[CONTRIBUTION FROM THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

ON THE DIGESTIVE ACTIVITY AND COMPOSITION OF DIFFERENT FRACTIONS OF THE PANCREAS. I.

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In a recent paper by one of us and Fenger,[1] it was shown that by the aid of the centrifuge it is possible to effect a mechanical separation of minced pancreas tissue in such a manner as to secure three layers of products in the centrifuge tubes possessing different properties. For this purpose we have employed a large laboratory centrifuge made by the International Instrument Company having eight tubes holding about 75 cc. each. These, or usually four of them, have been filled with the finely minced pancreas and rotated at a speed running up to 3500 revolutions per minute, through 45 minutes. The ordinary speed was about 3000 revolutions, because at the highest speed there is too great an elevation of temperature, which should be avoided.

By this centrifugal action the minced mass gradually separates into three layers. In the bottom of the tube we have an excess of protein substance more compact than the original minced gland. Above it there is a liquid layer which is distinct and nearly clear. Filtration is easy and the filtrate is perfectly bright. The top layer is composed of fat and a smaller amount of protein usually, with some water of course, as the separation of the latter is never complete. How far this might be effected by a higher velocity of rotation we do not know, but doubtless to a marked degree. The relative weights of these layers varies to a considerable extent with the speed and time of revolution, but in the majority of the mixed glands examined by us the lower layer made up 50%, or more, of the whole. In some cases the liquid and top layers were about 25% each.

To separate these three layers it is best to chill the tubes and contents. The fatty layer may then be removed with a spatula and the liquid layer poured off through a filter which makes it perfectly clear.

Separate examinations were made of the three layers, the results of which are given in the tables below. Most of the figures explain themselves, but for the starch digestion this must be said: The digesting power is measured by the number of parts of starch, employed in the form of a 1% paste, which may be digested to the colorless end point in 10 minutes, by one part by weight of the ferment mass. Thus, 1 cc. of the liquid in Col. B of the first table will digest 154 g. of starch, calculated as anhydrous, to the point where it will give no reaction with iodine after the mixture has been incubated 10 minutes at a temperature of 40°.

In the fibrin digestion the values given refer to the number of milligrams of amino nitrogen, as measured by the formaldehyde titration,

[1] THIS JOURNAL, 37, 2213 (1915).

liberated by one gram or cubic centimeter of the substance, when allowed to act on an excess of fibrin and incubated through 18 hours at 37°. The numbers have been corrected by subtracting the results of blank titrations where killed ferments were employed.

No attempts were made to compare the three fractions as to their lipase activity, but qualitative tests showed the liquid fractions markedly active in esterase.

In such centrifuge fractionations as we are concerned with here, some variation in the relative quantities of the layers is found. In most cases we obtained a better separation from the beef than from the hog or sheep pancreas. With pancreases from old sheep the separation seems to be difficult. In the tables below the means from four tests on hog pancreas, three tests on beef pancreas and from a number of partial tests on sheep pancreas are given. In this last case the numbers are therefore somewhat misleading. The letters A, B and C at the top of the columns refer to the upper, middle and lower layers, respectively.

TABLE I.

	Hog pancreas fractions.			Beef pancreas fractions.		
	A.	B.	C.	A.	B.	C.
Approx. wt., % of whole	36.3	17.2	46.0	16.0	23.4	60.6
Solids, % of fraction	49.3	15.0	31.2	60.0	11.4	29.6
Fat, % of fraction	34.0	....	6.8	50.7	....	8.3
Phosphorus, % of fat	0.27	....	0.27	0.26	....	0.27
Phosphorus, % of fraction	0.37	0.46	0.47	0.30	0.47	0.50
Nitrogen, % of fraction	1.49	2.22	3.42	1.16	1.87	2.95
P_H	....	5.541	....	....	5.537	....
F. p. depression	....	1.62°	....	....	1.12	....
Starch digestion	0.44	1.54	1.78	0.12	0.82	0.49
Fibrin digestion, amino N	0.029	0.017	0.036	0.016	0.015	0.029

	Sheep pancreas fractions.		
	A.	B.	C.
Approx. wt., % of whole	3.2	12.5	84.3
Solids, % of fraction	(90.0)	14.2	26.4
Fat, % of fraction	(85.0)	....	4.5
Phosphorus, % of fat	....	....	0.29
Phosphorus, % of fraction	....	0.40	0.51
Nitrogen, % of fraction	0.19	1.88	3.02
P_H	....	5.7	....
F. p. depression	....	1.13	....
Starch digestion	1 : 0.08	1 : 0.17	1 : 0.25
Fibrin digestion, amino N	....	0.013	0.019

