The observation of several investigators that vitamin C is increased when peas, lentils, and beans are germinated has been verified in the case of mongo. Ten grams of mongo as daily supplement to the scorbutic diet failed to protect guinea pigs from scurvy, while five grams of fresh togi as supplement to the same scorbutic diet cured three guinea pigs of the disease.

3 (1750)

Observations on pancreatic rennet.

By ALBERT A. EPSTEIN.

[*From the Department of Physiological Chemistry, Mt. Sinai Hospital, New York City.*]

Pawlow and Parastschuk,[1] Vernon[2] as well as Delezenne[3] have called attention to the presence of rennet in the pancreatic secretion of experimental animals. Wohlgemuth[4] claims to have found it in human pancreatic secretion, but not without some difficulty. Notwithstanding these observations some doubt seems to exist in the minds of a number of investigators in this field. Textbooks on physiology do not class rennet with the other pancreatic ferments.

Fresh or well-preserved dried preparations of pancreatic extract ordinarily do not show any milk coagulating ferment. When solutions of such extracts are permitted to deteriorate the rennet function comes into evidence. While studying the pancreatic ferments I have found that the presence of rennet in extracts of this organ may be demonstrated constantly in a number of different ways.

1. Rennet may be liberated by heating a solution of the extract from 50 to 65° C. for a period of about 10–15 minutes; the most favorable temperature being 60° C. Flocculation usually occurs upon heating, but the ferment remains in solution.

2. The addition of suitable amounts of hydrochloric acids reveals the presence of rennet.

[1] Pawlow, J. P., and Parastschuk, S. W., *Zeitschrift fur Physiologische*, 1904, xlii, 415.

[2] Vernon, H. M., *Journal of Physiology*, 1903, xxix, 302.

[3] Delezenne, *Soc. Biol.*, 1907, lxiii, 98.

[4] Wohlgemuth, *Biochem. Zeitschrift*, 1917, ii, 350.

3. By treating solutions of pancreatic extract with colloidal iron and other precipitants such as uranium acetate, alcohol, sodium sulphate and others. Calcium chloride solution in concentration accomplishes the same result.

4. The addition of products of peptic digestion, such as those of gliadin or Witte's peptone, to solutions of pancreatic extract also liberate the rennet.

5. Serum of a rabbit immunized by intravenous injections of pancreatic extracts, when added to solutions of pancreatic extract, liberate the rennet.

Whatever method of activation is used, in every instance, the rennet itself remains in solution, and some substance is precipitated, which before precipitation conceals the presence of the rennet.

I reported some of these results at a Section meeting of the American Chemical Society, held last September, and concluded at the time that the rennet in the pancreatic extract was probably present not as a pro-enzyme, but as an active enzyme mixed with substances which are antagonistic to its action. The conclusion is based on the foregoing experiments, the most significant of which is the one showing the effect of immunized serum on inactive pancreatic extract. Apparently the inactive solution of pancreatic extract is capable of producing in an immunized animal an antibody for the substance in the pancreatic extract, which is antagonistic to the rennet. The antibody thus produced is in the nature of a precipitin. The lack of any specific method of activation seemed to support this view that there is no pro-ferment. However, the proof is indirect, hence not final. Various attempts to recover the antagonistic substance in active form proved futile. The most that can be said about it is that it probably is a substance of protein nature which coagulates at a temperature between 60 and 65° C., is precipitable by sodium sulphate and other precipitants, and is capable of producing a precipitin in immunized serum.

These facts brought to mind the former controversy concerning the nature or state of the rennet in gastric mucosa.

You will recall that rennet is believed to exist in two states, that of an active enzyme (or rennet) and as a pro-enzyme (or

pro-rennet), and that under the influence of very small quantities of acid at the optimum temperature, the pro-rennet is rapidly transformed into the active rennet. This result is regarded as the product of true activation. Hedin[5], however, interprets the facts in another manner. He assumes that the pro-rennet is merely a combination of rennet with a substance antagonistic to it, and on the following grounds. If the pro-rennet be treated with dilute HCl, the rennet is set free and the antagonistic substance destroyed, hence, its inhibitory action is lost. On the contrary, a solution of pro-rennet, treated with very dilute ammonia at 37° C., loses all its rennet already free, while the antagonistic substance remains unchanged; so that, by adding active rennet to this treated liquor, the rennet is at once rendered inactive.

I applied Hedin's method of proof to the rennet in the pancreatic extract and found that the results were in accord with his. On closer analysis it became evident that Hedin's proof was insufficient and the conclusion erroneous.

Before proceeding to the evidence in substantiation of this, permit me to note the following concerning the pancreatic rennet. The content of this enzyme in pancreatic extract is very large, and goes absolutely hand in hand with the quantity of trypsin present. Means have not yet been found to separate rennet from trypsin. The two appear to be intimately associated functionally and chemically. A method has been devised for the quantitative recovery of the rennet-trypsin enzyme and for its purification. This will be presented at another time. Suffice it to say for the present that the trypsin-rennet combination constitutes about 1–2 per cent. of the total substance of dried pancreatic extract. It is of protein nature, is not precipitated by colloidal iron, is coagulated by heat (at 82–85° C.), is extremely hygroscopic, and of an acid character.

It is active only in the presence of Ca, which, however, must be available in ionizable form. In this fact seems to lie the fault in Hedin's proof.

I have stated that fresh or well-preserved pancreatic extract has no milk coagulating properties, but when a solution of it is

[5] Hedin, S. G., Harvey Lectures, 1914, p. 162.

treated by heating (up to 60° C.) or by means of colloidal iron and other precipitants, the rennet is set free. Now if some of the original extract is added to the activated preparation, no inhibition of the rennet action occurs. In other words, none of the substance which hinders the action of rennet is present in a free state in the pancreatic extract. When alkalinized with ammonia the original extract acquires the power to inhibit the action of active rennet preparations. The result, however, is not due to the setting free of the antagonistic substance (as Hedin believes), but to the fact that the calcium ion, which is essential for coagulation, is rendered inert by the procedure. On the other hand, if a solution of active pancreatic rennet is alkalinized with the hydrate or carbonate of ammonia or soda, the enzyme solution is rendered inactive. If, however, $CaCl_2$ in sufficient amounts is added to this liquor, the rennet is immediately reactivated. Neutralization or alkalinization of the active rennet solution by means of disodic phosphate, or calcium hydrate, does not inactivate the rennet.

It would appear from this that Hedin's result was not due to inactivation of the rennet by means of the antagonistic substance, but merely to the removal of the calcium ion from the sphere of action.

The experiments made thus far seem to indicate that the enzyme substance forms a chemical combination with calcium, in the nature of a salt, and only as such exerts its action. There appears to be some ground for the belief that rennet and trypsin reside in a single chemical unit of the pancreatic substance, and possibly represent two phases of one and the same ferment.

I might add in conclusion that, whereas the evidence is in favor of the view that the rennet in pancreatic substance exists as an active enzyme and not as a pro-rennet, definite proof for this opinion is still lacking.