A STUDY ON PROTEIN HYDROLYSATE WITH REFERENCE TO HISTAMINE AND HISTAMINE-LIKE SUBSTANCES. PART I. EFFECT ON DIFFERENT BIOLOGICAL SYSTEMS

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In the course of antigenicity tests of different batches of protein hydrolysate according to Dale's original technique it was observed in our laboratory that isolated non-sensitized normal virgin guinea-pig's uterus frequently contracts when brought in contact with protein hydrolysate of meat. Considering that the plain muscles of guinea-pig uterus also respond to histamine, a substance likely to be liberated from the degradation of tissues, it was considered worth while to study in detail the reaction brought about by protein hydrolysate in different biological systems in comparison with histamine.

Experimental

Action on uterus.—This was studied both on isolated virgin guinea-pig uterus and rat uterus. The uterii were mounted in a 50 c.c. organ bath containing oxygenated Ringer-Locke solution. The temperature of the bath was kept at 37·5°C. for guinea-pig uterus, and at 32°C. for rat uterus. Doses were given at regular intervals of time.

Action on isolated pieces of guinea-pig ileum.—The intestines were mounted in a 10 c.c. organ bath containing Tyrode solution at 38°C. For neutralization of choline-esters, atropinization was done at a concentration of 10^{-7}, each time before a test substance was put into the bath. For histamine inactivation pyribenzamine 5–10 μg. was used. Variation of calcium concentration was made, whenever necessary, to test the response at different concentrations of calcium.

Blood pressure in the cat.—For circulatory studies, chloralosed cats were put up (80 mg. per kg. intramuscularly). Comparison of the responses has been made with pure histamine diphosphate. For noting the effect of transfusion a dose of 10 c.c. per kg. was used, the rate of infusion being kept constant at 0·5 c.c. per minute. For detection of vasodepressor action, small doses were injected rapidly.

Protein hydrolysates from meat used for the study were prepared in three batches, carefully following the instructions given in the Indian Pharmacopoeial List (1946). Two batches of casein acid hydrolysate, similarly prepared, were also studied.

Results

Action on guinea-pig uterus.—The tests performed on isolated virgin guinea-pig uterii showed a high order of muscle-stimulating activity of protein hydrolysate. In some experiments even values similar to 10 μg. of histamine per c.c. were obtained (Fig. I). The presence of such a high quantity was considered alarming particularly when a chemical assay showed the presence of considerable quantity of substances in protein hydrolysate giving Pauly diazo reaction like histamine (Ganguly, 1954), varying from 10–14 μg. per c.c.

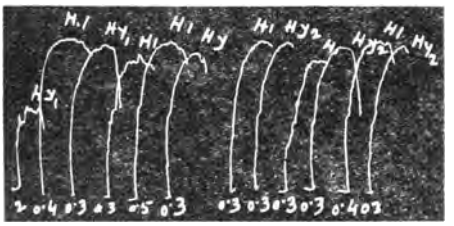

FIG. I

Guinea-pig uterus showing comparison of protein hydrolysate with histamine.
HI = Histamine solution (10 μg./c.c.).
Hy = Protein hydrolysate (two samples).

Action on blood pressure.—In order to test whether the stimulating factors had any depressor effect, experiments were put up with chloralosed cats for intravenous transfusion. It was found that rapid intravenous injections of small quantities of protein hydrolysate produced a depressor response similar to that given by histamine, which, though not so high as that shown by the guinea-pig uterus method, was still significant (Fig. II). Considering that a protein hydrolysate containing

FIG. II

Chloralosed cat (male), 2·7 kg., showing the depressor activity of three batches of protein hydrolysate (meat).
His = Histamine.
Hyd = Protein hydrolysate.

