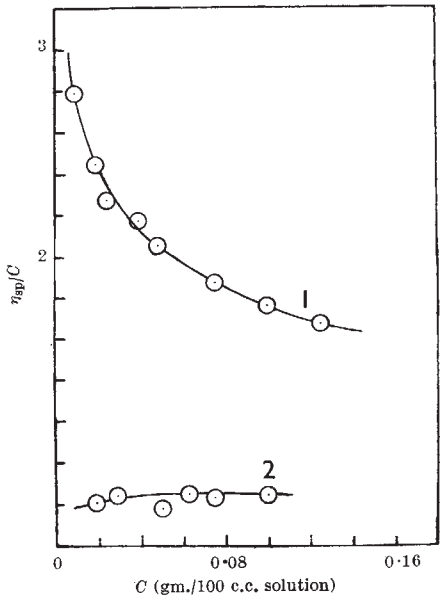
## Viscosity of Sodium Salt of Polyuronide Hemicellulose from Jute

It has been observed by Fuoss and Strauss[1] that the viscosity behaviour of a polymer such as polyvinylbutyl pyridonium bromide, containing ionizable groups, in water is not the same as that of a neutral polymer. The reduced viscosity ($\eta_{sp}/C$) of the former (a polyelectrolyte), unlike that of a neutral polymer, increases on dilution. A similar behaviour has been noted by Pals and Hermans[2] in the case of sodium pectinate. Basu[3] has studied the viscosity of sodium thymonucleate, which also behaves as a polyelectrolyte. The present communication describes the viscosity measurements of the sodium salt of methyluronic acid – xylan complex[4] (a jute hemicellulose fraction). As this uronic acid is believed to occur in a chain molecule, it was thought that the sodium salt of the complex in aqueous solution might also behave as a polyelectrolyte. This does not appear to have been studied hitherto.

The results of viscosity determinations are shown graphically; curve 1 shows that the reduced viscosity actually increases on dilution. In the presence of 0·1 *N* sodium chloride solution, the sodium salt behaves as a neutral polymer. These facts may be readily explained by the conception of polyelectrolytes put forward by Fuoss and Strauss[1]. Viscosities were measured in an Ostwald viscometer with an efflux time for water of about 165 sec. at 32° C.




Reduced viscosity of (1) sodium salt of methyluronic acid – xylan complex; (2) same in presence of sodium chloride

Defatted jute was delignified with 'Textone'[5]; the air-dry holocellulose thus obtained was treated with 9·3 per cent caustic soda solution (w/w) at room temperature for two hours and filtered through a sintered-glass filter. The filtrate was acidified with glacial acetic acid, and an equal volume of absolute ethyl alcohol was then added. The precipitate was filtered off and washed free from acetate with aqueous ethyl alcohol (1 : 1). The sodium salt of hemicellulose (acetic acid is too weak to decompose it) thus obtained was purified by dissolving in 4 per cent caustic soda solution at room temperature, filtering and washing the precipitate first with aqueous ethyl alcohol (50 per cent) and then with absolute alcohol. This was dried in a vacuum desiccator over concentrated sulphuric acid.

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## Biological Activity of some Enteramine-related Substances

The action of enteramine on various biological test objects has been established in previous investigations[1].

The present communication represents a preliminary contribution to the comparative study of the actions developed, on some of the most important and significant biological reactives of enteramine, by a number of enteramine-related indole derivatives.

The results obtained with indol- and hydroxyindolalkylamines on the diuresis of hydrated rats (antidiuretic action), on the blood pressure of the spinal cat (hypertensive action), as well as on the isolated œstrus-uterus of the rat, and the *in situ* urinary bladder of the dog (stimulant action), are summarized in the accompanying table.

The activity of enteramine is arbitrarily taken as 100; the activity of the other substances is expressed in percentage.

| | Hydrated rats' diuresis | Rat œstrus-uterus | Dog urinary bladder | Spinal cat blood pressure |
|---|---|---|---|---|
| Enteramine | 100 | 100 | 100 | 100 |
| Bufotenine | 6–7 | 10 | 5–10 | 50 |
| Bufotenidine | <0·3 | 0 | ? | 400–600 |
| Bufothionine | 0 | 0 | 0 | 0 |
| Dehydrobufotenine | 0 | 0 | 0 | 0 |
| Tryptamine | 0·3–0·5 | 1–1·5 | 1·5 | 12–16 |
| 1-Methyltryptamine | 0·1–0·2 | 1–3 | 1–1·5 | 10–12 |
| N-Methyltryptamine | <0·3 | <0·5 | <0·5 | 10 |
| N,N-Dimethyltryptamine | <0·3 | <0·5 | <0·5 | 6–8 |
| 5-Methoxytryptamine | 25–40 | 25–30 | 30–35 | 30–50 |
| Adrenaline | diuretic action | inhibition | inhibition | 2,000–3,000 |

The following compounds have proved wholly inactive on all the above biological reactives: indole, skatole, 5-methoxyindole, β-(indole-3)-acetic acid, β-(indole-3)-propionic acid, γ-(indole-3)-*n*-butyric acid, tryptophane, tryptophanol, hypophorine.

From the data in the table and from the study of numerous records, the following conclusions may be drawn.

(*a*) Among the indolalkylamines hitherto tested, enteramine possesses the most powerful action on the diuresis and the uterus of the rat, and on the urinary bladder of the dog. Moreover, the biological reactives are in no way damaged by the substance, as shown by the easily repeatable pharmacological response, and, when working with isolated organs,

by the prompt reversibility of this response, however intense it may be, after washing out.

This by no means occurs with bufotenine or with the methyltryptamines, which, on the contrary, may greatly damage the smooth muscle.