The table shows some points of interest. With equal periods of rotation in the centrifuge it is evident that a much larger weight is secured in the upper fraction in the case of the hog than is true of the other animals. But the sheep pancreases used for these first tests were from old animals, and different results may be secured in later tests with the organs of

younger animals. Fat is found in both the upper and lower layers, but much more in the former. The phosphorus content of all the fats is essentially the same and suggests a uniform composition. The amount is always low and this shows that the lipoid fraction present can not be over 7 or 8%, in any case, of the weight of the fat. The amount of phosphorus in the common distearyl lecithin is about 3.9%. It should be explained here that in all cases the fatty tissue attached to the pancreas was trimmed away as far as possible before grinding, so that the fat found is largely that in the organ itself.

It is plain that the amylopsin passes to a great extent into the liquid fraction, where it is especially abundant in the case of the hog. The four individual tests, pancreases of different dates, from which the mean was taken, gave the values, 156, 180, 156 and 125, and even these do not represent the maximum digesting power, since, as is well known, solutions of the ferment in question rapidly lose digesting activity on standing. The value 125 was obtained from a liquid which had stood about six hours before tests could be made with it.

This starch converting ferment is low in the fatty layer in all cases and it is interesting to note that it is abundant only in the pancreas of the hog. This fact is practically utilized by the manufacturers of the pancreatins of the market, but the discrepancy is not always as great as is here indicated.

In tryptic power the fractions all appear low, but the greatest activity is found in the bottom layer in all cases, and the lowest in the liquid. This is doubtless connected with the fact that trypsin tends to attach itself to proteins, and especially to suspended proteins. It is not clear just what degree of activity is indicated by the figures presented. The liberation of amino nitrogen has evidently not gone very far in any case. But the liquid fractions were always tested as to their action on flakes of fibrin and a digesting power noted in every instance. All of these tryptic digestions were carried out in a phosphate medium of which $P_H = 7.73$, which is but slightly alkaline and constant in reaction. The important point in this connection is, however, that through crushing of the pancreas cells the trypsinogen becomes activated by the coferment present in the tissue and exhibits its power at once. In a following paper more attention will be given to the extent of this tryptic action and also to the behavior of the lipase and esterase. The data here presented must be looked upon as preliminary to the discussion of the ferments in the fractions.

Attention must be called to the importance of the acid reaction found in this pancreas fluid of the third fraction in all the animals. The data on this point are more fully presented elsewhere[1] but it must be noted that, while in all laboratory digestions with amylopsin and trypsin the

[1] Long and Fenger, *Loc. cit.*

preferable reaction for activity is found to be slightly alkaline, the fluid itself in which the ferments are elaborated is distinctly acid. It seems to be true, in addition, that the stability of both ferments is greatest in the presence of weak acid. The degree of acidity of the fluid was found to be nearly the same for the three animals investigated, but there are doubtless fluctuations here, as in the blood, which call for fuller investigation later.

Our thanks are due to Dr. Frederic Fenger for his courtesy in aiding us in securing the pancreases, to Mr. William Johnson for the determinations of the starch-converting power and to Professor A. I. Kendall for the use of the centrifuge of the bacteriological laboratory.

Conclusions.

We have explained the method of separating a minced organ like the pancreas into three fractions by the aid of a powerful centrifuge. In the case of the pancreas these fractions have different properties, especially with reference to the distribution and amount of the ferments present. The general composition of the three fractions for the organ of the hog, beef and sheep is given. It is likely that the method can be applied with advantage to the study of the fluids contained in other tissues, and especially to the enzymes present.

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THE NEPHELOMETRIC ESTIMATION OF PURINE BASES, INCLUDING URIC ACID, IN URINE AND BLOOD.[1]

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I. Introduction.

The estimation of purine bases with the exception of uric acid, is at present a long and often inaccurate process. Recently Folin[2] and collaborators have developed a colorimetric reagent for uric acid, which does not react with the other purines, but gives reactions with phenols and allied substances. To circumvent the difficulty they precipitated the uric acid with Salkowski's reagent and, after washing by decantation, make the colorimetric estimation. This method is sensitive to small amounts of uric acid but the present methods for determining the other purine bases, xanthine, hypoxanthine, adenine and guanine require such large

[1] Read before the Am. Soc. Biol. Chem., Dec., 1914, St. Louis, Mo.

[2] Folin and Macallum, *J. Biol. Chem.*, **13**, 363 (1912); Folin and Denis, *Ibid.*, **14**, 95 (1913)