Summary.

1. It has been shown, in a new way, that invertase is colloidal in nature, and the reaction between the enzyme and cane sugar solution depends on the contact of two phases.

2. The activity of invertase (the product obtained from yeast and called invertase), is not affected whether or not the enzyme is adsorbed to a solid like charcoal, or to a colloid like saponin, serum, or egg albumin, distributed uniformly throughout the solution of the substrate.

3. Displacing the adsorbed invertase by a second colloid is without effect on the activity, contrary to the views held by many.

4. Invertase can be removed from an aqueous solution by adsorption to a solid, and again brought into solution by a second colloid suspended uniformly throughout the solution.

5. Eriksson's proof that cane sugar can liberate invertase adsorbed to charcoal is not valid.

NEW YORK, N. Y.

[FROM THE LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL AND THE RESEARCH LABORATORY OF ARMOUR AND COMPANY.]

ON THE REACTION OF THE PANCREAS AND OTHER ORGANS.

[SECOND PAPER.]

BY J. H. LONG AND F. FENGER.

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In a recent paper[1] we showed that the "press juice" obtained from the pancreas of the hog, sheep and beef by centrifugal separation, is characteristically acid, the degree of acidity being nearly constant. This unexpected result suggested the importance of further experimentation, as it has usually been assumed that the reaction of the fluids of the body is not far from that of the serum. But this assumption leaves out of consideration the fact that the external secretion of the pancreas is rather markedly alkaline. A compensating acidity should then be expected somewhere, and most naturally within the organ.

In our view the reaction might be due, in part at least, to the presence of acid phosphates in the juice, or possibly to acid organic compounds of phosphoric acid, since the acid is abundantly present. In the discussion following the presentation of the paper at the Seattle meeting, Sept. 1, 1915, the suggestion was made by several colleagues that the acidity might be due to ferment action through the lipase present. This and other points had already been considered by us, but in view of the importance of the phenomenon we have thought it desirable to present further evidence bearing on the question, this evidence being in the form of data collected since the first paper was published.

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

It was pointed out in that paper that the organs taken immediately from the animal after death exhibit an acid reaction with litmus. This was followed up in various ways. In the month of September the test was made on the organs of 160 sheep, 200 cattle and 120 hogs by one of us and assistants, the organs being cut open and tested while still warm. This of course precluded the possibility of acid formation through ferment action. An acid behavior was always found. The same fact may be shown in a very convincing form in this manner. A mixture of from 20 to 50 g. of the minced organ and some sodium bicarbonate is stirred up in the bottom of a beaker. Soon the mass begins to rise and may even flow over the top of the vessel, from the liberation of carbon dioxide. Certain proteins, it must be remembered, show the same behavior; casein for example. These proteins are acid in character.

It was evident from these and other observations that the acid reaction is present in the fresh organs and is not a consequence of bacterial decomposition, which might follow if the organs were allowed to stand some time before the tests were completed.

Since the tests recorded in the earlier paper were made on the juice of animals killed during the summer, mostly in July, it was thought wise to collect organs during the fall and early winter months for comparison. The results obtained under these conditions are given below. In the first of the observations the same Hasselbalch cells and platinized electrodes were used that were employed before. Experiments were frequently made with phosphate solutions of known hydrogen ion concentration for control. Then new electrodes with fresh deposits were substituted, tested by phosphate and other control solutions and used in the later work. In some of the observations toward the end of the series the cell recently described by one of us[1] was employed. The results obtained with it were always in close agreement with those from the first named cell. The data for the different pancreases tested will now be given, and in each case with date and number:

No. 123, Sept. 27. Beef pancreas. Organs placed in refrigerator immediately after removal from animal. Temperature 0°. Minced next morning and the juice separated by the centrifuge at once. Marked reddish color.

No. 124, Sept. 27. Hog pancreas. Condition the same as for No. 123.

No. 125, Sept. 29. Beef pancreas. Organs brought to laboratory and juice separated without delay.

No. 126, Sept. 29. Sheep pancreas. Conditions as in No. 125.

No. 127, Sept. 30. Sheep pancreas. Immediate separation of juice.

No. 128, Sept. 30. Hog pancreas. Immediate separation of juice.

