duction product of neutral red. Ammonia does not enter into the formation of the reduction product.

3. The Stokes neutral red medium is a convenient and reliable one for the detection of fecal contamination in water and is more sensitive than lactose bile.

NOTE.—It is unfortunate that, owing to the loss of a large amount of data in the burning of the Cornell University chemical laboratory, we cannot give a summary of the results obtained on a very large number of samples covering a period of five years. It is on a careful study of these that we have based our third conclusion. Nor are we able to give the data obtained with "synthetic media" bearing on our conclusions under II. This paper is presented in its unfinished condition since it would require several years more of work to again obtain data.

ITHACA, N. Y.

[CONTRIBUTION FROM THE LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

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

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Several papers have appeared from this laboratory on the subject of the behavior of fractions of the pancreas of certain domestic animals, secured by aid of strong centrifugal action on the finely minced gland. In the second of these,[1] by one of us and others, it was shown that the ferment activity is not uniform in different layers of the centrifugal mass and further that this activity differs greatly from one animal to another. Not much was said about the fat-splitting function of the different fractions, that property being reserved for fuller study. It was shown that the amylopsin value is very marked only in case of the hog pancreas, while for the beef and sheep organs it appears to be low.

When the minced pancreas is packed into centrifuge tubes and rapidly rotated, about 3000 revs. per min., the mass separates into three layers. When the tube comes to rest the top layer is found to contain a large amount of fat, the middle layer is a liquid which may be easily filtered, while the lower layer contains mostly protein. To effect a good separation the temperature of the centrifuge must get high enough to partially melt the pancreas fat, which, however, is accomplished without difficulty, as this is a very soft fat. The amylopsin is readily soluble and collects mainly in the liquid layer, but that is not the case with the other ferments. It was found, for example, that in testing the liquid layers from four hog pancreases, and the top and bottom layers under the same conditions, the following values were obtained. One gram of substance from each

[1] THIS JOURNAL, 37, 2427 (1915).

layer was found to be able to digest grams of starch, in 1% paste form, to the colorless end point, in 10 mins., as follows:

Top layer, 0.44 g.; Middle layer, 154.00; Lower layer, 0.078.

In other words, 1 g. of the liquid layer is capable of digesting 154 times its weight of actual starch, made up into paste form, in ten minutes, to the point where it gives no color reaction at all with iodine, which is a marked degree of activity.[1] With the beef and sheep organs much lower results were obtained, as follows:

	Beef.	Sheep.
Top layer	0.12	0.08
Middle layer	0.82	0.17
Lower layer	0.49	0.25

These figures suggested that in the pancreases of the ruminating animals the starch-digesting function is not important. It is likely that in such animals the work is done almost wholly by the saliva.

In the present investigation a much larger number of animals from the three groups have been examined and the lipase activity has been especially studied. In addition, the ferment activities in other directions have been more fully controlled than was possible in the first investigation. In the earlier papers referred to it has been shown that the relative weights of the three layers into which the minced mass may be separated depend on several factors, of which speed and duration of rotation and temperature are the most important. The temperature goes up with increased speed of rotation, but may be kept down to a point where there is no rapid change in ferment action by simply controlling this speed. In the machine employed by us in all the later work the velocity of rotation was maintained in the neighborhood of 3000 revs. per min. In a normal separation with this velocity maintained through about ninety minutes we obtained proportions like these:

Upper layer, solid	30% to 36%
Middle layer, liquid	18 to 27
Lower layer, solid	43 to 46

but in cases of too short rotation in the centrifuge the liquid portion has been found as low as 8%.

In a series of four determinations on hog pancreases the fat distribution was found as follows:

Upper layer	37.9%	25.6%	38.6%	33.8%
Middle layer	trace	trace	trace	trace
Lower layer	7.4	6.4	5.4	6.9

Above one-fourth of the upper layer is, accordingly, actual fat or other ether-soluble substance. In the same pancreases the protein content was found to be:

[1] Through a typographical error some of these figures were wrongly stated in the previous paper.

