quently, one cannot draw any very valid conclusions from his results as he only has one point on the curve.

Rosenthaler has suggested several other methods for separating the synthetic from the hydrolytic enzyme; for example, by treating emulsin with acid and then neutralizing with alkali, by partial precipitation with ammonium sulfate and copper sulfate. We have repeated some of these experiments but we cannot interpret the results correctly until we have made a further study of the effect of inorganic salts on the rate of reaction. There is, however, no evidence of a change in the equilibrium point though there is a marked change in the rate of the reaction.

Conclusions.

1. It is necessary to prepare a stock of pure benzaldehyde and to keep it over nitrogen in order to obtain comparable results in the synthesis of mandelonitrile.

2. There is no evidence of a variation in the equilibrium point in the synthesis of mandelonitrile, even though the oxynitrilase is obtained from widely diversified sources.

3. As far as investigated, there is no evidence of the possible separation of a hydrolytic from a synthetic enzyme in a preparation of oxynitrilase when treated according to methods suggested by Rosenthaler.

We are continuing the study of the general properties of oxynitrilase and expect to repeat all the other methods suggested by Rosenthaler for the separation of the hydrolytic from the synthetic enzyme.

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ON THE REACTION OF THE PANCREAS.

BY J. H. LONG AND F. FENGER.

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It is well recognized that the so-called pancreatic juice has a distinct alkaline reaction which has been expressed in different terms. The degree of alkalinity varies with the condition of the alimentary tract with reference to presence of food and with other factors as well. With lowered food ingestion the alkalinity decreases, as has been shown by the investigations of Glaessner,[1] Schumm[2] and others. The latter found the reaction of the human pancreatic juice from a temporary fistula to be equivalent, in the mean, to about 0.6% Na_2CO_3. The alkalinity found by Glaessner was, apparently, much less, and similar values have been reported by Ellinger and Cohn.[3] Observations made by Wohlgemuth[4]

[1] *Z. physiol. Chem.*, **40**, 465.

[2] *Ibid.*, **36**, 292.

[3] Ellinger and Cohn, *Z. physiol. Chem.*, **45**, 28.

[4] Wohlgemuth, *Biochem. Ztschr.*, **39**, 302.

on the flow from a pancreatic fistula gave a degree of alkalinity approximately that reported by Schumm. All authorities agree that the human pancreatic juice is distinctly or even strongly alkaline. In experiments on the reaction of the juice from a fistula made on a dog Auerbach and Pick[1] found the *true* alkalinity, as measured by the potential method, relatively lower than for the human juice. They suggest that the true reaction of the liquid which has not lost carbonic acid may be approximately neutral. By the use of the potential method Foa[2] has found the juice from a dog to be slightly alkaline. In the mean the hydrogen concentration was about $C_H = 0.0085 \times 10^{-7}$.

All investigations thus far available have dealt with a *secretion*, usually secured by aid of a temporary or permanent fistula. The reaction thus found is not necessarily that of the organ itself or the press juice from it. We have thought it worth while to determine the nature of the reaction in the fluid pressed out from the pancreas, or other organ, and below some results on the pancreatic fluids of several animals will be reported. The water content of this organ is about 75%, in the mean, but most of this can be separated only by a drying operation, since the cells hold the water very tenaciously.

When the fresh pancreas of the steer, hog or sheep is removed from the body immediately after death, cut open and tested with fresh litmus paper, a well-defined acid reaction is always observed. This is the case no matter how quickly the test is made, and even before the organ is removed, and it persists after the latter has been chilled. It appears to be normal.

We attempted to press out the liquid from the minced glands by the aid of a powerful laboratory press, but the product so secured was not clear and at best was more like a fine emulsion. In some trials the glands were ground up with sharp sand so as to more thoroughly disrupt the cell walls, but the results were unsatisfactory. Finally, it was found that a very good separation could be obtained by centrifugal action. Portions of the minced glands of about 75 g. each were packed in the large tubes of the high power machine employed, four or eight of these tubes being placed in the centrifuge at one time. By rotating at a speed of about 3000 revolutions per minute through 45 minutes the contents of each tube separated into three clearly defined layers, in this order; below a solid layer making up about 50% of the whole, above this a more or less reddish liquid layer, often quite clear, and making often as much as 25% of the whole contents, and finally a top layer containing much fatty matter along with part of the protein tissue of the gland. In amount this layer

[1] *Chem. Zentr.*, 1, 444 (1913).

[2] Foa, *Arch. di fisiol.*, 3, 390 (1906); through Neuberg's Handbook, II.

was sometimes in excess of the middle watery layer, and sometimes less. After removing the tubes from the centrifuge and chilling them in ice water it was found possible to separate the liquid layer from the other layers very completely. On filtration this liquid was secured in bright, clear condition. Some investigations as to the nature of the ferment activity in the three layers along with approximate composition are in progress, but in this place the question of the reaction of the liquid layer alone concerns us.