No. 129, Oct. 1. Sheep pancreas. The fat was thoroughly trimmed off and the organs thrown into boiling water. After boiling 15 minutes the glands were minced and centrifuged. It was found necessary to add a little water to aid in the separation. The reaction of the liquid now obtained was found to be slightly acid to litmus, more

[1] THIS JOURNAL, 38, 936 (1916).

acid than 6 sec. phosphate plus 4 prim. phosphate. The juice included some of the added water, and probably held more or less of the β-proteid of Hammarsten, to be discussed later.

No. 130, Oct. 1. Hog pancreas. Conditions as for No. 129.

No. 131, Oct. 6. Hog pancreas. The fresh juice was tested in parallel with a borate solution as control, with all conditions identical.

After making the observations on the liquids described above the platinum electrodes were cleaned and recoated. Before doing this, however, the potential values were found in a standard phosphate mixture as a control. This mixture was composed of equal volumes of molar/15th primary and secondary phosphates, as suggested by Soerensen. For the two cells we found $\pi = 0.7357$ and 0.7339, at 21°, or $P_H = 6.813$ and 6.783. After recoating we found the cells uniform with $P_H = 6.784$, which is a very satisfactory situation. More pancreas examinations were then made:

No. 141, Dec. 21. Hog pancreas. A large number of glands were minced and a mixture of juices from four centrifuge tubes taken for tests.

No. 142, Dec. 21. Hog pancreas. Juice obtained from another large lot of glands to secure averages. The liquids were separated in the forenoon and delivered for the potential determinations before 1.30 P.M.

The potential determinations on all these centrifugal juices are given below. It will be seen that they are fairly constant and agree pretty closely with the results of our former paper. It is believed that we have enough individual tests to properly cover the seasonal and other variations. The numerical values are given in Table I.

TABLE I.

No.	Animal.	Potential.	P_H.	C_H.
123	Beef	0.6691	5.691	20.3×10^{-7}
124	Hog	0.6690	5.689	20.5
125	Beef	0.6710	5.705	19.7
126	Sheep	0.6709	5.704	19.8
127	Sheep	0.6691	5.691	20.3
128	Hog	0.6690	5.689	20.5
129	Sheep	0.6970	6.170	6.7 boiled liquid
130	Hog	0.7261	6.670	2.1 boiled liquid
131	Hog	0.6679	5.685	20.7
141	Hog	0.6666	5.641	22.8
142	Hog	0.6633	5.591	25.6

Leaving out of consideration numbers 129 and 130, which are the liquids from the boiled organs and somewhat diluted, it will be seen that the results are remarkably uniform. The P_H values are a trifle higher than were those reported in our former paper for the hog and beef products, but a little lower than we found for the sheep, in the mean. Such variations follow, possibly, from differences in the completeness of extraction, which may depend, in part, on the temperatures reached in the centrif-

ugal machine. In any event they are almost within the limits of error in observation.

These results with the pancreas liquids suggested the importance of testing the juices from other organs, which, like body fluids in general, have all been assumed to have a nearly neutral reaction like the blood. The pancreas liquid is unquestionably acid; may not the same condition be found in other organs? We have made a number of examinations of the centrifugal juice from other minced organs, as follows:

No. 132, Oct. 15. Parotid gland of hog. A satisfactory separation could not be obtained, even after some hours of rapid rotation. The minced gland is rich in mucus which evidently interferes with such a separation as we secured from the pancreas. On mixing the minced mass with a little water, however, boiling and filtering, enough liquid was secured to make the potential tests. Similar efforts to secure liquids from the sub-lingual and sub-maxillary glands were not successful, as these glands furnish a slimy mass only, which could not be filtered.

No. 133, Oct. 15. Beef parotid gland. Conditions were similar to those for No. 132. A liquid was obtained in the same way.

No. 134, Oct. 19. Beef parotid. In this case the minced gland was ground with fine washed quartz and a little water. After the centrifugal action a clear filtrate was obtained. Through this filtrate a stream of pure hydrogen was passed to drive out carbon dioxide possibly absorbed in the long preliminary treatment. The liquid was then forced into the potential cell by means of hydrogen gas pressure, avoiding contact with air. The potential found remained constant overnight.