Upper layer	7.0%	16.6%	4.8%	8.7
Middle layer	12.9	16.5	14.7	13.4
Lower layer	18.5	22.9	21.9	22.0

The composition of the liquid part varies somewhat with the length of the centrifuge treatment. By long rotation more protein may be brought into this fraction at the expense of the upper and lower layers. The total solids of the liquid layer are thereby increased. In the paper presented by one of us and Fenger[1] an analysis of a composite is given in which the protein content of the liquid corresponds approximately to the mean of the values in the above table. We give here a new table based on the composite secured by centrifuging mixed hog pancreases through a somewhat longer period than was the case in the earlier work. The results are as follows:

Water	78.7%		
Solids	21.3		
Protein (N = 2.94)	18.35%	Calcium, Ca	0.018%
Ash	1.44	Magnesium, Mg	0.026
Phosphoric acid, PO_4	1.09	Potassium, K	0.353
Chlorine, Cl	0.004	Sodium, Na	0.097
Sulfuric acid, SO_4	0.000		

The chief difference between this and the former analysis is found in the higher proportion of extracted protein. The somewhat increased phosphoric acid is partly of protein origin. It appears that the first part of the liquid separated by the centrifuge is low in solids, both organic and inorganic, while a moderate action brings results about like the following, in the mean. The reaction of the liquid is always acid and carbonates do not appear to be present.

	1.	2.	3.	4.	5.
Total solids	11.51	12.62	16.60	15.93	18.24
Ash	1.24	1.48	1.28	1.46	1.46
Phosphoric acid, PO_4	0.96	1.00	0.86	1.07	1.09
PO_4, % in ash	67.9	67.2	67.4	73.5	74.5
Nitrogen	1.58	1.74	2.09	2.10	2.54
Nitrogen, % in solids	13.7	13.8	12.6	13.2	13.9

It is evident that the centrifugal juice is not of constant composition, even excluding the nitrogen fraction, but the variations in the amount of protein in the solids and in the phosphate in the ash are not very great. Sulfate is not present preformed, but is present in the ash in small amount from oxidation of protein sulfur. The chlorine content is always extremely small, which is worthy of notice. The salts present are essentially phosphates, and the above figures, taken in connection with those previously published, give a pretty good picture of the character of the press juice of the pancreas of the hog.

We have made some similar determinations on the liquids obtained

[1] Long and Fenger, THIS JOURNAL, 38, 1115 (1916).

from pancreases of sheep and cattle. The results were not essentially different from those found for the hog and need not be given in detail.

Digestive Activity.

Coming now to the question of enzymic power, we shall consider first the starch-converting capacity.

Amylolytic Activity. Pancreases of Different Animals.—Some digestive power is found in each of the three layers, as shown by the results of the previous experiments quoted above. This activity is extremely variable, not only in different species, but in animals of the same species. Our determinations were made in the usual manner followed in this laboratory, employing as substrate a 1% uniform paste of potato starch. To a series of flasks, each containing 50 cc. of the paste, different weights of the enzyme mixture were added and incubated. The observation was directed toward determining the weight of enzyme necessary to bring the paste to the colorless end point with iodine in 10 mins. The paste and fluid holding the enzyme were brought to the normal temperature of 40° before mixing. From the observed results the data of the following table were calculated. The numerical values given show the number of grams of real starch which may be digested to the colorless end point in the normal time, 10 mins., by 1 g. of the enzyme-holding tissue or liquid, the starch being brought to the condition of the normal 1% paste for the digestion. The results are not for single pancreases but for composites from a considerable number of glands secured on the same day.

	Hog pancreas composites.				
	1.	2.	3.	4.	5.
Upper layer	....	0.8	0.6	1.0	1.0
Middle layer	21.0	39.0	56.0	71.0	125.0
Lower layer	....	1.0	1.3	1.2	...

	Sheep composites.		Beef composites.	
	1.	2.	1.	2.
Upper layer	0.25	...	0.3	0.2
Middle layer	0.25	0.2	0.7	2.5
Lower layer	....	...	0.5	...

The marked activity of the liquid layer from the hog pancreas is very evident, while the upper and lower layers are weak. The small values found are due to the liquid retained by them, undoubtedly, but why these values are not greater is extremely puzzling in view of the fact that these layers still retain a considerable amount of water in loosely combined form. It is true that the inorganic phosphates pass very largely into the separated water and possibly may serve to activate the dissolved ferment. As far as the upper layer is concerned, it must be noted that the large fat content would not be without effect in masking the ferment activity.

