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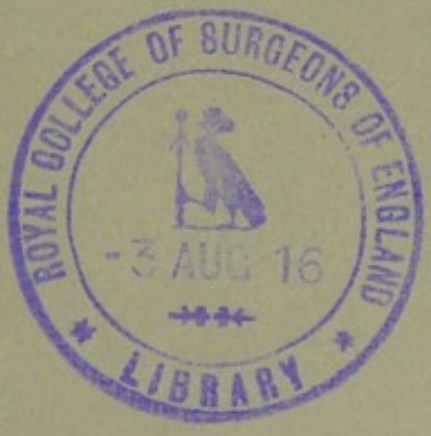


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# THE STIMULATING INFLUENCE OF SERUM ON PANCREATIC AMYLASE.

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In attempting to investigate the influence of normal and diabetic serum on pancreatic amylase, the authors had occasion to observe that even small quantities of serum had a marked stimulating effect on the amylolytic activity of the extract of a dog's pancreas. This confirmed the findings of Pozerski[1] who had noted, this fact, and had undertaken to investigate the manner of this action of serum on the starch-splitting ferment. Wohlgemuth[2] had also been aware of this interesting observation of Pozerski's, and had made some experiments tending in the same direction.

The following series of tests was instituted in the effort to add some facts to those already known which bear upon the mechanism of this phenomenon.

*Technique.* An extract of the pancreas of a dog was made as follows: A medium sized dog was bled to death; pancreas dissected clear of any surrounding tissues, cut into small pieces and ground up in a mortar with washed sand; extracted in 100 cc. of 0.03 per cent ammonia for twenty-four hours; precipitated by the addition of 2 per cent acetic acid, and filtered through soft filter paper while standing on ice over night; the filtrate was neutralized with ammonia and diluted with distilled water to strength desired.

Starch solution was prepared by making a paste of "Kahlbaum Soluble Starch" in cold water, mixing it well, and adding to distilled water brought almost to boiling, but not allowed to boil.

[1] Pozerski: *Compt. rend. soc. biol.*, lv, p. 29, 1903.

[2] Wohlgemuth: *Biochem. Zeitschr.*, xxxiii, p. 303, 1911.

The serum used was separated from the dog's blood, or from human blood or human ascitic or pleural exudate. For routine use the serum was diluted with distilled water, as desired.

The general method of estimation of amylase was the slightly modified method of Wohlgemuth, as used by one of us in previous work.[3]

EXPERIMENT 1. Human blood serum: digestion, 24 hours.

A. Serum, diluted 1 : 4, 0.5 cc. in each tube. 2 per cent starch solution in amounts of 1, 2, 3, 5, 8 and 10 cc. *No digestion.*

B. Pancreatic extract, diluted 1 : 10, 0.5 cc. in each tube. 2 per cent starch solution in amounts of 0.5, 1.0, 1.5, 2, 4, and 6 cc. *Digestion = 1.75 cc.*

C. Serum, diluted 1 : 4, 0.5 cc. in each tube. Pancreatic extract, diluted 1 : 10, 0.5 cc. in each tube. 2 per cent starch solution in amounts of 2, 4, 6, 8, 10 and 12 cc. respectively. *Digestion complete in all tubes.*

This experiment demonstrates (1) the inactivity of the amylase of this particular specimen of serum; (2) an activity of pancreatic extract equivalent to 1.75 cc.; (3) an activity of the combined pancreatic extract and inactive serum equal to complete digestion of 12 cc., *i.e.*, at least seven times greater than that of pancreatic extract alone.

EXPERIMENT 2. A comparison of three sera (L1, K2, and K3), using in this case a fresh extract of dog's pancreas diluted 1 : 100 and 4 per cent starch solution. Digestion, 24 hours.

| | L1 | K2 | K3 |
|---|---|---|---|
| | cc. | cc. | cc. |
| A. Serum alone, diluted 1 : 4, 0.5 cc | 0.5 | 0.75 | 0.5 |
| B. Pancreatic extract alone, diluted 1 : 100 | 5.0 | 5.0 | 5.0 |
| C. Combination of A and B | 13.0 | 11.0 | 12.0 |

In this experiment we note (1) the comparative inactivity of serum amylase. (2) The constant value of pancreatic extract for amylase. (3) That the almost inactive serum combined with the pancreatic extract gives results which are much greater than the sum of the two acting separately.