No. 135, Oct. 26. Beef parotid. The glands were removed on the evening of the 25th and quickly frozen, remaining so overnight. They were then ground fine and centrifuged without addition of sand or water. A red liquid was obtained which evidently contained hemoglobin. Hydrogen was applied as in the last case before filling the potential cell.

Sub-lingual and sub-maxillary glands collected at the same time and treated in the same way gave a distinct acid reaction with litmus but could not be made to yield a clear juice.

No. 136, Nov. 1. Hog bile. Clear. Some of the liquid was tested immediately and some after standing 24 hours in the cold. This latter bile showed an increased alkaline reaction.

No. 137, Nov. 1. Hog bile. Markedly turbid. It was tested at once.

No. 138, Nov. 2. Sheep liver. A centrifugal separation from the freshly removed livers is rather easily secured. In this case the liquid was blood red. It was tested at once.

No. 139, Dec. 17. Hog thyroid. The centrifugal liquid was secured on the evening of the 16th and kept near the freezing point until the next morning when the test was made.

No. 140, Dec. 17. Hog liver. The liquid was separated on the evening of the 16th. The separation was comparatively easy.

No. 143, Dec. 22. Hog thyroid. Minced organ centrifuged in the morning and the reddish liquid obtained examined for the potential in the early afternoon.

No. 144, Dec. 22. Hog spleen. Treated and tested as 143.

No. 145, Dec. 22. Hog liver. Treated and tested as 143.

No. 146, Jan. 5. Hog spleen. Separation easy, treated and tested at once.

No. 147, Jan. 5. Beef spleen. Separated and tested at once.

In practically all the above cases, it is seen, the centrifugal liquid was not allowed to stand more than the time necessary to transfer from the centrifuge to the potential cell. Where the separation was made in the laboratory of the medical school this was but a few minutes, and where made in the laboratory of Armour & Company it was not above an hour. There was no time allowed in which fermentation changes could take place of sufficient importance to affect the potential results. The potential determinations are given in Table II.

TABLE II.

No.	Organ.	Potential.	P_H.	C_H.
132	Hog parotid	0.7339	6.804	1.57×10^{-7}
133	Beef parotid	0.7064	6.332	4.66
134	Beef parotid	0.7187	6.545	2.85
135	Beef parotid	0.7169	6.512	3.07
136	Hog bile	0.7491	7.042	0.91
Same, after standing		0.7668	7.369	0.43
137	Hog bile	0.7592	7.215	0.61
138	Sheep liver	0.7180	6.510	3.09
139	Hog thyroid	0.7505	7.089	0.81
140	Hog liver	0.6959	6.151	7.06
143	Hog thyroid	0.7524	7.099	0.80
144	Hog spleen	0.7093	6.361	4.35
145	Hog liver	0.6981	6.169	6.78
146	Hog spleen	0.7223	6.563	2.73
147	Beef spleen	0.7215	6.550	2.82

The examination of the liquids from other organs than the pancreas discloses the very interesting fact that a number of them are very distinctly acid, although not to the same degree that was found in the pancreas. In general, these juices do not appear to be as rich in the phosphates as we find for the pancreas juice.

The press juice from the salivary glands all appear to be slightly acid, although the saliva itself is usually described as alkaline. However, this alkaline reaction is never strong and is by no means constant. After a meal the flow of the mixed saliva may even be slightly acid. The reaction seems to vary with the gland from which the flow is secured. This being the case, we should expect to find at times an acid reaction in the gland cells to balance the corresponding alkaline flow to the mouth of the saliva proper. The same condition doubtless prevails here that we find in the pancreas. The nearly neutral blood furnishes the juice which appears both in the saliva and in the external excretion of the pancreas, the so-called pancreatic juice. When these are alkaline we should properly expect the return flow to the blood to be acid.

The reaction of the bile appears to be neutral, or very slightly alkaline. The numerical values obtain for a temperature of 20°, from which it appears that the observed reaction is about of the neutral order. We have

more than once observed that the bile becomes slightly alkaline on standing, and this change may be responsible for the view usually held. It is true that the bile is alkaline, in the sense that it contains salts of weak acids which by double decomposition are able to neutralize hydrochloric acid from the stomach, but free alkali is doubtless not present. According to Foa,[1] however, the bile may be at times appreciably acid. This might be expected when the conditions of the formation of bile are kept in mind.

