A STUDY ON PROTEIN HYDROLYSATE WITH REFERENCE TO HISTAMINE AND HISTAMINE-LIKE SUBSTANCES. PART II. DETECTION OF ANTIDIURETIC ACTION AND ITS COMPARISION WITH 5-HYDROXY TRYPTAMINE, PITUITARY POSTERIOR LOBE EXTRACT AND HISTAMINE.

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In modern therapeutics, transfusion of protein hydrolysate is used in various disorders, such as, malnutrition, hypoproteinemia, cirrhosis of liver, gastro-doudenal ulcers and anaemia. It is valuable for emergency transfusion in surgical shock, and as an aid to rapid convalescence after acute abdominal operations. Being a material for intravenous alimentation, considerable work has been done on its analysis and on the interaction between the different constituents (Basu, Sen and Bose 1946 ; Basu and Ray 1946 ; Ray 1949 ; Basu, Bose and Ray, 1950). Rigid tests for its preparation (Dyson and Bavin 1950) and standardization have also been laid down to eliminate the presence of undesirable substances (U.S.P. XV).

In the course of a pharmacological study on infusions of protein hydrolysate, Bose (1955) observed that considerable plain-muscle stimulating activity was associated with the preparation whether derived from meat or casein. While the activity resembled histamine in some respect, transfusion of large quantities of such hydrolysate did not produce any undesirable effect like that given by the infusion of a low strength solution of histamine. It was further found that, unlike histamine, rat uterus was powerfully stimulated by such hydrolysates from meat. Considering that enteramine or 5-hydroxy tryptamine (5-HT), a powerful stimulant of rat uterus, is likely to be liberated from the tissues during hydrolysis, it was thought to be of interest to make a comparative study of the responses with protein hydrolysate, enteramine, pituitary posterior lobe extract and histamine. A preliminary study suggested (Bose 1956) that protein hydrolysate from meat possesses some very powerful antidiuretic factor. The present paper is concerned with a more detailed and comparative study of protein hydrolysates from different sources with respect to anti-diuretic and other systemic actions.

MATERIALS

1. *Protein hydrolysate* :—The samples studied were prepared by enzymic hydrolysis of meat, using both papain and trypsin for digestion. The degree of hydrolysis lay between 50 to 55%, and the material was adjuvated with

glucose (5%) and sodium chloride (0.85%). The pH of the solutions varied between 6.7 to 6.9, and the nitrogen content was adjusted to 1%. The samples passed all the tests laid down in the Pharmacopoeia of India (1956).

2. *Casein hydrolysate* :— This was prepared by sulphuric acid digestion from three sources : (i) from freshly prepared casein ; (ii) from a variety of technical casein ; and (iii) by dissolving Difco's casamino acids. The strength in terms of nitrogen was adjusted to be kept the same as in protein hydrolysate.

3. *Histamine solution* :—Pure Histamine diphosphate (B.D.H.) was dissolved in sterile normal saline.

4. Grammine and pyribenzamine in fresh solutions were used to note the possible antagonism to 5-HT and histamine respectively.

METHODS

Anti-diuretic action :—This was studied on in-bred unselected albino rats, mostly male and weighing 200-300 g. The hydration procedure was similar to the method of Erspamer and Correale (1955) with slight modifications. It consisted in (1) water-rich food in the evening preceeding the day of experiment, (2) first preparatory water-load of 2.5 ml. per 100 g. of body weight in the morning, and watching for the first voiding of urine ; (3) and thereafter a second water load of 6 ml./100g. followed immediately by the subcutaneous injection of test materials. In some later experiments a heavy hydration procedure was adopted by feeding the animals water-soaked bread 24 hours prior to the experiment.