The low activity of the amylopsin in the different fractions of the beef and sheep pancreases is sharply shown in these composites. It is some-

what greater in the liquid layers than in the others where it was too weak for accurate measurement in several instances. As these glands are rich in phosphate salts no further activator was employed in any case. In all experiments with fractions of the minced gland it was found that the mean of the fraction values was far greater than the digestive value secured from the whole mass. This value for the whole gland was never much above that found for the top or bottom layer, which suggests the possibility that the protein or fat of the gland may exert some inhibiting power on the digestive function that comes strongly into evidence only in the juice or solution freed from the disturbing influences. This applies, of course, to the hog glands as well as to the others.

The low starch-digesting power of the sheep or beef pancreas is probably perfectly normal. These animals are the best representatives of the group of ruminants in which the stomach has a complex structure, the object of which is to facilitate a prolonged salivary digestion. It is extremely likely that in these animals a pancreatic amylase is not really necessary as the whole work of starch digestion may be performed by the salivary secretion rendered available through the cud-chewing process.

It has been shown that the hog pancreases themselves vary greatly in their activity. The factors on which this variation depends we have not been able to discover; it may have some relation to the stage of digestive activity when the animal is killed. The juices studied were composites from hogs killed, however, at the same time. Seasonal variations have been noticed by manufacturers of digestive ferments, but in our work these cannot be factors of any moment as far as the amylolytic function is concerned.

Proteolytic Digestion.

In proteolytic power there are not the differences between the glands of different animals that were noted for the starch digestion. Some of the mixtures employed in the above tests were used in the determination of proteolytic power. The several fractions required no preliminary activation, because on standing a short time after being ground the full activity seemed to be reached. The situation here is quite different from that noted in the fistula juice which calls for the aid of an activator to bring the zymogen to its normal value. Other substances in the minced organ seem to be sufficient to furnish all the activation required.

Tryptic Activity. Pancreases of Different Animals.—In the measurement of tryptic activity portions of 2 g. from the upper and lower layers obtained by centrifugal action and 5 g. of the middle liquid layer were incubated through three, five or sixteen hours with an excess of fibrin, enough to make either 1 or 2 g. of the anhydrous substance. The digestions were carried out in a phosphate mixture, 50 cc., so as to maintain a pretty constant P_H value of 7.7.

A control set of experiments was made by employing the same weights of fibrin and gland products in which the ferments of the latter had been destroyed by heat before mixing. After digesting all of these at 40° for the desired time the phosphates were removed by barium hydroxide and the volumes made up to 100 cc. Aliquot parts were taken for the formaldehyde titration. The figures in the following tables give the net results for the amino acid nitrogen in the weight taken, the nitrogen of the blank control being already subtracted. The figures here actually given are mg. of nitrogen from the volumes of 0.2 *N* sodium hydroxide required in the titrations. For comparison they are all reduced to the basis of 1 g. samples. In all digestions the liquids were fully protected by toluene from bacterial action.

		Hog pancreas products.				
		2.	3.	4.	5.	6.
Upper fraction	3 hrs.	....	18.7	7.0	18.5	5.0
	5 hrs.	....	23.1	12.6	44.0	10.5
	16 hrs.	....	....	....	51.0	....
Middle fraction	3 hrs.	....	8.4	....	20.2	10.0
	5 hrs.	....	16.8	....	....	....
	16 hrs.	18.4	28.0	23.6	36.0	....
Lower fraction	3 hrs.	....	22.9	11.2	23.0	10.0
	5 hrs.	....	32.2	19.6	32.0	18.0
	16 hrs.	....	....	....	....	....
Whole pancreas	3 hrs.	....	20.5	12.6	19.0	12.0
	5 hrs.	....	21.9	19.6	21.5	19.0
	16 hrs.	....	....	....	57.0	....

		Sheep products.		Beef products.		
		6.	8.	9.	10.	11.
Upper fraction	3 hrs.	7.0	18.0	7.5	12.0	5.5
	5 hrs.	9.0	19.0	20.0	19.0	8.5
	16 hrs.	25.0	50.0	35.0	63.0	....
Middle fraction	3 hrs.	3.4	12.4	6.4	9.0	5.6
	5 hrs.	5.0	16.2	9.8	11.2	7.8
	16 hrs.	9.6	29.0	21.6	....	....
Lower fraction	3 hrs.	5.5	21.0	18.0	19.5	11.5
	5 hrs.	7.0	22.5	26.5	25.0	15.0
	16 hrs.	39.0	54.5	58.0	70.0	....
Whole pancreas	3 hrs.	17.5	31.0	24.0	19.0	8.0
	5 hrs.	28.5	31.5	38.0	27.5	12.5
	16 hrs.	48.5	74.5	67.5	77.5	....