In degree of acidity the liver appears to stand next to the pancreas. The juices from the two hog livers examined show an acidity about one-third of that noted in the pancreases, but the causes of the reaction in this case are doubtless different from what they are in the case of the latter organ. The work of the liver is so largely chemical, involving the production and combination of so many groups of acid character, that the resulting reaction may well be acid. It must be kept in mind, further, that the nucleoproteins abundantly present may play a part here as some of them are slightly acid. It is probable that the degree of acidity in the liver will be found to vary with the time of taking food.

The two specimens of thyroid juice obtained are practically neutral and in this respect resemble the bile. As the thyroid possesses no system of external secretion it is practically necessary that the reaction of its press juice should be very close to that of the blood. Of the essential work of the thyroid but little is known; however, any products formed must be contained in the press juice or must go back to the blood and the sum of the reactions cannot be well changed.

The spleen, on the contrary, discloses a slight degree of acidity, not greatly different from that of the parotid gland. In view of the relation of this organ to the blood and the absence of any clearly defined excretory system it is surprising that a reaction should be found which is in any essential different from that of the blood. One of the main functions of the spleen, perhaps the important function, is connected with the elaboration of the white blood corpuscles. It is possible that this operation involves the liberation of a slight trace of an acid fraction from the complexes from which the white cells are produced. If these cells themselves should be found to be on the slightly alkaline side the rejected material might well be slightly acid. In the building up of new cells from the amino acid complexes available such a rearrangement might possibly occur. It will be necessary to examine a much larger number of organs before the constancy of the acid behavior can be definitely affirmed.

The slight degrees of acidity or alkalinity observed in some of the cases discussed above are so near the neutral point that errors of experi-

[1] See "Neuberg's Handbuch," II, p. 1601.

ment must be always kept in mind. We have therefore made frequent controls of the apparatus and have used different types of cells in the progress of the work. In practically all cases the final readings were made by aid of a delicate D'Arsonval galvanometer as well as by the use of the capillary electrometer. To secure greater delicacy the cell and measuring apparatus were mounted on blocks of paraffin. A temperature of 20° was maintained as closely as possible throughout the experiments.

It is known that the removal of carbon dioxide from blood serum by a current of hydrogen causes an appreciable increase in the apparent (OH) concentration, which is in turn reduced by restoring the displaced gas. In our experiments the possible effect of carbon dioxide, absorbed from the laboratory air during manipulation, was tested by noting the changes in dilute potassium chloride solutions. Made up with fresh distilled water the P_H values of these solutions were always close to 7 at 20°. By exposing the potassium chloride solutions in beakers into which the breath was blown it was found possible to perceptibly reduce the potential and reach P_H values ranging from 6.8 to 6.9, indicating slight degrees of acidity. But the amount of contamination in such cases was far greater than would be possible in our usual experimentation, from which we conclude that an acidity error from the absorption of carbonic acid must be of a very low order and not sufficient to account for any of the values obtained.

In a very considerable number of cases the potential cells were allowed to stand overnight after the constant readings had been obtained. In the morning, at the same temperature, these readings were generally found to be quite unchanged, showing that reaction changes from ferment or other kind of activity were inappreciable. On the whole, we are of the opinion that the values recorded represent the actual normal reactions of the organs in question.

Source of the Acid Reaction of the Pancreas.

We do not intend in this place to attempt an explanation of the cause of the acidity observed in any case except that of the pancreas. From the preliminary tests made it was suggested in our earlier paper that the explanation of the pancreas reaction may be found possibly in the relation of the various phosphates in the centrifuge juice, that is in the proportions of the primary and secondary alkali phosphates. This can be determined only by a complete analysis of the inorganic substances in the liquid and allowance for the phosphorus organically combined. We have carried out such an analysis, to be given below.

It must be recognized, further, that some of the organic phosphorus compounds of the type of the nucleoproteins have an appreciable acidity

and the presence of such bodies may be a factor of importance. This point will be considered in what follows.