As the filtered liquid always possessed a more or less reddish or yellowish red color it was not possible to measure the reaction by one of the indicator methods. Besides, the protein content was high in most of the juices and this, also, would have added to the uncertainty of the indicator action. As it is possible to secure plenty of the separated juice the potential method of measurement was found to offer the easiest solution of the problem, and in the work we employed Hasselbalch cells along with a calomel electrode maintaining a temperature of 20° as nearly as possible throughout the observations. The cells used were frequently controlled by aid of phosphate mixtures of known potential value. With a mixture of equal volumes of Soerensen's primary and secondary phosphates we found P_H values amounting to 6.803, in the mean. For different cells the extreme values were 6.784 and 6.822. Occasionally two hydrogen electrodes were employed for comparison but work with the $N/10$ calomel electrode gave the most satisfactory control. For work at 20° we used the reduction formula

$$P_H = \frac{\pi - 0.3379}{0.0582}.$$

Observations have been carried out on liquids from beef, hog and sheep pancreases, the glands being removed immediately from the slaughtered animals and worked up without delay. No time was given for any ferment or other change as the centrifuge separation and the potential measurements were carried out within an hour or two after death of the animal. It was found, however, that the potential remained constant through many hours in the cell, in fact over night in some trials made. This is a consideration with some bearing on the explanation of the reaction, as we believe we observe here the true normal reaction of the organ.

Beef Pancreas Juices.

Samples were secured and examined on the dates given below. It is possible that a seasonal variation may be found in the examination of pancreases of animals slaughtered in the winter, and such a test we have in mind. The filtered juices in all these cases remained perfectly clear during the tests, although they occasionally deposited a protein precipitate later, which precipitate is soluble in weak salt solution.

Date.	P_H.	C_H.
June 11	5.525	29.8×10^{-7}
17	5.469	33.9
18	5.451	35.4
23	5.578	26.4
29	5.602	25.0
30	5.597	25.3
	Mean, 5.537	29.3×10^{-7}

Hog Pancreas Juices.

These resemble the beef juices in most respects, but are lighter in color. The same difference shows in the minced glands and, but not as distinctly, in the whole glands. Collections and tests were made on the following dates:

Date.	P_H.	C_H.
June 10	5.609	24.6×10^{-7}
11	5.548	28.3
17	5.447	35.7
18	5.489	32.4
23	5.595	25.4
29	5.574	26.7
30	5.552	28.1
July 6	5.530	29.5
7	5.524	29.9
	Mean, 5.541	28.9×10^{-7}

It is plain that the hydrogen concentration values for the two types of pancreases are nearly constant and about the same for the two animals. We have reason for believing that the values for P_H on June 17th and 18th are a little low for both sets of observations, because of a slight error in the potential reading which was not recognized until too late to make a proper correction. We are certain that with this correction the results would be much like the others for these days. In digestive activity, however, and other properties the two kinds of glands show no similarity.

Sheep Pancreas Juices.

The digestive activity of the pancreas of the sheep has been but little investigated, and in our experiments we have found the action on starch rather weak. The tryptic power appears to be stronger. No commercial use is made of these pancreases. The juice pressed out or separated by a centrifuge is in general higher in color than was found for the other glands, and appears to be richer in solids. For this reason the preparation of a clear liquid is attended with greater difficulties, and the potential tests must be made with greater dispatch because of the rather rapid separation of a protein precipitate. We add a few results obtained from this type of juice.

Date.	P_H.	C_H.
July 6	3.575	26.6×10^{-5}
7	5.913	12.2×10^{-7}
14	5.602	25.0×10^{-7}
14	5.623	23.8×10^{-7}
27	5.976	10.5×10^{-7}
27	5.976	10.5×10^{-7}

In these few liquids the hydrogen concentrations are not as regular as in the cases of the hog and beef. For two of the dates the values are approximately the same as found for the other juices, but the sample of July 6th shows a high acidity. In this case very little fat was present and in some other points the behavior was abnormal. The liquid was not clear as in the other juices examined, and this may have had a bearing on the result. The two juices secured on July 14th are much alike and were obtained in perfectly clear condition. These resemblances to the hog and beef juices are very striking. The two juices of July 27th are from young lambs, and while they are practically identical in freezing point, —1.14 and —1.15, and in acidity, they differ slightly from the earlier samples. But all of the acidities, excepting the first, are of the same order, and are probably related to the blood in the same general manner.

In the centrifugal separation of these fluids some temperature is developed in the machine because of the very high speed of rotation. Care must be observed to keep this down by frequent opening of the centrifuge, to avoid an alteration in the digestive power of the fluids or a possible change in their composition. In the preparation of muscular juices for study Fletcher and Hopkins[1] have observed that the acidity varies greatly with the process followed in grinding the pulp and especially by the temperature obtaining. In this case the acid developed is largely lactic acid, and while the situation is quite distinct from that in our liquid, it is to be recognized that the temperature *might* be a factor. The fact of the rather marked constancy in the results speaks against any very appreciable heat effect. In any event the temperature should not be allowed to go above the normal body temperature.