The amount of fluid injected per 100 g. body weight of the animal was kept constant by adjustment, which depended mainly on the total volume of protein hydrolysate to be administered. Hourly samples of urine were collected up to 5 hours from the time of the second water load. The rats were kept in individual all-glass metabolism cages. The percentage excretion was calculated on the basis of the total intake of fluid (quantity of second water load + the volume of the fluid injected), and the hourly output of urine. Each dose of a test solution was tested several times, and the average results plotted on graph paper.

Comparisons were made with meat hydrolysate, casein hydrolysate, enteramine picrate, serotonin, histamine and pituitary posterior lobe extract. Grammine and pyribenzamine were used as antagonistic agents. Sterile pyrogen-free distilled water was used in the controls.

Action on isolated guineapig ileum and on cat's blood pressure :—Studies were made according to the procedure already reported (Bose 1955). Chloralose in a dose of 0.11 g./kg. was used for anaesthetising the cats. Tyrode solution at 37.5°c. was used for the intestinal strips.

TABLE I

Showing the comparative effect of Protein hydrolysate and entermine on water diuresis. Each experim
based on the average from 3 rats.

Substance	Dose injected subcutaneously per 100g	Average percentage excretion of urine at hours. 1	2	3	4	
Protein hydrolysate	0·5 ml.	21.7±10·0	36·4±22·6	42·6±20·8	51·4±15·8	56·6
	1·0 „	28·5±10·3	43·1±2·4	52·0±3·3	96 8±6·9	58·3
	2·0 „	19·5±8·0	26.0±8·4	32·3±9·4	35·4±9·0	42·6
Enteramine picrate	0·375 mg.	31·2±6·5	61·5±3·5	70·8±2·5	73·6±6·5	81·0
	0·75 „	51·5±15·9	66·7±7	76·4±8·6	79·1±4·7	80·3
	1.50 „	30·0±5·9	52·5±4·3	60·9±3·6	67·7±3·7	71·3
	3·00 „	23·3±4·9	43·3±8·4	59·5±6·6	64·3±0·07	68·7
	6·00 „	24·3±17·4	32·4±21·3	50·4±24·8	61·9±10·6	67·4
Re-distilled water (Control)	1·0 ml.	29·5±16·6	57·3±14·5	75·6±23·8	83·6±21·7	89·9
	2·0 „	27·8±9·3	56·9±8·7	72·4±8·7	81·0±2·1	86·4

Mean values ± Standard deviation

RESULT

The results of all experiments on rat diuresis showing a comparison between protein hydrolysate and enteramine are summarised in Table I. Similar comparisons between enteramine and serotonin, the effect of casein hydrolysate and histamine are given in tables II, III & V. The influence of grammine on the anti-diuretic activity is recorded in Table III.

TABLE II

Comparison of Enteramine picrate and Serotonin on rat diuresis. Figures averages from 3 experiments.

Expt. No.	Snbstance	Dose injected	Percentage excretion at hours				
			1	2	3	4	5
I	Enteramine picrate	3.0 mg./kg.	19·8	31.3	54·8	64·3	68·3
	Serotonin (5 hydroxytryptamine creatinine complex)	3·0 mg./kg.	20·2	47·0	61·2	69·0	69·0
	Control (redistilled water)	20 ml./kg.	35·0	68·0	73·7	79·3	83·1
II	Entermine	6·0 mg./kg.	24·2	32·4	56·4	62·0	67·4
	Serotomin	6·0 mg./kg.	26·9	37·3	45·2	50·5	57·8
	Control (redistilled water)	20 ml./kg.	22·7	49·2	71·3	83·6	94·6

Action on rat diuresis

From the results of all experiments (Table I) it can be seen that protein hydrolysate exerts considerable anti-diuretic activity in rats, when injected parenterally. The activity of a dose of 20 ml. per kg. is remarkably greater than enteramine (5–hydroxytryptamine picrate) even at a dose of 6 mg/kg. As a matter of fact, the significant anti-diuretic effect of small doses of enteramine, as reported by Erspamer and Ottolenghi (1953) could not be elicited in any one of our experiments. That the substance (a picrate of 5–HT) did not differ from serotonin (a creatinine complex of 5–HT) in biological activity, could be found from a comparative study of both (Table II). At the dose level of 6 mg./kg., enteramine showed some noticeable effect particularly during the early period of diuresis. This confirms to some extent Erspamer's observation of early inhibition of diuresis. With heavy hydration procedure, however, the anti-diuresis could be increased, but not to the same extent as that of protein hydrolysate.