As regards the relation of the acid and basic groups among the salts of the extracted liquid it is probably true that, while the concentration of the extract may vary with the length of time given to the centrifugal action, the character of the reaction will depend mainly on the *relation* between the ions of the extract. The ions of the alkali phosphates form readily soluble salts which probably extract in equal degrees of rapidity. In the determination of the various ions present we examined a composite made from a portion of each fluid used in the potential measurements on the hog pancreas. The individual liquids making up the composite vary somewhat in their properties but the mixture undoubtedly well repreents the average character of the press juice. In a former paper[1] it was shown that the solids in the liquid from the hog pancreas made up about 15% of the whole, while in the beef pancreas juice the solid part was somewhat less. We are concerned with the hog pancreas juice alone here, and the centrifugal actions may be described as of moderate length, and so conducted as to avoid much rise in temperature. To show the effect of temperature on the extract some other composites were made. The results from this large composite are here given in percentage form, that is as parts of the whole liquid.

Water	83.7%		
Solids	16.3%		
Proteins (N = 2.1)	13.09	Calcium, Ca	0.026
Ash	1.32	Magnesium, Mg	0.020
Phosphoric acid, PO_4	0.982	Potassium, K	0.328
Chlorine, Cl	0.012	Sodium, Na	0.121
Sulfuric acid, SO_4	0.000		

As the ash must contain the phosphates in condensed type, meta- or pyrophosphates, its weight appears small in comparison with the sum of the weights of the phosphoric acid and the metals. The composition of this liquid is somewhat remarkable. While the total solid matter is less than is found in the blood, for example, the salt fraction is unusually high, apparently much higher than is found in any other of the body fluids. The relatively high content of potassium is to be noted. It was separated from the sodium by chloroplatinic acid, and determined, also, indirectly a number of times.

The phosphoric acid is the total acid, that contained in the nuclein form as well as in combination with metals. While the liquid contains a trace of sulfur, it appears to be all in protein combination, and not in the oxidized form as sulfate. The trace of sulfur in the ash is not calculated as sulfate. The low chlorine content is noteworthy and was reached

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

in a number of determinations, as well as with different composites. The amount found corresponds to about 0.02% of sodium chloride.

Press juice from lean meat furnishes, possibly, the nearest approach to this in composition. In respect to mineral matters, and especially with reference to potassium and phosphoric acid the pancreas juice and the muscle juice appear to be much alike. But we have here a far higher amount of protein and other organic substances. It is of course possible that beside the protein nitrogen this element may be present in some other form, in a protein derivative, as a nucleic acid, for example. But this point we have not investigated.

It is evident that the inorganic salts present are essentially phosphates of potassium and sodium, with small amounts of calcium and magnesium phosphates. There are no very exact data in the literature from which the phosphoric acid held in other forms may be calculated, but it may be assumed that a portion of the soluble protein in the juice is the α-proteid of Hammarsten. This point we have looked into. It is not likely that a lecithin body is found in the juice, because it is perfectly clear and the lecithins go with the fat fraction in the centrifugal separation.

We have identified the α-proteid[1] in a number of tests and have made some effort to estimate the amount in the press juice. The phosphorus content of the body is about 1.7%. On boiling a solution containing this protein it breaks up, splitting off a fraction called by Hammarsten β-proteid which contains about 4.5% of phosphorus. This fraction has been purified and analyzed by several chemists. Steudel found as a mean of several trials on different fractions[2] 4.74% of phosphorus in the substance which yields guanylic acid on splitting. In some of this work as much as 2% of the weight of the organ was secured as α-proteid, but in our preparations it is not likely that a correspondingly complete extraction was secured, but the protein which passed into solution was rich in phosphorus and acid in character.

By boiling this liquid, as in the separation of the β-proteid, we obtain a coagulum which is possibly split off from the original soluble protein. By estimating the nitrogen in this and in the filtrate we find that the 2.1% of total nitrogen is divided between 0.23% in the coagulum and 1.87% in the soluble portion. If all of this nitrogen were assumed to exist in the form of α-proteid the phosphorus corresponding would be very much below what we find in the analysis given above, suggesting that the larger part of the phosphorus must exist in some other combination. Some of the organic phosphorus bodies are possibly of acid character.