One fact appears immediately from this table and that is that the activity is not confined to the liquid layer. The values for the digestions secured from the gram samples of the upper and lower layers are, with a few trifling exceptions, markedly higher than the values from the liquid.

The behavior of the trypsin is clearly distinguished from that of the amylopsin in this way. The tryptic action of the whole organ is even more marked. In most cases we find the greatest activity here, and this shows particularly in the beef and sheep pancreases.

It must be noted that the hog pancreases, which are very strong in starch-converting power, are in general no stronger than the organs of the other animals in proteolytic behavior. In sheep pancreas composite No. 8 and in beef products Nos. 9 and 10 there was often a complete solution of all the fibrin taken, showing a degree of activity in excess of that found in most of the commercial concentrates which we have examined. It is a singular fact that the tryptic activity here persists better in the whole material than in the fractional separations, while with the amylopsin the reverse in the case.

The preformed amino acid nitrogen, that which shows in the blank titrations, is higher in the liquid than in the other fractions. The blanks in these cases were always high, and this was noted in all samples.

Lipase and Esterase Splitting.

Probably the greatest interest in this investigation attaches to the lipase and esterase distribution. It is practically a very difficult matter to secure a permanent dry pancreas preparation which has much action on fats, as the lipase is not stable in the degree that obtains for the other ferments. In the fresh gland, however, the activity of the lipase, and esterase also, may be very marked, sometimes showing in high degree.

The pancreas contains the two distinct enzymes of this type, the one working on fats proper and the other on simpler esters. In the centrifugal separation very little of either enzyme passes into the liquid fraction, as will be brought out by the following figures.

Lipolytic Activity. Pancreases of Different Animals.—In making determinations of this splitting power we have tried most of the methods which appear to have value, but finally followed this as a routine process: 15 cc. of a neutral olive oil emulsion were rubbed up in a mortar with 1 g. of the tissue or liquid fraction to be examined and after the addition of toluene the mixture was incubated at 40° through the desired time. The amount of fatty acid split off was measured by titration with 0.1 N or 0.5 N sodium hydroxide, after dilution to 50 cc. with neutral alcohol. A blank test was made in every case by incubating the emulsion and killed pancreas in the same manner and this result was used as a correction on the first titration. The pancreas material has itself a considerable acid value which could not be overlooked. This is much stronger in the alcohol solution than in water. The net results are given in the following tables in terms of alkali used, and for the same composites that were used in the tryptic measurements.

		Hog pancreas products.				
		2.	3.	4.	5.	6.
		Cc. of 0.5 *N* NaOH used in titration.				
Upper fraction	30 min.	...	...	...	3.9	3.8
	60 min.	...	...	7.0	5.6	4.8
	180 min.	6.4	6.9	6.7	8.6	7.0
		Cc. of 0.1 *N* NaOH used in titration.				
Middle fraction	30 min.	...	...	...	2.8	1.5
	60 min.	...	...	1.5	2.9	1.5
	180 min.	2.0	8.0	2.2	2.6	1.5
		Cc. of 0.5 *N* NaOH used in titration.				
Lower fraction	30 min.	...	...	...	3.0	4.4
	60 min.	...	...	6.0	3.6	4.7
	180 min.	7.0	6.9	7.0	...	5.9
		Cc. of 0.5 *N* NaOH used in titration.				
Whole pancreas	30 min.	...	...	...	3.6	3.7
	60 min.	...	...	8.0	5.6	4.4
	180 min.	6.5	8.0	7.2	8.8	8.7

