that this method of estimating the diastasic activity of pancreatic extracts is open to considerable objection.

By this method the activity of the ferment is measured by the rapidity of dextrin formation, not of sugar formation. It would be assumed from an examination of the figures in the above table that pancreases of the Otter and Wallaby possessed a very active diastasic ferment, but it was found from experiments in flask digestion that the conversion of starch into sugar was brought about very slowly.

In both cases to 50 c.c. of the standard starch, 5 c.c. of pancreatic extract were added and the mixture placed in an incubator at a temperature of 36° C. At the end of 24 hours no evidence of sugar could be obtained, but at the end of 70 hours there was evidence of the presence of a considerable quantity of sugar.

It was also observed that the presence or absence of a blue colour on the admixture of the iodine solution and the enzymosing fluid depended to a certain extent on the relative amount of the two fluids used. Thus by adding 1 c.c. of the enzymosing fluid to 2 c.c. of dilute iodine (1·200), no colour would be seen, though if 2 c.c. of the enzymosing fluid were employed and only 1 c.c. of iodine solution, a distinct blue colour would be produced.

It is to be noted that when the achromic point is reached not only is there no blue colour, but the natural yellow colour of the iodine solution is lost and the mixture is quite colourless. This decolourization of the weak iodine solution does not indicate that there is no starch present, but it indicates that a new substance (probably dextrin) is produced in sufficient amount to decolourize the iodine and prevent it giving a blue colour with starch. We had abundant opportunity of observing the mutual relations of quantity and time in the action of the diastasic ferment. Sir W. Roberts says, "The fundamental rule which governs the mutual relations of quantity and time in the action

of an enzyme is that of inverse proportion. That is to say double the quantity of an enzyme will do a given amount of work in half the time and that half the quantity will require double the time." In many of our experiments this relation did not seem to hold good: *e.g.*

(1) 10 c.c. of Starch mucilage + 90 c.c. of water + 4 c.c. extract (Paradoxure).
Achromic point reached in 5 minutes.

(2) 10 c.c. of Starch mucilage + 90 c.c. of water + 2 c.c. extract (Paradoxure).
Distinct blue colour after 12 minutes.

(3) 1 c.c. of Starch mucilage + 9 c.c. of water + 2 c.c. extract (Seal).
Achromic point reached in 4 minutes.

(4) 1 c.c. of Starch mucilage + 9 c.c. of water + 1 c.c. extract (Seal).
Pale blue colour after 10 minutes.

Tryptic power. For estimating the proteolytic activity of the pancreatic extracts the method suggested by Sir W. Roberts was employed. "When milk is subjected to digestion with pancreatic extract, a striking change takes place in it at an early stage of the process—the milk acquires the property of curdling when boiled. The onset of this reaction occurs at an earlier or a later period, according to the activity of the extract and the quantity of it employed, and it is possible to fix the time of its advent with considerable accuracy—sufficient accuracy to serve as the basis of a method of measuring the proteolytic activity of pancreatic extracts."

This body which curdles on simple boiling has been named by Roberts metacasein, and the property of curdling when boiled is spoken of as the metacasein reaction.

"In principle the method of trypsimetry here proposed, consists in ascertaining how many cubic centimeters of milk can be changed to the onset point of the metacasein reaction in five minutes by 1 c.c. of the extract to be tested at a given temperature." Roberts pointed out that by diluting the milk with an equal quantity of water the metacasein reaction is postponed, but the inequalities of the milk were minimised and "strike" of the reaction was more sharply defined.