Grammine has been reported to be an antagonist of 5–hydroxytryptamine (Gaddum, 1954). But in the present investigation, it was found that grammine was unable to modify to any great extent the powerful anti-diuresis caused by protein hydrolysate (Table III). Rather, it tended to potentiate the effect.

TABLE III

Showing the effect of grammine on the anti-diuretic action of protein hydrolysate and 5-hydroxytryptamine.

Figures are based on the average from 3 experiments.

Expt. No.	Substance	Dose injected	Percentage excretion of urine at hours				
			1	2	3	4	5
I	Protein hydrolysate Batch 23855	20 ml./kg.	27	27·0	36·7	36·7	42·0
	5–hydroxytryptamine	6 mg./kg.	21·4	31·9	42·3	54·4	62·7
	Control (redistilled water)	20 ml./kg.	22·0	55·0	74·0	80·3	87·5
II	Protein hydrolysate+ Grammine	20 ml./kg. + 3 mg./kg.	9·4	14·2	19·8	32·1	32·1
	5–hydroxytryptamine+ Grammine	6 mg./kg. + 3 mg./kg.	2·3	2·3	25·4	32·3	46·6
	Control (redistilled water	20 ml./kg.	9·3	35·8	66·0	75·8	82·8

It was interesting to enquire whether histamine itself has anything to do with the causation of anti-diuresis, particularly when vaso-depression is associted with injection of protein hydrolysate. It was, however, found that inspite of heavy dosage (2 mg. per kg.) histamine did not cause any anti-diuretic effect (Fig. 1); and combination of histamine with the histamine-antagonist, pyribenzamine, was ineffective in modifying the action (Table IV). Pituitary (posterior lobe) extract, however, exerted a powerful and prolonged anti-diuresis. (Table IV).

TABLE IV

Showing the effect of histamine, histamine + pyribenzamine and pituitary posterior lobe extract on rat diuresis in comparison with Enteramine.

Heavy hydration procedure.

Substance	Dose injected	Percentage excretion (average of 3 rats)				
		1	2	3	4	5
Enteramine	0·75 mg./kg.	62.7	71·2	82·4	82·4	82·4
Histamine	2 mg./kg.	50·2	70·2	70·2	72·7	72·7
Histamine+Pyribenzamine	2 mg./kg. + 2 mg./kg.	58·8	67·7	81·1	81·8	81·1
Pituitary (Post. lobe) Extract	5 I.U./kg.	21·7	26·1	35·0	37·0	42·4
Control (redistilled water)	20 ml./kg.	25·1	60·6	77·8	79·4	100·6