		Sheep products.		Beef products.		
		7.	8.	9.	10.	11.
		Cc. of 0.5 *N* NaOH used in titration.				
Upper fraction	30 min.	0.8	1.1	0.6	...	0.5
	60 min.	2.0	2.6	1.3	0.6	1.0
	180 min.	2.6	4.6	2.0	1.8	2.6
		Cc. of 0.1 *N* NaOH used in titration.				
Middle fraction	30 min.	1.8	1.0	0.0	0.0	0.0
	60 min.	2.0	1.0	0.1	0.2	0.0
	180 min.	2.8	1.0	0.8	0.5	0.0
		Cc. of 0.5 *N* NaOH used in titration.				
Lower fraction	30 min.	0.6	1.6	0.2	0.4	1.1
	60 min.	1.0	2.7	0.7	0.8	1.0
	180 min.	1.9	4.1	1.4	1.7	1.6
		Cc. of 0.5 *N* NaOH used in titration.				
Whole pancreas	30 min.	0.8	0.5	0.4	0.4	1.0
	60 min.	1.0	0.9	0.6	0.7	1.6
	180 min.	1.7	1.9	1.2	1.6	2.3

It is apparent from these results that the fat-splitting power of the hog pancreas is greatly in excess of that obtaining with the sheep and beef organs. This is perfectly consistent with the physiological requirements of the three classes of animals, as the feed of the hog will always naturally contain much more fat than is true of the other animals. In some cases the extent of the hydrolysis reaches quite appreciable figures in the three-hour incubation periods. If we consider the olein molecule as completely hydrolyzed the alkali required in titration would correspond to over 1 g. of actual fat, while if we consider the reaction as carried to the production of diolein, only, the hydrolysis would correspond to

over 3 g. in the time. This is all accomplished without any partial neutralization of the acid liberated, which, however, is rather inert under the conditions obtaining.

It is further evident that extremely little of the lipase passes into the liquid layer. The titrations in this case are with 0.1 *N* alkali, it must be remembered, and only in one case, hog composite No. 3, is the volume used comparable with the others. Some of the solid tissue must have passed into the liquid in this case. Numerous other observations on this fraction in our various separations confirm our view that the lipase is practically absent in the liquid layer. It would appear that the lipase must cling to the solid tissue, and practically as well to the top layer holding much fat, as to the lower layer rich in proteins. The enzyme does not readily leave these substances to go into solution. However, these tissues do not hold the lipase in such a manner that it cannot exert its normal action when they are incubated with fat substrate.

We have spoken of the liquid layer as weak in its lipolytic action. Yet, as compared with the values reported in the literature for somewhat related preparations, it shows a fair degree of activity. This applies, for example, to the enzymes of the central nervous system discussed by English and MacArthur[1] recently, and to soy-bean and castor-bean extracts described by Falk and others.[2] In our work the liquids examined were absolutely fresh, not concentrated in any way and not activated by any addition. It is not clear from the work of the first-named investigators just how much original material is represented in the extracts finally employed.

Our findings are consistent with the statement of Connstein,[3] who sums up the results of many studies on the so-called gastric lipase and on aqueous or glycerol extracts of the pancreas with the remark that the activity of extracts seems to be without quantitative importance. The lipase appears to have but a low degree of solubility, and therefore not important in solutions obtained by digesting tissues in water or other solvent. Kanitz, on the other hand,[4] appears to consider it easy to secure glycerol extracts of the pancreas, by long maceration, which show appreciable reactivity. Probably the long time given to the extraction here, weeks to months, is the important factor in accounting for his findings and later ones, inasmuch as in this time through a partial autolytic digestion of the mass of gland and glycerol more of the ferment could become liberated from its union with the protein. The Kanitz extracts were finally filtered through muslin and could not properly be termed

[1] THIS JOURNAL, 37, 653 (1915).

[2] *Ibid.*, 37, 217, 649 (1915).

[3] "Ueber fermentative Fettspaltung, Ergebnisse der Physiologie," 3, 194.

[4] *Z. physiol. Chem.*, 46, 482 (1905).

solutions. It is likely that in these glycerol extracts the ferment was held in an emulsion-like form with cell material. Our centrifugal liquids held coarse suspended particles at the outset which were readily filtered out by paper.

On the other hand, the activity of the lipase in the upper and lower layers must be considered as relatively strong. We have taken these small portions of pulp exactly as separated from the mass in the centrifuge tube without activation or modification in any manner, as it was our desire to secure results without the introduction of any complicating factors. The addition of bile salts, for example, was found to work a great increase in the activity, and doubtless this plays a part in the change in the fraction which finally finds its way into the intestine through the pancreatic juice. Our data represent the distribution of the lipase in the original tissue and in normal strength.