In our experiments we followed exactly the mode of proceeding thus described by Sir W. Roberts. "50 c.c. of fresh milk were diluted with 50 c.c. of water, less the quantity of extract intended to be added. The diluted milk was then warmed to 40° C., and maintained exactly at that temperature until the close of the experiment. The intended quantity of the pancreatic extract, say 1 c.c., was then added, and the time exactly noted. At the end of each minute a portion of the digesting

milk was withdrawn and boiled for a few seconds in a test-tube, inclining the test-tube to one side after boiling, to observe the effect. As soon as distinct curdling occurred on boiling, the experiment was considered finished; the time was recorded and the number of minutes which had elapsed from the commencement of the experiment were reckoned. The object of the experiment was to ascertain how many c.c. of milk can be changed to the onset point of the metacasein reaction by 1 c.c. of extract in a period of 5 minutes at the temperature of 40° C. The tryptic value or T was calculated from this first expression of the results of an experiment in exactly the same way as for diastase. If p be made to signify the quantity of pancreatic extract added to the milk, and m the number of minutes which were required to reach the onset point of the metacasein reaction, then the value of T was obtained by the following formula:—

$$\frac{50}{p} \times \frac{5}{m} = T."$$

The following results were obtained:—

TABLE V.

Tryptic value of the pancreatic extracts.

Dog (recently fed)	T = 4·464
Dog (starving)	T = 15·625
Lion	T = 3·333
Axis Deer	T = 2·0
Brown Bear	T = 2·0
Wallaby	T = 6·25
Otter	T = 15·625
Drill Baboon	T = 10·416
Horse	T = 62·5
Seal	T = less than 1
Rhea	T = less than 1
Eagle	T = 2·777

In the preceding notes of their experiments, it will be seen that the authors have not been able to find any fixed rate as to the amount or activity of the pancreatic ferments in different classes of mammals. It has seemed to them remarkable that such irregular results should have been obtained. This irregularity might have been due to the condition of the animal at the time of death, whether healthy or unhealthy, and whether in good condition or wasted, and the time which had elapsed

since its last meal, but it could not have been due to any difference in the method of preserving the pancreases or of the method of preparing the extract for comparison.

It will be noticed that of the four ferments dealt with, the tryptic ferment is by far the most universal and hardy, next to that we should put the rennet ferment, next the diastasic, and lastly, as being that which is, if present universally in the pancreases of animals (and this we are strongly inclined to doubt), most easily destroyed by preserving the pancreas in spirit, the fat-splitting ferment. We are quite unable to find proof of any special fat emulsifying ferment in any pancreas with which we have experimented.

Section II.

As regards the question as to whether there is any constant relation between the activity of the various ferments of the pancreas of an animal and of its food, we may say that the results of our experiments touching the pancreases of the carnivorous animals, the lion, the leopard, the serval, and the ocelot are of interest.

Large quantities of brine, glycerin and spirit extracts of lion's pancreas[1] were added to weak starch solution and the mixture placed in an incubator at a temperature of 40° C. for 24 hours. At the end of that time iodine solution produced a deep blue colour, and there was no evidence of sugar.

The same extracts were found to digest fibrin with great rapidity. No coagulation of milk occurred on adding 5 c.c. of brine extract to 15 c.c. of milk.

Leopard. The following mixture was placed in the incubator at a temperature of 40° C. for 24 hours.

1 c.c. of 1 % starch solution.
9 c.c. of 1 % Sod. Carb. solution.
1 c.c. of 25 % spirit extract of pancreas.

There was no evidence of sugar at the end of 24 hours.
Decolourization of weak iodine solution (1—200).
Milk—very slight and imperfect coagulation.

Ocelot. After 24 hours' digestion with starch solution in an incubator at a temperature of 40°C., there was a well-marked blue colour with iodine solution.

No sugar reaction, as indicated by the copper reducing test.
No clotting of milk.
Rapid action on fibrin.

[1] We have had two opportunities of obtaining the pancreas of the lion.

Serval. Blue colour with iodine after 24 hours' digestion. Very slight reduction of copper. On adding 5 c.c. of extract to 15 c.c. of milk no clot formed at the end of forty minutes. After several hours a slight and imperfect clot appeared.

This would seem to shew that the pancreases of the above-named carnivorous animals have little or no power of converting starch into sugar and also do not possess any rennet ferment.

The tryptic power of all these extracts was unusually active.