such a high amount of histamine-like substance might give rise to a transfusion shock, a preparation of protein hydrolysate, which had shown previous stimulation of guinea-pig uterus and depressor response in cat, was transfused in large quantity (10 c.c. per kg.) and at a slow rate into an adult anaesthetized cat with normal blood pressure. Instead of a fall of blood pressure, the transfusion was without effect on either the blood pressure or the rate of heart beat. Histamine in dose of 2 μg. was however enough to lower the blood pressure. Further transfusions made on cats after lowering the blood pressure by repeated injections of histamine and/or by carbachol showed a satisfactory recuperation of the hypotensive state. Instead of any vasomotor shock, the blood pressure of the animal began to increase slowly and the heart, which was profoundly depressed by the histamine or carbachol injections, recovered its tone and began beating more forcefully (Fig. III). Transfusion of histamine diphosphate solution (5 μg./c.c.) and that of protein hydrolysate containing added histamine (5 μg./c.c.) however caused a gradual fall of blood pressure and a steady lowering of vasomotor tone. A transfusion of histamine solution (0·5 μg./c.c.) or of protein hydrolysate containing 0·5 μg./c.c. of histamine was without any significant effect.

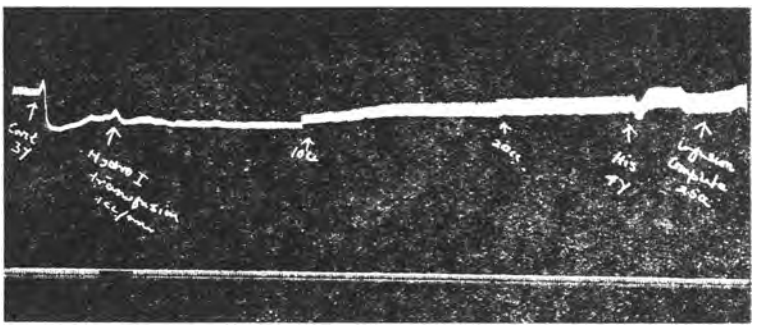

FIG. III.

Cat I. Chloralosed (male).

Shows the increase in vasomotor tone with transfusion of protein hydrolysate after carbachol depression.

Carb = Carbachol.

Action on isolated ileum.—The results of the experiments on cat blood pressure made it necessary to determine the histamine content of protein hydrolysate on isolated guinea-pig's ileum, as this method is considered more specific for histamine. The ileum was suspended in various modifications of Tyrode solution, with normal or diminished concentration of calcium, with or without atropine and/or pyribenzamine. In several batches the spasmogenic activity as detected without atropinization was found to vary from 0·1 to 0·5 μg. of histamine per c.c. It is significant to note that casein hydrolysate also showed similar presence of histamine-like substances. Since liberation of acetylcholine might be a factor in causing contraction of non-atropinized ileum, the presence of histamine was further checked by full atropinization of the ileum preparation, which became non-sensitive to carbachol, but responded to both histamine and protein hydrolysate (Fig. IV). Pyribenzamine

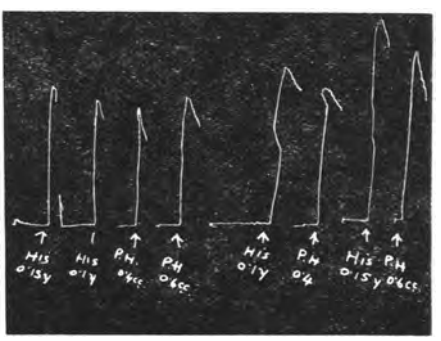

FIG. IV

Atropinized guinea-pig ileum in Tyrode solution.

His = Histamine.

P.H. = Protein hydrolysate.

in 5–10 μg. doses was able to neutralize fully the effect of both histamine and protein hydrolysate (Fig. V). Considering the known biological behaviour of histamine, it would be justifiable to infer from these experiments that the muscle-stimulating factor in protein hydrolysate, however small it may be, is likely to be