(*b*) 5-Methoxytryptamine is the product which most closely approaches to enteramine, both qualitatively and quantitatively; bufotenine (= N,N-dimethylenteramine) does it to a much lesser degree.

It seems, therefore, that for the appearance of the specific antidiuretic and uterus-stimulating actions the peculiar structure of the lateral chain of enteramine (chain with two carbon atoms, with a primary amino-group attached to the terminal carbon atom) is as important as the presence of a free phenolic hydroxy group in position 5.

(*c*) A satisfactory agreement exists between the antidiuretic action of the different substances and their uterus- and bladder-stimulating action. No relationship exists, on the contrary, between the antidiuretic action and the hypertensive action in the spinal cat.

I wish to thank Prof. R. H. Manske for the methyltryptamines.

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## A Bacterial Growth-Factor Synthesized by a Soil Bacterium

EARLIER studies in this laboratory[1] have indicated the presence in soil of bacteria dependent upon growth-factors provided by soil extract but not by yeast extract nor by combinations of amino-acids and vitamins (not including $B_{12}$). More recently it was shown[2,3] that for many of the indigenous soil bacteria dependent upon essential growth-factors supplied by soil extract, the nutrilite effect of the latter could be replaced by vitamin $B_{12}$. Other micro-organisms, however, remained dependent upon unidentified growth-promoting substances in soil.

In a study of the growth requirements of certain bacterial strains for the nutrition of which vitamin $B_{12}$ was unable to replace soil extract, it was found that a number of bacteria isolated from soil and having simple nutritional needs were able to synthesize a factor nutritionally equivalent to soil extract. Using a selected test organism requiring the growth-factor (No. 88) and one capable of synthesizing it (No. *B*89), both pleomorphic bacteria of the 'soil diphtheroid' type and apparently undescribed species, more detailed study was made. With a basal medium of inorganic salts, sugar and yeast extract ('Difco'), the test organism gave no response to the addition of any of the following: thiamine, riboflavin, niacin, pyridoxin, pyridoxal, pyridoxamine, pantothenic acid, biotin, folic acid, vitamin $B_{12}$, *p*-aminobenzoic acid, inositol, choline, combinations of B vitamins, cytosine, thymine, uracil, adenine, guanine, xanthine, cystine, β-alanine, ribonucleic acid, deoxyribonucleic acid, oleic acid, tomato juice filtrate, casamino-acids, enzymatic casein digest and soil extract ash. On the other hand, growth resulted from additions of small amounts of the culture filtrate of organism No. *B*89, grown in a simple nutrient medium containing only inorganic salts and sugar (see accompanying table). Partially purified material from the metabolic liquid

GROWTH RESPONSE OF ORGANISM NO. 88 (WASHED CELLS)

| Addenda to basal medium* | Optical density (aver. of 4 tests) |
|---|---|
| None | 0·005 |
| Soil extract | 0·132 |
| B vitamins, purines and pyrimidines | 0·005 |
| 0·00001 ml. culture filtrate *B*89/ml. | 0·007 |
| 0·00002 ,, ,, ,, ,, | 0·014 |
| 0·00003 ,, ,, ,, ,, | 0·044 |
| 0·00004 ,, ,, ,, ,, | 0·083 |
| 0·00005 ,, ,, ,, ,, | 0·105 |
| 0·00007 ,, ,, ,, ,, | 0·149 |
| 0·00010 ,, ,, ,, ,, | 0·180 |
| 0·001 ,, ,, ,, ,, | 0·196 |
| Liver extract ( 0·37 mμ gm. $B_{12}$/ml.) | 0·007 |
| ,, ,, ( 3·75 ,, ,, ) | 0·075 |
| ,, ,, (37·5 ,, ,, ) | 0·228 |
| ,, ,, (75·0 ,, ,, ) | 0·194 |
| Crystalline vitamin $B_{12}$, 0·37 mμ gm./ml. | 0·003 |
| ,, ,, 3·75 ,, | 0·003 |
| ,, ,, 37·5 ,, | 0·004 |
| ,, ,, 75·0 ,, | 0·003 |

* Salts, glucose and yeast extract.

gave observable growth response at approximately 0·1 p.p.m. concentration.

It was further found that a growth-factor effect equivalent to that given by filtrate *B*89 was obtained on addition of injectable "15 unit" liver extract. The effect was not attributable to vitamin $B_{12}$ as indicated in the table, showing results with different amounts of liver extract and equivalent concentrations of crystalline vitamin $B_{12}$. Growth stimulation of *Lactobacillus leichmannii* by a factor in liver extract which promotes growth in excess of that attributable to vitamin $B_{12}$ has been reported[4], while concentrates from liver have been found[5] to contain a factor required by *Leuconostoc citrovorum* which appears to be identical with 'folinic acid'[6]. Since these factors were reported to be present in yeast or yeast extract, there is reason for assuming that the factor produced by organism *B*89 is a different substance. This latter is believed to represent a nutrilite required for the growth of a group of indigenous soil bacteria and to contribute to the growth-promoting properties of soil extract. It is considered a matter of interest that it is nutritionally equivalent (for the test bacterium) to a factor in liver extract and that it is a product of microbial synthesis.

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## Inhibitors of Hyaluronidase

AN unsolved problem in the study of rheumatic diseases is whether or not hyaluronidase plays any part in the typical changes in the connective tissue, particularly of the interfibrillar cement substance, which is believed to contain hyaluronic acid. Guerra[1], for example, claimed that sodium salicylate significantly inhibits the spreading power of hyaluronidase injected into the skin, and therefore ascribed the value of salicylate in rheumatic fever to an action of the drug on the hyaluronidase–hyaluronic