We have attempted to separate the protein bodies holding the phos-

[1] Hammarsten, *Z. physiol. Chem.*, **19**, 19 (1894); Bang, *Ibid.*, **26**, 133 (1898); Steudel, *Ibid.*, **53**, 539 (1907).

[2] *Loc. cit.* and *Ibid.*, **68**, 40.

phorus and roughly determine the amount of each. For the separation of the α-proteid we extracted a kilogram of minced beef pancreas with physiological salt solution at a low temperature, repeating the process a number of times. The liquids obtained were mixed and precipitated by weak acetic acid, as recommended by Hammarsten and his co-workers. The weight of precipitate obtained was considerably below what was expected from the accounts in the literature, but this may be due in part to the rather marked solubility in the wash water. In order to remove the last traces of acetic acid and to avoid putrefactive changes the last washings were made with water saturated with toluene. Something will be said below of the acid behavior of the so-prepared protein body. In appearance and general behavior our product corresponds closely to the substance described by Hammarsten and others. From our own results and those just cited it appears that vigorous extraction with salt solution will yield the α-protein in amount about 1.5% of the weight of the original moist organ. The PO_4 content corresponding is about 0.077% of the weight of the organ, and this probably represents the larger part of the α-proteid phosphorus in the whole moist organ.

Our centrifugal extraction is far from complete with respect to the total phosphorus. In one experiment with 375 g. of moist pancreas we secured 0.627 g. of P_2O_5 in the top layer, 0.888 g. of P_2O_5 in the liquid layer, and 1.128 g. of P_2O_5 in the lower layer, *exclusive* of the phosphorus found in the fat of the upper and lower layers. This makes 0.705% of P_2O_5 for the whole organ, or 0.943% as PO_4, an amount not very different from what we secured in our composite liquid. This is a further suggestion that the α-proteid phosphorus must make up but a small part of the whole phosphorus.

It has been stated that the α-proteid gives rise to the β-proteid on decomposition and that the phosphorus can be most accurately estimated in the latter. That the larger part of the phosphorus combined with soluble protein is held in this form is suggested by another test independent of those made on the composite liquid. In this case 130 cc. of liquid were secured from a mass of minced pancreas representing about 5 kg. of the fresh organs, the weight centrifuged being about 600 g. 50 cc. of this liquid, weighing 53.2 g., furnished 9.705 g. of dry solids, or 18.24%. 75 cc. of the liquid were mixed with an equal volume of water and heated to boiling. A marked precipitate was formed which was well washed, dried and weighed. The weight was 2.25 g. equivalent to 2.82% of the weight of the liquid or 15.4% of the weight of the solids. The phosphorus content of this residue was 0.61%, as P_2O_5, and this must represent the phosphorus split off from the α-proteid in making the β-proteid.

The filtrate from this coagulum was evaporated to dryness and tested for phosphorus, yielding 5.13% of the dry weight of P_2O_5, a part of which

is inorganic phosphorus, and a part protein phosphorus. These determinations may be condensed as follows:

PO_4 in total solids.....	5.990%	PO_4 of coagulum as part of liquid..	0.023%
PO_4 as part of whole liquid.	1.093	PO_4 in dried filtrate from coagulum.	6.86
PO_4 in dry coagulum.....	0.82	PO_4 of filtrate as part of liquid.....	1.058

These results are slightly different from those of the large composite sample. The solids are somewhat higher and we have found the solids of the coagulum higher. The difference is probably due to the fact that here in the centrifugal action the temperature was permitted to run higher than was the case before, where it was kept down low enough to prevent any change in the ferments of the liquid. Elevated temperature favors a somewhat more perfect extraction. But the results are alike in showing that the larger part of the phosphorus is in the soluble fraction, that is in the fraction holding the β-proteid as well as the inorganic salts.

In several portions of this filtrate an attempt was made to throw down the β-proteid so as to secure a separation of the nuclein phosphorus from the inorganic. This was done by precipitating the liquid with dilute acetic acid and alcohol as described in the papers of Hammarsten[1] and Steudel.[1] The precipitation is not strictly quantitative, but a close approximation is reached. The determination of phosphorus in this precipitated β-proteid gives an amount of PO_4 equal to 0.037% of the liquid weight, as against 1.056% not thrown down. Different trials give essentially the same result, from which we conclude that the larger figure must represent the portion of the phosphorus not combined as a nuclein but combined in the inorganic form in the press juice.