It will also be noted by reference to Table I., that in the extracts of the pancreases of several more carnivorous animals, the diastasic power was slight, but the horse's pancreas gave an irregular result, it was very inactive as regards amylolytic power and very active as regards its tryptic or proteolytic ferment.

It will also be noticed with reference to Table II., that the tryptic ferments of several carnivorous animals were only slightly active.

The fat-splitting ferment is not commonly present in pancreatic extracts.

No conclusions can be drawn with reference to the rennet ferment (Table III.) except that it is more often present than not, and that in some pancreases it is extremely powerful.

We may notice, in passing, as an interesting fact, that only the following animals appear in the first class as regards the activities of all of their ferments; the pig, the ox, the man, and sea-eagle[1].

Section III.

Our experiments to test the activity of human pancreatic ferments in diseased conditions have not been as numerous as could be wished. The main reason of this has been the difficulty of obtaining the gland at a sufficiently short time after death to preclude the possibility of incipient decomposition. So far[2] we have carefully gone into the ferment activity of pancreases from one case of phthisis, one case of surgical disease causing death, one of morbus cordis and one case of leucocythemia, and were fortunate to obtain, by the kindness of Mr Anthony Bowlby, the pancreas of a strong muscular healthy man who was killed at a fire which took place in Cloth Fair, E.C., in

[1] We have had opportunities of obtaining several specimens of pancreas from different kinds of eagles; that of the sea-eagle is by far the most active.

[2] We are continuing these experiments (April, 1892).

October 1890, the extract from which we used as one type of a healthy human pancreas, and compared the extracts from the other human pancreases therewith.

Case I. Patient died of surgical disease, nature not stated, but not producing any marked wasting.

(*a*) *Amylolytic ferment.* The brine extract and the 10 % chloride of ammonium extract were moderately active in converting starch mucilage into sugar; 3 c.c. converting 1 grn. of dry starch, and producing in 24 hours 0·15 grains of sugar at 38° C.

The diluted acid spirit extract was much less powerful and took 3 days to perform about half the work.

(*b*) *Tryptic ferment.* This ferment was powerful both in brine and acid spirit extract. 4 c.c. completely dissolved 0·44 grains of boiled fibrin in 16 hours.

(*c*) *Rennet ferment.* Was present in any extract tried to a very slight degree.

Case II. Patient, girl, aged 18, died of leucocythemia—not much wasted.

(*a*) *Amylolytic ferment.* About two-thirds the activity of Case I. and one-half that of Case III. The amylolytic ferment was thus only slightly active.

(*b*) *Tryptic ferment.* Was present and active.

(*c*) *Rennet ferment.* Was absent.

Case III. Case of healthy man suddenly killed.

(*a*) *Diastasic ferment.* 5 c.c. of a brine extract completely converted 1 grain of dry starch into sugar in half the time taken by extract from Case II.

(*b*) *Tryptic ferment.* Very active.

(*c*) *Rennet ferment.* Very active, 2 c.c. of any extract was able to coagulate 20 c.c. of skimmed milk in 2 minutes.

In passing we may mention that the extracts from this patient's gland were only second in activity to the extracts of the pig's pancreas.

Case IV. From a patient with morbus cordis (aortic and mitral valvular disease, and no other disease).

(*a*) *Diastasic ferment.* The acid spirit extract was very feeble, the extract was used according to Roberts's method and the value of *D* in terms of dry starch = ·033 grains.

(*b*) *Tryptic ferment.* Very active.

(*c*) *Rennet ferment.* (No note can be found of this experiment, probably omitted by mistake.)

(*d*) *Oil-splitting ferment.* Absent.

Case V. From a case of advanced phthisis.

(*a*) *Diastasic ferment.* The diastasic value of *D* in terms of dry starch was 0·02, or nearly a third less powerful than in Case IV., scarcely any power of converting starch into sugar.

(*b*) *Tryptic ferment.* Very feeble, in Roberts's terms *T* = less than 1.