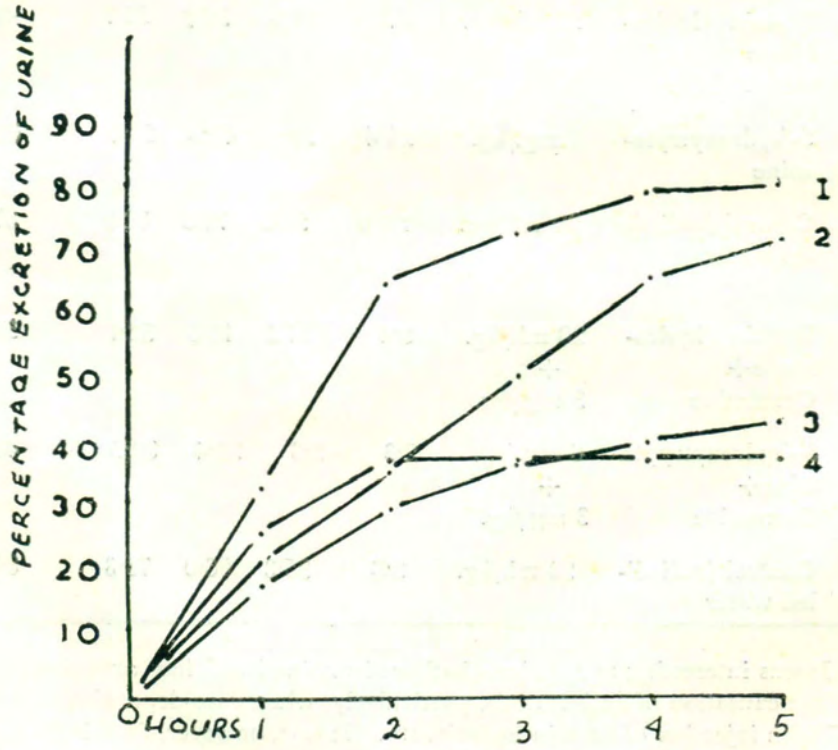


Figure I. Showing the comparative anti-diuretic effect of protein hydrolysate; enteramine picrate, and pituitary (posterior lobe) extract.

1 Control (re-distilled water) 20 ml. per kg.
2 Enteramine picrate 6 mg. per kg.
3 Protein hydrolysate 20 ml. per kg.
4 Pituitary (Post. lobe) Extract 2 I. U. per kg.

The anti-diuretic properties of protein hydrolysate was similarly associated with freshly prepared casein hydrolysate as well as solution of dehydrated hydrolysate of casein (casamino acid, Difco) suggesting thereby that possibly hydrolysis, and not the individual type of protein, was responsible for the production of such activity (Table V).

TABLE V

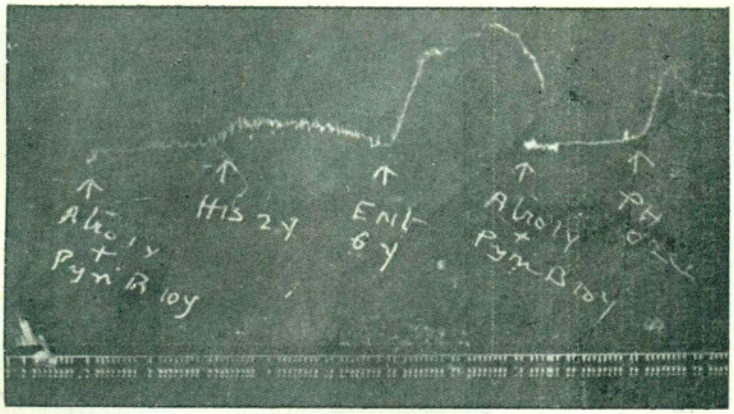
Showing the effect of casein hydrolysate (freshly prepared) Casamino acid solution (Difco)

Animals put under heavy hydration procedure.

Substance.	Dose injected ml./kg.	Percentage excretion (average from 6 rats) of urine at hours				
		1	2	3	4	5
Casein hydrolysate (from freshly prepd. material)	20	23·3	43·9	53·5	62·8	65·8
		13·9	32·0	33·8	33·8	33·8
Casamine acid (Difco) solution	20	0	10·5	10·5	12·9	27·0
Control (redistilled water)	20	38·0	66·7	83·1	86·1	105·3

Action on isolated ileum

Both enteramine and protein hydrolysate stimulated the intestine, even when atropinised (Curve 1.a). Pyribenzamine failed to antagonise the tone of both the substances, while neutralising completely the stimulation by histamine (Curve 1.b).