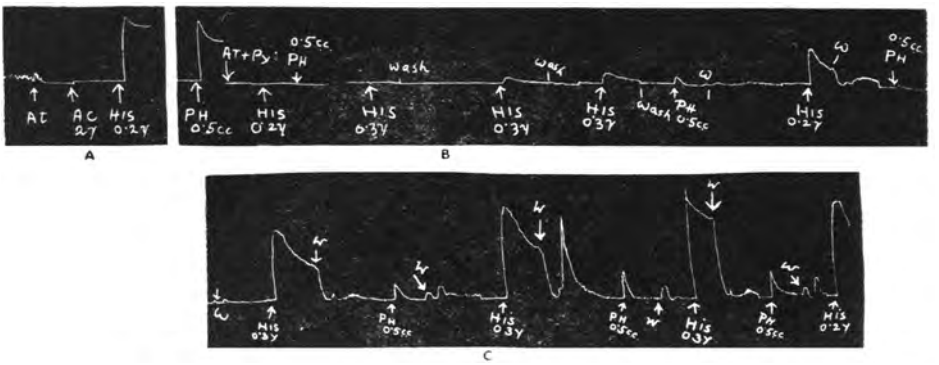

FIG. V

Atropinized (10^{-7}) guinea-pig ileum. A shows the antagonism of acetylcholine (AC) by atropine (At), but non-antagonism of histamine (His) action. B and C are continuous records, showing the differential return of response to histamine and protein hydrolysate, after contact with atropine + pyribenzamine (AT + Py).

W = Wash.

similar to histamine. But the results of work with ileum mounted in low-calcium Tyrode do not warrant such a sweeping conclusion (*vide infra*).

Isolated ileum in low-calcium Tyrode.—It is known that lowering the calcium content of Tyrode makes the ileum less sensitive to stimulation (Gaddum, Peart, and Vogt, 1949; Dalgliesh, Toh, and Work, 1953). Further experiments with $\frac{1}{2}$ calcium and $\frac{1}{4}$ calcium showed that preparations of protein hydrolysate, which contained originally a definite amount of histamine-like substance in comparison with histamine (not less than 0·2 μg. per c.c.), was showing a very low response in calcium-deficient medium (Fig. VI). It appears likely from these experiments

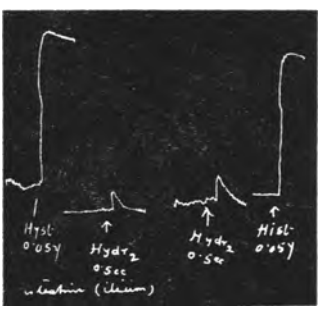

FIG. VI

Guinea-pig ileum in Tyrode with $\frac{1}{4}$ calcium. Shows negligible activity of protein hydrolysate (Hydr).

Hist = Histamine.

that most of the contents of the plain muscle-stimulating factors present in protein hydrolysate might be different from true histamine.

This suggestion of qualitative difference of the histamine-like substances from histamine also receives additional support from the observation with pyribenzamine. It is found that after contact with a mixture of atropine and pyribenzamine for some time and then washing out, the sensitiveness of the ileum preparation returns towards histamine more rapidly than that towards the histamine-like

substances of protein hydrolysate (Fig. V), though both were given in equivalent or near equivalent dosage in term of histamine.

Results on rat uterus.—Rat uterus has been reported by Gaddum to be non-responsive to histamine (Gaddum and Hameed, 1954). Experiments were, therefore, put up with rat uterus on the hypothesis that if the plain muscle-stimulating factor of protein hydrolysate be histamine, it will fail to stimulate the rat uterus. Contrary to this expectation it was found that while histamine showed negligible stimulation of the rat uterus, several batches of protein hydrolysate indicated the presence of factors causing powerful stimulation of the rat uterus (Fig. VII).

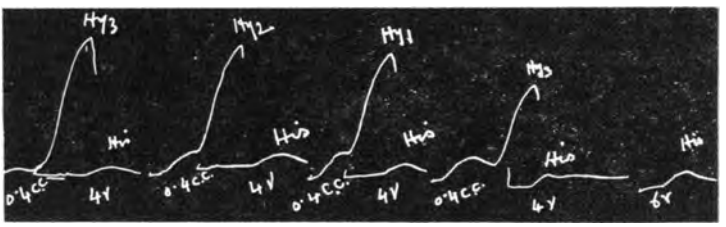

FIG. VII

Rat uterus in Ringer-Locke solution. Shows the selective stimulating property of three batches protein hydrolysate (Hy) compared to histamine (His).
Figures His in μg.
Hy in c.c.