[3] Crohn: *Amer. Journ. Med. Sci.*, cxiv, p. 393, 1912.

Experiment 3. Comparison of sera from pathological conditions in their ability to stimulate the amylolytic activity of pancreatic amylase.

| | A SERUM ALONE DILUTED 1:4, 0.5 cc. | B PANCREATIC EXTRACT DILUTED 1:100, 0.5 cc. | COMBINATION OF A AND B |
|---|---|---|---|
| | cc. | cc. | cc. |
| 1. Pneumonia | 0.5 | 5 | 13 |
| 2. Diabetes mellitus (urine sugar-free) | 0.5 | 5 | 12 |
| 3. Chronic nephritis | 0.75 | 5 | 11 |
| 4. Uremia | 0.5 | 4 | 13 |
| 5. Pleural fluid, pleurisy with effusion | 0.5 | 6 | 12 |
| 6. Pleural fluid (new growth of lung), non-hemorrhagic | 0.5 | 6 | 13 |
| 7. Pleural fluid (new growth of lung), non-hemorrhagic | 0.5 | 6 | 13 |

In this experiment the addition of 0.5 cc. of diluted serum more than doubled the activity of pancreatic extract. The origin of the serum did not seem to play a rôle for the activating power of all specimens appeared to be the same, barring slight fluctuation due to possible imperfection in technique.

Experiment 4. Activating effect of serum on human pancreatic secretion (duodenal contents, obtained by the Einhorn duodenal tube).

A. Duodenal contents, normal case (dilution 1 : 10) 0.25 cc. used alone ..... 3 cc.
The same + human serum diluted 1 : 4, 0.25 cc. used ..... 20 cc.
B. Duodenal contents, cholelithiasis (dilution 1 : 10) 0.25 cc. used ..... 6 cc.
The same + human serum diluted 1 : 4, 0.25 cc. used ..... 30 cc.

This experiment shows marked activation of human pancreatic secretion by even a very small amount of human serum.

Experiment 5. Effect of varying the quantity of serum used, on its activating power. In each tube, 0.5 cc. of pancreatic extract, dilution 1 : 100, having a constant digestive value of 5 cc. of 2 per cent starch solution was used.

| | CONTROL | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|
| Amount of serum added | 0 | $\frac{1}{128}$ cc. | $\frac{1}{64}$ cc. | $\frac{1}{32}$ cc. | $\frac{1}{16}$ cc. | $\frac{1}{8}$ cc. | $\frac{1}{2}$ cc. |
| 2 per cent starch solution digested | 5 | 6 | 7 | 8.5 | 9 | 13 | 20 |

This experiment indicates that the activating power is proportionate to the quantity of serum used. Diluting the serum diminishes its activity in a like manner up to a certain limit of dilution.

EXPERIMENT 6. Effect of boiling the serum (sterilization) upon its activating power. Dilution of serum was 1 : 4; of pancreatic extract, 1 : 100. The results are expressed as cc. of 2 per cent starch solution digested in twenty-four hours.

| | |
|---|---|
| 1. 0.5 cc. serum, unboiled | 1.5 |
| 2. 0.5 cc. serum, boiled 10 minutes | 0 |
| 3. 0.5 cc. pancreatic extract | 4.5 |
| 4. 0.5 cc. serum, unboiled, + 0.5 cc. pancreatic extract | 15.0 |
| 5. 0.5 cc. serum, boiled 10 minutes + 0.5 cc. pancreatic extract | 16.0 |

This experiment shows that boiling serum in no way diminishes its activating power. The slight difference after boiling may be due to increased concentration from evaporation.