Esterase Activity. Pancreases of Different Animals.—These several fractions of the pancreas not only hold the lipase proper, capable of splitting olive oil and other fats, but also notable quantities of the so-called esterase, capable of splitting such esters as ethyl butyrate. The same

		Hog pancreas products.					
		1.	2.	3.	4.	5.	6.
Upper fraction	30 min.	...	....	....	....	9.6	5.0
	60 min.	6.0	10.9	11.4	12.9	10.5	9.0
	180 min.	...	....	....	....	13.0	10.0
Middle fraction	30 min.	...	....	....	....	1.0	1.0
	60 min.	2.6	3.5	4.8	2.5	2.2	1.8
	180 min.	4.0	6.0	10.2	6.0	4.0	3.3
Lower fraction	30 min.	...	....	....	....	8.6	8.7
	60 min.	7.0	10.0	11.5	11.0	10.4	11.7
	180 min.	...	....	....	....	15.2	14.6
Whole pancreas	30 min.	...	....	....	....	8.4	9.7
	60 min.	6.5	10.1	11.8	12.5	11.3	11.2
	180 min.	...	....	....	....	14.8	16.2

		Sheep products.		Beef products.		
		7.	8.	9.	10.	11.
Upper fraction	30 min.	7.5	4.3	7.6	4.2	6.2
	60 min.	8.5	5.5	8.5	5.4	7.7
	180 min.	....	7.5	....	....	...
Middle fraction	30 min.	1.3	0.6	0.1	0.0	...
	60 min.	2.6	2.2	0.2	0.0	...
	180 min.	5.8	4.0	0.3	0.0	...
Lower fraction	30 min.	7.3	4.0	6.6	5.8	5.4
	60 min.	8.3	4.4	9.2	7.4	6.4
	180 min.	14.6	8.3	12.4	12.0	...
Whole pancreas	30 min.	7.8	6.1	6.7	4.8	2.2
	60 min.	9.3	7.4	8.8	6.1	5.2
	180 min.	12.0	8.9	12.7	10.0	...

fractions of pancreas composites as were employed in the lipase tests were used here and the activities were observed by measuring the acid liberated by the action of 1 g. portions on a substrate of 50 cc. of saturated aqueous ethyl butyrate solution. The results given above are the net titration values obtained by subtracting the results of the blanks made as in the case of the lipase. In this case the titrations are made with 0.1 *N* alkali throughout, and not for the liquid layer only, as with the lipase.

Keeping in mind that the lipase values for all but the liquid layer are expressed in terms of 0.5 *N* alkali while the esterase values are in terms of 0.1 *N* alkali it is plain that the activity of the former is much more marked than is that of the latter in the upper and lower layers for the hog products. In the upper layer for the three periods the lipase activity, measured by the acid liberated, is about three times as great, while in the lower layer it is about twice as great as the other. In the liquids, however, the esterase is distinctly the higher. In the cases of the sheep and beef pancreases the esterase activity is generally higher than the lipase. These relations are important in showing that we are dealing with two different enzymes. A number of observations made on the pancreases of cats gave lipase values fully twice as great as were found for the esterase.

The data shown above for the esterase values in hog pancreases were secured from composites. To indicate somewhat more distinctly the variations in single organs 8 pancreases were brought to the laboratory, minced and examined under identical conditions. Gram portions of the whole organ were incubated 3 hrs. with 50 cc. of the ethyl butyrate solution. The titrations with 0.1 *N* alkali gave these values in cubic centimeters:

1.	2.	3.	4.	5.	6.	7.	8.
16.7	11.0	10.5	15.5	13.0	12.8	9.4	14.1

The apparent activity increases rather rapidly in the minced mass if it is allowed to stand just above the freezing point. One of the composites tested above when examined in gram portions with the same butyrate substrate on different days furnished these titrations:

Oct. 21, 10.5 cc.; 23, 14.6; 25, 16.7.

The incubations were carried through 3 hrs. here without addition of toluene, which was used in all other cases. As the material was not actually frozen there remains the possibility that the increase in splitting power of the esterase may be only apparent, and that bacterial action may be really responsible for the increased acidity. Under certain conditions bacteria have this action on fats and other esters.[1]

This increase does not appear to follow in the prolonged incubation itself, in presence of toluene, as indicated by the following experiment:

[1] See Connstein, "Ergeb. der Physiol.," 3, 194, for literature.