This finding also suggests that the plain muscle-stimulating properties of protein hydrolysate, particularly the factors which stimulate the uterine muscles, may be due to substances other than histamine itself.

DISCUSSION

From the evidence so far presented, it appears reasonable to assume the presence of some definite plain muscle-stimulating factors in protein hydrolysate in fairly large quantities. Though the guinea-pig ileum method suggests that a part of such activity might be due to histamine, yet it could neither account for t he high spasmogenic activity on both guinea-pig and rat uterus, nor could it explain the vasodepression with small doses. Infusion of small quantities at a rapid rate to anaesthetized cats with high blood pressure, shows a typical depressor response like a large dose of histamine; but that the product does not really contain histamine to a significant amount can be judged by observing the results of the transfusion of a large amount of the same substance. The question of explaining the vasodepressor action of small doses of protein hydrolysate will, therefore, have to be related to factors other than histamine. In a muscle extract, various metabolites are present which can effect capillary dilatation. From large scale transfusion experiments it also appears reasonable to infer that histamine even if it is present in traces as low as 0·1–0·5 μg. per c.c. is likely to be tolerated in any transfusion of protein hydrolysate.

But the main question, arising out of the findings of these experiments, is related to the nature of the plain muscle-stimulating factors present in protein hydrolysate, particularly the factor causing stimulation of rat uterus and the differential behaviour towards pyribenzamine. Apart from histamine, a plain muscle can be stimulated by other tissue metabolites, such as acetylcholine, adenosine triphosphate, creatin phosphate, adenylic acid, inosinic acid, tryptamine or 5-hydroxytryptamine. It remains to be seen which of these factors will remain stable in an

hydrolysate of muscle protein, after the drastic treatments required for its preparation. A recent paper (Gaddum and Hameed, 1954) has shown that 5-hydroxy-tryptamine causes contractions of both the rat uterus and the guinea-pig uterus. The behaviour of protein hydrolysate towards the rat uterus, as noted in this paper, suggests similarity of action with that of 5-hydroxy-tryptamine. Further confirmation may be had if the activity is found to be neutralized by gramine (3-dimethyl-amino-methyl indole) which acts as a specific antagonist of 5-hydroxy-tryptamine (Gaddum and Hameed, 1954).

It thus appears that further work is necessary to characterize the plain muscle-stimulating factors liberated during protein hydrolysis. Whether done by acid or enzymic digestion it is quite possible that, in a carefully prepared protein hydrolysate from meat, these factors would be found predominant as stimulants of plain muscle than the much-feared substance, histamine. Whether these are connected in any way to adenosine which has vasodilator properties (Sexton, 1953) and adenylic acid system, or tryptamine and 5-hydroxy-tryptamine will have to be worked out. Work on these lines is in progress.

Summary

1. Hydrolysate of protein, particularly from muscle, contains considerable quantity of substances, which stimulate the plain muscles of the uterus and the intestine.

2. Rapid injections of very small doses of such hydrolysate produce a transient vaso-depression similar to histamine. Transfusion of large quantities to both normotensive and hypotensive cats however show no vasodepressive effect. On the contrary the maintenance of a slow and steady rising tone of the vasomotor system proves that majority of these factors are probably not histamine.

3. Experiments on atropinized guinea-pig ileum preparation suggest the presence of histamine in small quantity, which is neutralizable with an antihistaminic. But experiments with Tyrode solution of low-calcium content suggest that the majority of the histamine-like substances, as detected by the guinea-pig's ileum suspended in normal Tyrode solution, is probably not histamine.

4. Rat's uterus responds powerfully to protein hydrolysate, but not to histamine, thus showing that the plain muscle-stimulating factors are probably not histamine but may be similar to 5-hydroxy-tryptamine, or adenosine, or adenylic acid.

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