EXPERIMENT 7. Effect of incubating serum and pancreatic extract, separately and together, on amylolytic activities. Dilution of serum was 1 : 4; of pancreatic extract, 1 : 100. Results are expressed as cc. of 4 per cent starch solution digested in 24 hours.

| | | | |
|---|---|---|---|
| A. 0.5 cc. serum, unheated | | 0.5 | |
| B. 0.5 cc. pancreatic extract, unheated | 5 | | 5 |
| C. 0.5 cc. serum, incubated 24 hours at 38°C. | | 0 | |
| D. 0.5 cc. pancreatic extract incubated 24 hours at 38°C. | | 0 | |
| 0.5 cc. pancreatic extract incubated 1 hour at 38°C. | 4 | | 3 |
| E. A + B | 16 | | 13 |
| F. C + D, after incubation together 24 hours | 11 | | 11 |
| C + D, after incubation together 1 hour | | 13.0 | |
| G. C + D, after incubation separately 24 hours | 0 | | 8 |
| C + D, after incubation separately 1 hour | | 11.0 | |

The following conclusions may be drawn from this experiment: 1. Pancreatic extract when incubated alone loses part of its amylolytic strength. 2. Serum when incubated alone loses part of its amylolytic strength. 3. When mixed together and incubated there is some loss of amylolytic strength. 4. When incubated separately and then mixed there is marked loss of amylolytic strength. 5. The presence of serum during incubation of pancreatic extract prevents in part the loss of amylolytic activity of the latter. 6. Serum after incubation is just as strong an

activating agent as before, although it loses its own natural amylolytic power.

EXPERIMENT 8. Effort to isolate the factor in the serum which activates the pancreatic extract.

A. Serum was boiled, precipitated with dilute acetic acid and filtered.

1. Whole serum, 1 : 4, + pancreatic extract, 1 : 100, digests 14 cc. 4 per cent starch solution in 24 hours.

2. Filtrate from boiled serum, 1 : 4, + pancreatic extract, 1 : 100, digests 12 cc. 4 per cent starch solution in 24 hours.

3. Protein precipitate from boiled, acidified serum has absolutely no activating power.

B. Dialysis experiments.

1. Undiluted pancreatic extract was dialyzed against normal salt solution for 24 hours. No amylase could be detected in the dialysate.

2. Human serum, diluted with equal volume of water was dialyzed against distilled water.

*a*. Pancreatic extract, 1 : 100, + distilled water digests 3 cc. of 2 per cent starch solution.

*b*. Pancreatic extract, 1 : 100, + 0.5 cc. of dialysate (24 hours) digests 5.5 cc. 2 per cent starch solution.

*c*. Pancreatic extract, 1 : 100, + 0.5 cc. of dialysate (48 hours) digests 7 cc. of 2 per cent starch solution.

*d*. Pancreatic extract, 1 : 100, + 0.5 cc. of serum (1 : 2) which had been dialyzed for 72 hours digests 11 cc. of 2 per cent starch solution.

*e*. Pancreatic extract, 1 : 100, + 0.5 cc. normal (not dialyzed) serum, 1 : 2, digests 20 cc. of 2 per cent starch solution.

3. Human serum was dialyzed against running water for 24 hours.

*a*. Control serum (not dialyzed) + pancreatic extract digests 11 cc. of starch solution.

*b*. Dialyzed serum + pancreatic extract digests 9 cc. of starch solution.

The results of experiment 8 show (1) that the activating substance in serum is not a coagulable protein but is present in the incoagulable fluid portion: (2) that amylase is not dialyzable: (3) that the activating substance is dialyzable.

These results directed our attention to the salts of serum which are the constituents which would probably be most easily removed from the serum by dialysis. They have been held by Wohlgemuth[4] to be responsible for the phenomenon as first noted by Pozerski. Experiment 9 was carried out to determine the activating power of the salts of serum.

[4] Wohlgemuth: *loc. cit.*

Experiment 9. Determination of activating power of salts of serum.

