XCIII. THE ACTION OF GASTRIC JUICE ON BEEF MUSCLE-GLOBULIN, WITH REFERENCE TO ANAEMIA.

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PERNICIOUS anaemia results from a failure of the supply of healthy red cells to the circulating blood. No way was known of restoring this lost power of regeneration of healthy red cells until Minot and Murphy [1926] observed that by feeding liver to persons suffering from this type of anaemia increased numbers of reticulocytes, or young red blood cells, appeared in the circulation, with the result that the blood was gradually restored to its normal condition.

Following on this discovery, Castle [1929; Castle and Townsend, 1929] announced that changes in the blood, similar to those produced by liver feeding, could be caused by daily administration of raw beef which had previously been digested by normal gastric juice, either in the stomach of a normal person or *in vitro*. Neither beef nor gastric juice was effective alone or when fed successively. Castle suggested that pernicious anaemia is a deficiency disease of a new type; that it is due to a lack of some unknown substance or principle produced from protein during normal digestion; and that the gastric secretion in pernicious anaemia is deficient in a factor necessary for the production of this principle.

According to this work there are, therefore, two factors involved in the maintenance of the proper supply of red blood cells: (1) an intrinsic factor in the gastric juice, and (2) an extrinsic factor contained in protein. By the interaction of these factors the haematopoietic principle is produced. Normally this principle is concentrated in the liver, hence the efficacy of this organ in the treatment of anaemia.

In his later work Castle *et al.* [1930] demonstrated that the protein of beef muscle, precipitated at p_H 6 and washed with saline of the same reaction, could act as the extrinsic factor: washed caseinogen and wheat gluten were unsuitable. The principle could be obtained by digesting this precipitated beef protein with normal gastric juice at a neutral reaction. The intrinsic factor appears to be neither HCl, nor pepsin, nor trypsin; it is confined to the gastric juice and is destroyed by boiling and behaves like an enzyme [Castle *et al.*, 1931].

In view of what is known of the intrinsic factor, its resemblance to an enzyme, *etc.*, we felt that it would be of value to study more closely the interaction of gastric juice and muscle-protein with the object of obtaining chemical evidence of the production of the active principle. Up to this time the test for the presence of the haematopoietic principle in digests, and incidentally, for the presence or absence of the intrinsic factor in a given sample of gastric juice, has been a reticulocyte response after feeding the products of digestion to cases of pernicious anaemia.

There is evidence that the active principle produced during the digestion of muscle-protein by gastric juice is a water-soluble nitrogenous base, not precipitated by trichloroacetic acid [Cohn *et al.*, 1930]. We therefore planned to determine whether a non-protein nitrogenous body results from such digestion by normal gastric juice *in vitro* near the neutral point, and if so, whether the gastric secretion of cases of pernicious anaemia shows a diminished activity in this respect.

EXPERIMENTAL.

Gastric juice (obtained 1 hour after subcutaneous injection of histamine, or following ingestion of 5 % alcohol) was incubated with muscle-protein in a buffered medium of approximately p_H 6. The unchanged protein was removed by means of trichloroacetic acid and the N in the filtrate determined.

A difficulty which we early encountered was the occasional presence of minute amounts of trypsin in the gastric juice: such tryptic activity was exceedingly small but was sufficient to cast doubt upon our findings. Experiments with commercial trypsin (Liquor Pancreaticus (Armour) precipitated with alcohol, the precipitate washed with alcohol, dried and taken up in saline) showed, however, that the amount of soluble N produced from beef-muscle by this enzyme is 50 % less at p_H 6 than at p_H 8. We were able, therefore, by determining the extent of digestion at p_H 8, to make a suitable correction for tryptic activity when it was present. In doing this we were obviously neglecting the possibility of digestion such as we were seeking occurring at the alkaline reaction, a circumstance which could not be excluded and which, in any case, does not minimise the significance of our findings.

The activity of pepsin under the conditions of our experiments becomes negligible at p_H 3.5, *i.e.* well removed from the region in which we were working.

Method. The gastric juice was freed from solid particles and its reaction adjusted to p_H 6 with alkali or acid as required. 5 ml. were measured into each of three test-tubes: to one of these tubes were added 5 ml. of $N/5$ HCl; to the others 5 ml. of $M/20$ phosphate buffer solution of p_H 6 and 8 respectively. The contents of the tubes were mixed and the reaction checked with the glass electrode. 4 ml. portions of this mixture were separately transferred to test-tubes containing 50 mg. of muscle-protein (this was probably the globulin of muscle and was prepared according to the directions of Castle *et al.* [1931], dried and powdered). The remaining fluid was heated to the boiling-point, cooled, and 4 ml. portions were similarly put up with protein as controls. All the tubes were incubated at 40° for 3 hours and gently agitated 3 or 4 times an hour. When incubation was complete 2 ml. of 25 % trichloroacetic acid were added to each tube, and the tubes after shaking were allowed to stand overnight. Finally, the contents of the tubes were filtered and the N in 4 ml. of the filtrates determined by the micro-Kjeldahl method, using $N/100$ acid. The results represent the differences between the N values of the two digests, calculated as for 100 ml. of gastric juice.

RESULTS.