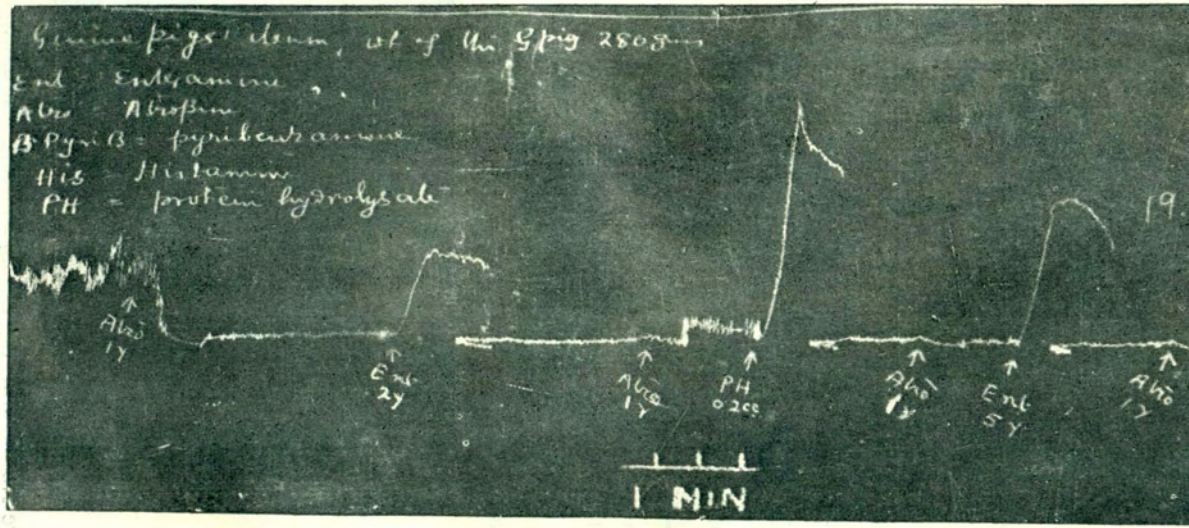


Curve 1.b—Guineapig ileum, showing stimulation by enteramine (Ent) and protein hydrolysate (PH), even after atropine (1μg.) and pyribenzamine (10μg.) in the bath. Histamine (His) action is antagonised.

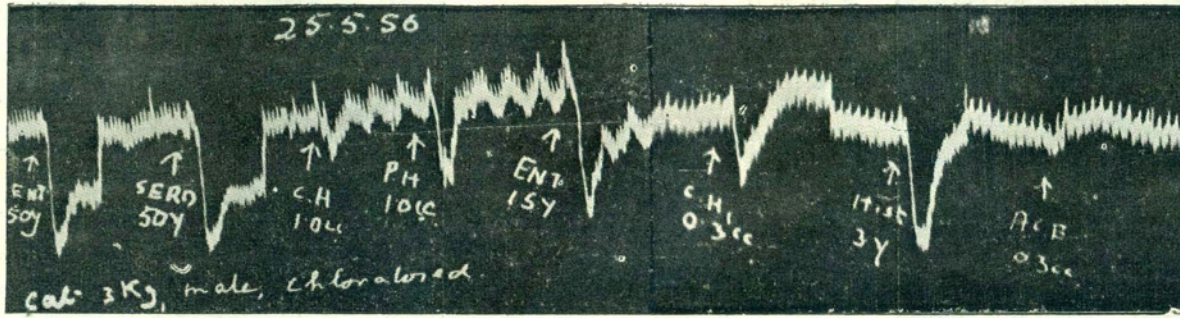
Action on blood pressure

In chloralosed cat with or without atropinisation, protein hydrolysate, casein hydrolysate, en solution of Difco Casamino acid gave typical hypotensive effects (Curve 2). Freshly prepared casei however, showed some pressure instead of a depressor response (Curve 3) and an acetone extract of p lysate failed to give the usual hypotensive effect (Curve 2). In some experiments, a biphasic type of r initial pressor followed by a depressor response) was noticed with both enteramine and protein particularly with the former. With heavy doses of enteramine, however, the response tended to be in effect (Curve 4).