A. 1. Pancreatic extract + 0.5 cc. 0.9 per cent NaCl digests 6 cc. of 4 per cent starch solution.

2. Pancreatic extract + 0.5 cc. distilled water digests 6 cc. of 4 per cent starch solution.

B. 1. Pancreatic extract + 0.5 cc. Ringer's solution digests 5 cc. of 4 per cent starch solution.

2. Pancreatic extract + 0.5 cc. distilled water digests 3 cc. of 4 per cent starch solution.

C. Serum was dried, ashed and the residue taken up with water. The amount of this solution used in (1) corresponded to 0.5 cc. of serum.

1. Pancreatic extract + serum salts digests 7 cc. of 4 per cent starch solution.

2. Pancreatic extract + distilled water digests 3 cc. of 4 per cent starch solution.

From the results of this experiment we conclude that sodium chloride *per se* is not responsible for the activation: that a combination of chlorides of sodium, potassium and calcium as in Ringer's solution have activating power: and lastly that the salts derived directly from the serum account for a large part of the phenomenon.

Experiment 10. Determination of the activating power of other possible constituents of serum.

A. 1. Pancreatic extract + distilled water digests 3 cc. of 4 per cent starch solution.

2. Pancreatic extract + 0.1 cc. adrenalin, 1 : 1000, digests 3 cc. of 4 per cent starch solution.

3. Pancreatic extract + 0.5 cc. serum, 1 : 4, digests 7 cc. of 4 per cent starch solution.

4. Pancreatic extract + 0.5 cc. serum, 1 : 4, 0.1 cc. adrenalin, 1 : 1000, digests 8 cc. of 4 per cent starch solution.

B. 1. Pancreatic extract + distilled water digests 4 cc. of 4 per cent starch solution.

2. Pancreatic extract + 0.5 cc. 0.1 per cent lecithin solution digests 3 cc. of 4 per cent starch solution.

This experiment indicates that adrenalin may slightly increase the activating power of serum and that lecithin may exert a slight inhibiting action.

GENERAL SUMMARY.

The results of these experiments may be summarized as follows: Small quantities of serum possess the power of increasing the amylolytic action of pancreatic extracts and pancreatic secretion by two, three or more than four-fold. This power is not diminished by boiling the serum nor by incubating it at 38°C. for many hours. The activating power of serum is lessened by dialysis. The salts of serum, in the proportion in which they exist in it, are largely responsible for the phenomenon: sodium chloride alone is not. Adrenalin may have a slight positive influence; lecithin has not. The origin of the serum plays no rôle.

If we attribute the greatest part of the activating power to the mineral salts, then the constancy of the phenomenon in different pathologic sera becomes intelligible, inasmuch as the mineral content of such sera varies but little. Roger[5] has found a similar ability, though to a lesser degree in sterilized saliva; Roger[6] also found the same for white of egg; Pozerski[7] for intestinal secretions. Probably in all the instances, the mineral content was the causative factor. Wohlgemuth[8] further has demonstrated that the serum after digestion is only slightly more activating in its effect than in the fasting person, also that the activating power of sera of different animals varies slightly in the following order: dog, sheep, rabbit, man, rat, horse. Nor is there any material difference between specimens of blood from different veins and different areas of the body. Wohlgemuth also ascertained that serum has the same influence on pancreatic amylase which it has on the amylase derived from other sources in the body, *e.g.*, organ extracts, saliva, etc.

Practically from the viewpoint of the physiologist, these facts are of great interest, as it has been found by one of us (Crohn)[9] that the pure duodenal contents have an amylolytic activity, which

[5] Roger: *Arch. de maladie de tube digestif et de la nutrition*, iii, p. 509, 1909.

[6] Roger: *Compt. rend. soc. biol.*, lxiv, p. 16, 1908.

[7] Pozerski: *Maly's Jahresbericht*, 1902, p. 400.

[8] Wohlgemuth: *loc. cit.*

[9] *Loc. cit.*

if estimated for the hydrolysis of the average carbohydrate intake of a normal man seems barely sufficient to digest it. But if we increase this coefficient two, three, or four times, by the presence of serum salts through the exudation of intestinal secretions, or the saliva, we can satisfactorily answer the query and explain why so very rarely starch appears in the stools, and why the pancreatic digestion so infrequently becomes insufficient for carbohydrates.