In Table I our findings in a series of experiments on the gastric juice from normal and pernicious anaemia and other patients are set out. Where the samples of juice contained trypsin, as evidenced by digestion at p_H 8, the amounts of soluble N produced at p_H 6 have been corrected by subtraction of one-half the values obtained at p_H 8.

It will be seen that in all but two cases in the normal group, N amounting to 5–17 mg. per 100 ml. of juice was produced at p_H 6. There is no apparent reason

Table I. *Action of gastric juice on muscle-protein.*

mg. of soluble N per 100 ml. of juice. (Values at p_H 6 are corrected for trypsin.)			
	p_H 1-2	p_H 6	p_H 8
Normals	146	14	9
	227	17	34
	234	13	0
	164	17	11
	200	0	2
	151	7	1
	157	5	2
	174	7	6
	153	11	0
	192	5	0
	156	6	0
	155	11	0
	160	0	0
Pernicious anaemia	78	0	3
	128	0	5
	31	5	6
	30	4	14
	42	13	23
	24	0	5
	*203	1	0
	61	1	3
	24	0	8
Achlorhydric secondary anaemia	99	22	0
	61	4	0
	66	10	5
	—	6	2
Sub-acute combined degeneration of spinal cord	123	8	6

* Free HCl after histamine.

for the failure of two cases to show digestion, and no explanation is possible at this stage.

Turning now to the findings in the collection of cases of pernicious anaemia, with one exception, digestion at p_H 6 was very considerably less than in the normal group: comparison of the two groups clearly shows a difference in the behaviour of the anaemic and normal secretions in regard to the quantities of soluble N produced under the conditions of the experiments.

Of four cases of achlorhydric secondary anaemia, two conformed to the normal and two to the pernicious type.

The amount of N produced at p_H 6 bears no relation to the magnitude of either tryptic or peptic digestion. The juice in pernicious anaemia was weak in peptic activity under these conditions: this is in agreement with the findings of other workers using different substrates.

One case in the pernicious group was found to have free HCl in the gastric juice after histamine and peptic activity above the normal average. The occurrence of free HCl in the gastric secretion in this disease is extremely rare, and it is particularly interesting that in this case there should have been practically no evidence of activity by our method. The blood of the patient, a female, showed R.B.C., 1,400,000; Hb., 36 %; and C.I., 1.3.

In addition we have made some observations of a preliminary nature on the effect of p_H on the production of the soluble N. Samples of normal gastric juice

were allowed to act upon muscle-protein, in a buffered medium, at various reactions. Two curves are reproduced in Fig. 1. In the same figure are plotted curves for the action of pepsin and trypsin under similar conditions.

Fig. 1. Action of pepsin, trypsin and gastric juice on muscle-globulin.

Castle *et al.* [1931] found that commercial pepsin was devoid of the intrinsic factor: we assume that the behaviour of the enzyme preparations used by us (pepsin scales, B.P., and Armour's pancreatic extract) is in accordance with that of the gastric enzymes. It will be observed that just as the action of pepsin becomes negligible at p_H 3.5, trypsin ceases to produce any N from muscle-protein at about p_H 5.5, with the result that there exists a range from p_H 3.5 to 5.5 over which neither enzyme yields demonstrable amounts of N under the conditions of our experiments. In regard to gastric juice, however, it is clear that even at reactions within the range p_H 3.5–5.5 definite activity exists, and it would seem to be an advantage in future study to concentrate on this region and so eliminate altogether the participation of pepsin and trypsin.

At this stage we can do little more than record these findings in the hope that subsequent work will reveal their true significance.

We have had in mind the possibility that a bacterial agency might be responsible for the effects obtained. Control experiments in which toluene was

used to inhibit bacterial activity still gave positive results. Further, it is in the anaemias in which one finds a rich bacterial flora in the stomach owing to the absence of free acid that the low results were obtained.

If, as is possible, the nitrogenous substance produced in our experiments during digestion of muscle-protein by gastric juice near the neutral point is related to the haematopoietic principle of Castle, then we have demonstrated in a direct manner the relative absence of the intrinsic factor from the gastric secretion in pernicious anaemia. In consideration of the finding of a certain degree of activity among the anaemias, it must be remembered that even if the gastric juice in pernicious anaemia had all the characteristics of normal juice it is highly probable that its normal functions would be disturbed by a deficiency of secretion, the amount of gastric secretion being so small in this disease. When the secretion is not only scanty in amount but poor in quality it can easily be understood that serious impairment of its function may result.

In regard to the findings in achlorhydric secondary anaemia, it is of much interest, in view of the possibility of a transition from this condition to true pernicious anaemia, that some cases show little activity.

We hope that continuation of this work with improved methods of investigation will lead to a fuller understanding of the significance of Castle's observations and of the rôle of gastric secretion in the workings of the body.

SUMMARY.

1. Small amounts of soluble N are produced when normal gastric juice is incubated with beef muscle-protein at p_H 6.
2. The amounts of this N produced by the gastric secretion from cases of pernicious anaemia were found to be diminished.
3. In the few cases of achlorhydric secondary anaemia studied the results were variable.
4. Pepsin and trypsin play no part in the process.
5. It is possible that the intrinsic factor (Castle) of the gastric juice is concerned in the production of the nitrogenous substance.

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