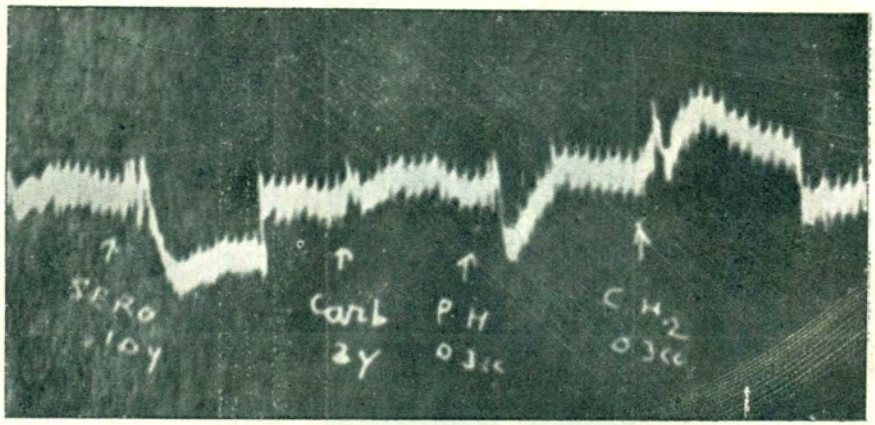
There was some difference in the type of depressor response, shown by enteramine and protein With the former, the depressor effect was more prolonged (Curves 2 & 3).



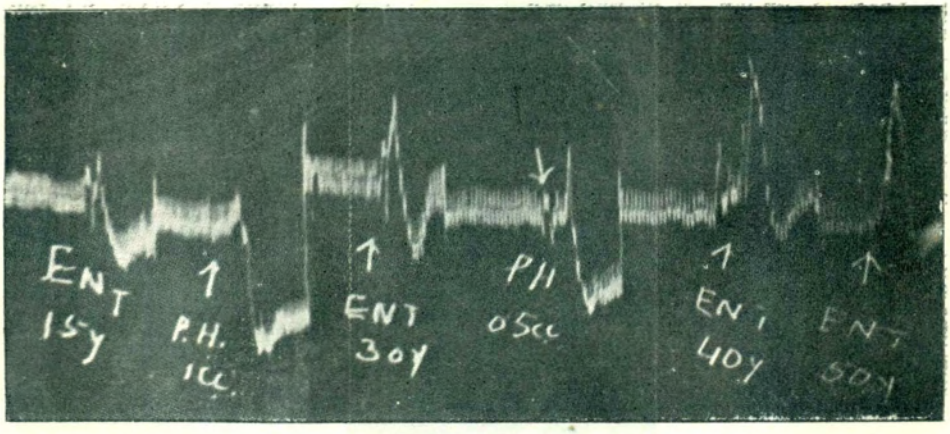
Curve 1a. Guineapig ileum (atropinised), showing stimulation by protein hydrolysate (PH) an (Ent). 0.2 ml of PH appears almost equivalent to 10 μg. of enteramine.



Curve 2.—Effect of enteramine (Ent), serotonin (SERO), casein hydrolysate, technical, (C.H1), prot
(PH) histamine (Hist), Difco casaminoacid (CAD) and an acetone extract of protein hydrolysate
blood pressure of a chloralosed cat. All show hypotensive effect, except ACE. The identical action
and serotonin is noteworthy.



Curve 3.—Records of blood pressure tracings of a chloralosed and atropinised cat (2.5 kg. male), showing no response with 2 g. carbachol (Carb), pressor response with 0.3 c.c. of freshly ppt. casein hydrolysate (CH2), and depressor response with protein hydrolysate (PH) and serotonin (SERO). Note the prolonged action of serotonin.



Curve 4.—Blood pressure tracings of chloralosed and atropinised male cat (2.8 kg), showing biphasic reaction with enteramine (Ent) and protein hydrolysate (PH), tending to be more pressor with heavier dosage of Enteramine.