Going back now to the composite liquid from which the larger table was made, we found, after separating a coagulum, and treating the filtrate with acetic acid and alcohol, an amount of phosphorus in the precipitate which, as PO_4 is equal to 0.023% of the weight of the original liquid. The coagulum formed by boiling in this case contained PO_4 equivalent to 0.0045% of the original liquid. The PO_4 in both amounted to 0.027% of the weight of the original liquid, from which it appears that the organic phosphorus must be very low, or, in other words, that the combination of this element is largely with metals.

This being the case will the acid and base content, as exhibited by the table, account for the reaction of the liquid? Probably not. If we combine the calcium and magnesium with phosphoric acid to form salts of the $CaHPO_4$ type, and the chlorine with sodium, we have left 0.841 PO_4 and sodium and potassium equivalent to 0.520 K. Taking out, further, the phosphoric acid which seems to be held in protein combination, we have remaining 0.814 PO_4 to be combined with alkalies. Cal-

[1] *Loc. cit.*

culation shows that these amounts of acid and alkali are sufficient to form a salt of the type KH_2PO_4 holding 148 parts of potassium, and a salt of the type K_2HPO_4 holding 372 parts of potassium or its equivalent, which proportions correspond closely to 4 molecules of KH_2PO_4 and 5 molecules of K_2HPO_4. A mixture in these proportions would be slightly acid, but not as strongly acid as the potential measurements indicate.

It remains now to test the behavior of the α- and β-proteids with respect to reaction. Our various preparations of the latter compound yielded amounts of phosphorus between 4.79% and 4.94%, with nitrogen varying from 17.20 to 17.58%. Hammarsten gave 4.48%, and Steudel, in a number of preparations, found between 4.45 and 4.86% of phosphorus, with nitrogen in about the range we found. It is evident then that we are dealing with the same preparation which is known to have an acid behavior and which breaks up easily, yielding about half its weight of guanylic acid. A solution was made of one of our preparations containing 250 mg. in 100 cc. of pure neutral 0.2 N KCl. The potential value at 20° was found to be $\pi = 0.6754$, from which $P_H = 5.797$. This value is not greatly different from those we found in the centrifugal liquids.

We have made this determination on the β-proteid because it is obtainable in a relatively pure condition. The α-proteid is more likely to be the phosphorus-holding complex in the centrifugal juice and we have therefore examined this product also, although it cannot be as readily secured in pure form. Following the methods already referred to we have made a number of preparations which are clearly acid in behavior. Great care must be taken to wash out all the acetic acid used in the precipitation of the substance from the extractive liquid, which was in some cases physiological salt solution and sometimes a stronger salt solution. It is possible that in the organ juice the α-proteid exists in combination with part of the alkali metals, rather than in the free state. Several reactions strongly suggest this, but in our calculations above we have assumed that the alkali is combined with phosphoric acid. If held with the protein the effect would be to leave the reaction of the phosphates more strongly acid. Gamgee and Jones[1] have given some experiments on this protein and suggest that it acts as a dibasic acid which yields an acid ammonium salt soluble in water. For the purpose of measuring the optical rotation of the substance they dissolved it in water plus a small amount of ammonia. They recognize in the α-proteid a body of distinctly acid character.

At any rate, by the addition of the dilute acetic acid the protein acid is set free and thrown down as a flocculent precipitate which may be washed by decantation rather easily, using toluene to prevent decomposition. It is finally washed on a filter in the same way. A little calcula-

[1] *Beit. Chem. Phys. Path.*, 4, 10 (1904).

tion shows that the acetic acid can soon be reduced to a dilution where it will give no measurable reaction in the concentration cell.