(*c*) *Rennet ferment.* Present but to only a very slight degree—2 c.c. of the spirit extract required 65 minutes to clot 20 c.c. of milk (neutral in reaction and of Sp. Gr. of 1032).

So few cases are insufficient to justify any very definite conclusions, but Cases I. II. and V. appear to allow the general statement that the activity of the human pancreatic ferments may be considerably diminished in wasting disease. Case V. is remarkably illustrative, in the extract used three of the ferments were present but to a comparatively slight extent.

Section IV.

Some experiments were made with pigs' pancreases to discover whether any inversive ferment was present which has the power of converting cane sugar into dextrose and lævulose.

Inversive ferment. Neither active brine extracts of pancreas nor fresh pig's pancreas has the power of inverting cane sugar in an alkaline medium.

Cane sugar kept in contact with 2% solution of HCl reduces copper readily at the end of 15 minutes.

At the end of 24 hours it still, however, blackens on the addition of pure H_2SO_4.

Some experiments were also made with raw starch to ascertain whether pigs' pancreatic extracts have any action upon it to convert it into sugar.

Experiment I. Raw starch grain 1 + Water 50 c.c. + Sod. Bicarb. grain $\frac{1}{2}$.

This was placed in an incubator for 24 hours at a temperature of 32° C. Violet colour with iodine solution. No evidence of sugar on boiling with KOH and $CuSO_4$.

Experiment II. Raw starch grain 1 + Water 50 c.c. + Sod. Bicarb. grain $\frac{1}{2}$ + fresh pig's pancreas, pounded.

Placed in incubator at a temperature of 32° C. for 24 hours.

No evidence of sugar on boiling with KOH and $CuSO_4$.

After boiling, cooling and adding iodine solution, there was a reddish colour which rapidly faded. All the starch appeared to have been converted into erythro- and achroo-dextrin.

EXPERIMENT III. Raw starch grain $\frac{1}{2}$ + solution of HCl 1 % 50 c.c.

Placed in incubator at a temperature of 90° F. for 24 hours.

No sugar reaction. After boiling, cooling and adding iodine to some of the solution, a pure blue colour appeared.

The acidity of the solution was neutralized and rendered alkaline to the extent of 1 % Sod. Carb.

Some fresh pig's pancreas previously pounded in a mortar was added and the mixture was replaced in an incubator for 24 hours.

On again testing the mixture there was no evidence of the presence of sugar. After boiling, and cooling, iodine solution was added. A reddish colour appeared which rapidly disappeared.

Fresh pig's pancreas appears therefore to have the power of converting raw starch into dextrin.

Effect of Antiseptics on Starch Digestion.

Two flasks were taken.

A. 50 c.c. (1 % Starch solution).
1 % Sod. Carb.
5 c.c. of Brine extr. (Ox).
10 c.c. water.

B. 50 c.c. (1 % Starch solution).
1 % Sod. Carb.
5 c.c. of Brine extr. (Ox).
10 c.c. of 5 % Solution of Acid Carbol.

Proportion of Acid Carbol. = 1—130.

Placed in incubator.

At end of 24 hours faint reddish colour in both flasks, on adding iodine.

Sugar estimated by Pavy's method shewed an equal quantity in both flasks.

A. 50 c.c. Starch solution (1 %) + 1 % Sod. Carb. + 5 c.c. Brine Pig's.

B. 50 c.c. Starch solution (1 %) + 1 % Sod. Carb. 5 c.c. Pig's + Hyd. Perchlor. gr. 1/20.

Incubator 24 hours.

Sugar estimated and found equal in both flasks, therefore conversion of starch into sugar not hindered by presence of acid carbol. 1 in 130 of Hyd. Perchlor. 1—1000.

These and similar experiments, with reference to the other ferments proved conclusively that antiseptics do not interfere with the action of the unorganized ferments of the pancreas and confirm the results of former experiments by one of us, reported in a former volume of this *Journal*[1].

[1] Harris and Tooth. Vol. IX. p. 220.