DISCUSSION

The plain-muscle stimulating activity, the selective action on rat uterus, the effect on the blood pressure and isolated intestine, all suggested that the activity of protein hydrolysate might possibly be related to 5–hydroxy-tryptamine (5–HT). But the comparative tests on rat diuresis definitely show that hydrolysates of protein (meat or casein) possess a powerful anti-diuretic action, which cannot be explained on the basis of 5–HT or histamine contaminations. Even a large dose of histamine fails to produce any anti-diuretic effect, while the action of a heavy dose of enteramine (6 mg/kg.) is much lower than that of protein hydrolysate. It is particularly to be noted that grammine was unable to neutralise, to any extent, the powerful anti-diuretic action of protein hydrolysate. The findings on guineapig ileum or cat's blood pressure also fail to throw any discriminating light in this respect. But the fact that freshly prepared casein hydrolysate, though exerting significant anti-diuresis in rats, causes a pressor instead of a depressor response is worthy of note, and the observation needs further investigation. It is likely that the liberation of such an anti-diuretic factor takes place on account of some decomposition which may be associated with the use of technical casein, or meat stored for a long period. The presence of traces of extraneous matter in the form of remnants of enzyme does not seem to be of significance, since acid hydrolysates are equally active in exerting anti-diuresis. The liberation of polypeptides similar to pituitary posterior lobe principle also does not appear likely, considering that protein hydrolysate exerts a more depressor than pressor activity on the circulation.

Whatever may be the nature of the anti-diuretic substance in protein hydrolysate, it is certain that it is heat and time stable. It would therefore be interesting to isolate and purify the substance by chromatograhic methods and to see whether it has any relationship with substance P, substance R, (Gaddum, 1953), or Kalikrein (Werle and Berk, 1950). Work on these lines is in progress.

SUMMARY

1. A study of protein hydrolysate from different sources (meat, freshly precipitated casein, technical casein, and dehydrated casein hydrolysate) has been made in comparison with enteramine (5–hydroxy tryptamine picrate), serotonin (5–hydroxy tryptamine creatinine complex), histamine, and pituitary posterior lobe extract.

2. In all types of protein hydrolysate, a powerful anti-diuretic action in rats is noticed, which is much greater than that exerted by enteramine or serotonin, but appears to be similar to pituitary posterior lobe extract.

3. From the studies on blood pressure, isolated intestine, and rat diuresis, it appears that the powerful anti-diuretic activity of protein hydrolysate cannot be due to either 5–hydroxy tryptamine, histamine, or pituitary posterior lobe extract.

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REFERENCES

1. Basu, U. P., Sen, S. N. and Bose, A. N. (1946) : *Pharmaceutical J.*, **157**, 409.
2. Basu, U. P. and Ray, N. (1946) : *Ibid*, **157**, 151.
3. Basu, U. P., Bose, A. N. and Ray, N. (1950) : *Ibid*, **165**, 130.
4. Bose, A. N. (1955) : *Proc. Nat. Inst. Sci. (India)*, **21B**, 195.
5. Bose, A. N. (1956) : *Science & Culture*, **22**, 240.
6. Dyson, G. M. and Bavin, E. M. (1950) : *Chemistry & Industry*, p. 215.
7. Erspamer, U. and Correale, I. (1955) : *Arch. int. pharmacodyn.* **101**, 99.
8. Erspamer, U. and Ottolenghi. (1953) : *Arch. int. pharmacodyn.*, **93**, 177.
9. Gaddum, J. H. (1953) : *Brit. J. Pharmacol.*, **8**, 321.
10. Gaddum, J. H. (1954) : *Brit. J. Pharmacol.*, **9**, 240.
11. Pharmacopoeia of India (1955), p. 322.
12. Ray, N. (1949) : *Science & Culture*, **14**, 386.
13. United States Pharmacopoeia XV (1955): p. 591.