The α-proteid does not appear to be as soluble in weak potassium chloride solution as was the other product. For our tests we shook up small portions with the chloride and obtained solutions which showed a marked acid behavior in the cell. But the concentration of the solutions was always low. The acid character of the preparation is better shown in another way. We found that it dissolves in solutions of secondary sodium phosphate readily, about as it does in weak soda solution or ammonia. If this solution follows by reason of the formation of a salt at the expense of part of the sodium of the phosphate a mixture of primary and secondary phosphates would be left and the fact would be shown by an altered potential value in the cell. Our experiments confirmed this view.

We dissolved an amount of the moist α-proteid equivalent to 0.78 g. of the dry substance in 15 cc. of molar/15 Na_2HPO_4 plus 20 cc. of water (including the water held in the moist precipitate), and found for the solution at 20° a value of $\pi = 0.7330$, from which $P_H = 6.788$, or $C_H = 1.63 \times 10^{-7}$. Fifteen cc. of the same phosphate solution plus 20 cc. of water gives a mixture from which $P_H = 8.982$ was found, or $C_H = 1.03 \times 10^{-9}$. The phosphate solution has become strikingly more acid after being mixed with the protein and the final solution has about the P_H value of a mixture of 4.5 volumes of molar/15 secondary phosphate and 5.5 volumes of the corresponding primary phosphate. This change corresponds to the removal of a certain amount of sodium from the secondary phosphate solution, and practically the equivalent of 0.842 g. per liter, or 12.6 mg. for the 15 cc. used in dissolving the protein.

It is evident, therefore, that this so-called α-proteid is a distinctly acid substance. In our centrifugal juice it may act as an acid or it may hold part of the sodium in salt form. Probably the latter is the true condition, and the original combination in the organ must be an alkali salt of the nucleoproteins present. The effect of this would be to leave more of the phosphorus combined as a primary phosphate and increase the acidity of the press juice. It must be recalled that besides the α-proteid the pancreas is known to contain other similar bodies which are not readily separated from the solvent liquid. If these have the same markedly acid character as this one, we have a further explanation of the acid reaction of the organ.

We wish to acknowledge in this place our indebtedness to Mr. R. A. Nelson and Miss Mary Hull for valuable help in the preparation of material and in the analysis of solutions described.

Summary.

1. We have shown by a large number of qualitative experiments on individual organs of freshly slaughtered animals that the normal reac-

tion of the pancreas is distinctly acid. This confirms and extends the conclusions of our former paper regarding the pancreases of the hog, beef and sheep.

2. The quantitative relations were studied on organs collected from September to December to cover possible seasonal or feeding variations, and results were found which show that the reactions of the pancreases of the three animals are essentially as reported before, with the P_H values running between 5.5 and 5.7. There appears to be no seasonal effect, and the condition of the animal was evidently without influence. This ion concentration is then nearly a constant and is undoubtedly a factor of physiological importance.

3. Similar experiments were made on the organs of other animals. It was found that the parotid glands, the liver and the spleen were acid in reaction, but not to the extent found for the pancreas, while the bile was slightly alkaline or neutral and the thyroid gland practically neutral, as might be expected from the location and relations of the gland.

4. In searching for the cause of the reaction of the pancreas a complete analysis of the salts of the press juice was made. The juice is rich in phosphates, while sulfates are absent and chlorides present only in traces. Of the metals, potassium is the most abundant and the relations of all the metals to the phosphoric acid is such as to give rise to a slightly acid reaction.

5. In addition to this reaction of the inorganic salts it is found that the nucleoproteins present have an acid reaction, and that one of them, at least, the α-proteid of Hammarsten, goes readily into solution with secondary sodium phosphate, in which solution the reaction of the phosphate changes from alkaline to acid, apparently from the formation of primary phosphate.

6. It appears that the marked acid reaction of the pancreas, which is stronger than found for other organs, may be accounted for through the presence of acid phosphates and acid nucleoproteins. The alkali salts of the nucleoproteins are readily soluble in water and therefore pass into the centrifugal or press juice.

CHICAGO, ILL.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF WISCONSIN.]

THE INFLUENCE OF SOME SOLVENTS ON THE RATE OF ACTION OF SODIUM WITH ISOAMYLBROMIDE.

BY PAUL WHITTIER CARLETON.

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Three types of solvents, ether, tertiary amines, and hydrocarbons, have particular interest because of their relation to the formation of the