The acid reaction of the pancreas is undoubtedly the normal one, and it has been overlooked because the duct flow from a fistula is so plainly alkaline. This reaction is what should be expected when it is recalled that the source of both liquids is the nearly neutral blood with its high content of primary and secondary phosphates. In the normal activity of the gland the work of the cells results in sending an excess of one phosphate to the intestine while an excess of the other is retained to make the gland tissue slightly acid. We find that this acid liquid is very rich in acid phosphate, while the ordinary pancreatic juice contains an excess of the secondary phosphate, which is partly responsible for the reaction

[1] *J. Physiol.*, **35**, 247.

usually ascribed to carbonates. The amount of carbonate in the press juice is small.

Similar liquids may be separated from other organs and the reaction is frequently acid. This we have found in the liver. The bile from the liver is commonly spoken of as alkaline but this is not always the case, and perhaps not normally. Much depends on the age of the examined bile as far as the reaction is concerned, and the fresh bile is nearly neutral or even acid, when examined by the potential method. We are making a more thorough study of the reactions of a number of the fluids which may be separated from various organs by the centrifugal method.

The normal blood reaction is approximately $P_H = 7.52$, which may be easily duplicated by proper mixtures of primary and secondary phosphate, or by combination of these with sodium bicarbonate, as shown especially by L. J. Henderson.[1] With a mixture of about 8.5 volumes of molec./15 secondary phosphate with 1.5 volume of primary phosphate of the same concentration we have approximately the blood reaction, while 9 volumes of the primary phosphate and 1 volume of the secondary phosphate, each of the molec./15 concentration, gives about the reaction we find in the liquids from the pancreases. A reversal of these last proportions furnishes a reaction such as we sometimes find in the fistula juices. It is evident that a change in the proportions in which the two phosphates are mixed would account for the various degrees of acidity or alkalinity we note in the two kinds of pancreas liquids.

A selective secretion from a mixture of secondary phosphate and bicarbonate would lead to the difference in reaction observed between the liquid retained and that excreted by the pancreas. The reaction of the fistula juice is that of a normal carbonate, rather than bicarbonate, which may be accounted for through the cell activity working in this manner.

$$Na_2HPO_4 + HNaCO_3 = NaH_2PO_4 + Na_2CO_3,$$

the primary phosphate resulting being more largely retained within the organ while the carbonate is thrown to the duct or fistula. The freshly cut surface of the pancreas, or the separated liquid, will usually liberate carbon dioxide from solution of bicarbonate added, just as we have a liberation of the gas when we mix properly diluted solutions of bicarbonate and primary phosphate. The centrifugal liquid yields 1.5% or more of ash, over half of which is in the form of P_2O_5, and this phosphoric acid content is apparently nearly constant in the various juices examined. This matter will be discussed elsewhere, as will also the general question of the composition of the several fractions referred to above, and their behavior. It may be remarked here that a very sharp amylolytic activity was found in the liquid fraction, as well as characteristic tryptic

[1] *Ergebnisse der Physiol.*, **8**, 254.

behavior, without the addition of any form of activator. The minced gland itself furnishes all that is necessary to activate the trypsinogen.

Conclusions.

We have shown that it is possible to separate the true press juice from the minced pancreas, and best by aid of a powerful centrifuge operating at a speed of 3000 revolutions, or over, per minute. In the centrifugal separation the minced mass divides into three layers of distinct properties. The lower layer is largely protein, the middle layer a clear liquid, and the upper layer solid with much fat present.

The liquid layer has a marked acid reaction, as distinct from that of the so-called pancreatic juice, which reaction is nearly a constant for beef and hog glands. The juice is rich in phosphates and the reaction seems to be due to acid phosphate. This reaction may be as constant and characteristic as is that of the blood and may be expressed, approximately, by the relation,

$$C_H = 29 \times 10^{-7}.$$

CHICAGO, ILL.

[CONTRIBUTIONS FROM THE LABORATORY OF SOIL CHEMISTRY OF THE IOWA STATE COLLEGE EXPERIMENT STATION.]

AMINO-ACID NITROGEN OF SOIL AND THE CHEMICAL GROUPS OF AMINO ACIDS IN THE HYDROLYZED SOIL AND THEIR HUMIC ACIDS.

BY R. S. POTTER AND R. S. SNYDER.

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This investigation was undertaken to correlate, if possible, the amounts of the various chemical groups[1] (1) in the soil with its humic acid, (2) in the soil and its humic acid with the kind of organic fertilizer previously applied to the soil, (3) in the soil and its humic acid with similar groups found in pure proteins, and (4) to compare the amounts of amino acid nitrogen, as such, in the soil with that found by hydrolysis. No study of just this nature has ever been made. Several investigators, in recent years, subjected the solution obtained by boiling the soil with strong acids to the Osborne and Harris-Hausmann method. Kelley and Thompson[2] analyzed the alkali extract and the humic acids of several soils by this same method.

Suzuki[3] analyzed three humic acids rather extensively. Humic acid A was a Merck product, origin unknown to Suzuki. Humic acids B and C were prepared, respectively, from an unmanured soil and a compost

[1] Van Slyke, *J. Biol. Chem.*, **10**, 15 (1910).

[2] THIS JOURNAL, **36**, 438 (1914).

[3] *Bull. Col. Tokyo*, **7**, 513 (1907).