Five fractions of the same minced gland, of 1 g. each, were mixed with a substrate of 50 cc. of saturated ethyl butyrate solution and 25 cc. of water and placed in the incubator at the same time, all protected by toluene. Titrations were made on one of these at the end of an hour and again at the end of two, four, twenty-four and forty-eight hours. With the other fractions the titrations were begun later as shown. The tabulated results are clear and speak for themselves. They are the net values for titrations with 0.1 N alkali. The amount of the ester split in two days appears to be independent of the accumulated free acid. In other words, the neutralization at intervals does not here bring about any greater final splitting, and the increases in the acidity are regular enough to point to prolonged esterase action rather than to any bacterial splitting which would not be as uniform as are these increases. But in any event it is desirable to perform all these operations in presence of toluene and without delay.

PERIOD OF INCUBATION AND TITRATION VALUES.

Fraction.	1 hour.	2 hours.	4 hours.	24 hours.	48 hours.	Total.
1	15 cc.	8 cc.	5 cc.	2 cc.	1 cc.	31.0 cc.
2	..	16.7	7	3.8	2	29.5
3	..	...	20	6.5	2	28.5
4	..	...	..	25.0	3	28.0
5	..	...	..	...	29	29.0

Summary.

This investigation shows the extent to which the substance of the minced pancreas may be separated into fractions by strong centrifugal action. A high velocity is necessary for the purpose.

The distribution of fat and protein in the three layers or fractions as secured in the centrifuge tubes is shown for a number of samples. The distribution may be made fairly constant with uniform conditions. The middle layer is liquid holding suspended particles, but it gives readily a perfectly clear filtrate.

The inorganic salts of this filtrate have been determined and the results agree well with those previously reported from this laboratory. The variation in this salt composition with change in external conditions is shown for a number of cases.

The enzymatic activity of this liquid fraction has been studied. In the liquid from the hog pancreas amylopsin is very strong, while in the beef and sheep liquids it is weak. Lipase is weak in all three liquids, while esterase is somewhat stronger. In tryptic power the liquid from the hog pancreas is fully as active as are the solid fractions, but for the beef and sheep organs the liquid is markedly weaker.

The distribution of enzymic power in the solid fractions has been noted. The activity of the starch-digesting ferment in the upper and lower fractions is extremely small. While these are moist and hold much water,

it is remarkable that most of the amylopsin goes into the main liquid portion. Trypsin is found in the solid parts as well as in the liquid, while lipase is found mainly bound to the solids. This union is not so fixed, however, that when mixed with other substrates the ferments cannot readily separate and attack these.

The pancreas of the hog is distinguished from those of beef and sheep mainly in the power of starch digestion. It is suggested that in the last-named animals amylopsin may not be physiologically necessary because the starches can be digested by the ptyalin of the saliva through the aid of the prolonged cud-chewing process.

Lipase and esterase values do not vary in the same manner in the different fractions with the different animals. These variations are striking enough to indicate that the properties are associated with distinct enzymes.

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THE ISOLATION OF PARA-HYDROXYBENZOIC ACID FROM SOIL.

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In an investigation of a sandy soil from Florida an aromatic acid has been isolated and its identity with *p*-hydroxybenzoic acid established.

The compound was isolated in the following way: Twenty-three kilograms of soil were extracted with about 75 liters of an aqueous 2% solution of sodium hydroxide at room temperature for twenty-four hours. The extract was made slightly acid with sulfuric acid and filtered. The acid filtrate was then extracted with ether and the ether extract was concentrated to a volume of about 200 cc. and shaken up with a concentrated solution of sodium bisulfite to remove aldehydes or other substances which combine with this reagent. The bisulfite solution was drawn off and extracted several times with fresh ether. The combined ether extracts were then slowly evaporated on the surface of a small volume of warm water. The water solution was heated to boiling and filtered while hot to remove an insoluble oily residue. From the cold, concentrated aqueous solution a crystalline compound separated. This compound was purified by repeated crystallization from water. It persistently retained a slight tinge of color and only after many crystallizations and boiling with a small quantity of purified bone-black accompanied by much loss of material, could it be freed from color. The aqueous solution was then subjected to steam distillation to remove benzoic acid or any other volatile