On the presence of beta-imidazolethylamine in the intestinal wall : with a method of isolating a bacillus from the alimentary canal which converts histidine into this substance / by Edward Mellanby and F.W. Twort.

Contributors

Mellanby, Edward, Sir, 1884-
Twort, Frederick William, 1877-1950.

Publication/Creation

[Place of publication not identified] : [publisher not identified], [1912?]

Persistent URL

https://wellcomecollection.org/works/c62fj4a8

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

[*Reprinted from the Journal of Physiology,*
Vol. XLV. Nos. 1 & 2, *August* 2, 1912.]

ON THE PRESENCE OF β-IMIDAZOLETHYLAMINE IN THE INTESTINAL WALL; WITH A METHOD OF ISOLATING A BACILLUS FROM THE ALIMENTARY CANAL WHICH CONVERTS HISTIDINE INTO THIS SUBSTANCE.

By EDWARD MELLANBY (*Beit Memorial Research Fellow*), AND F. W. TWORT (*Superintendent of the Brown Institution, London University*).

(*From the Physiological Laboratory of St Thomas's Hospital, and the Brown Institution, London University.*)

β-IMIDAZOLETHYLAMINE, the base produced by splitting off carbon dioxide from histidine, has recently become of great interest, in the first place, because of its potent physiological properties, and secondly, because of its peculiar distribution in nature. Barger and Dale(1) showed in their investigations on the physiological action of ergot that, of the various substances isolated which cause contraction of the uterus, this histidine base is by far the most powerful. Kutscher(2), at the same time and independently, isolated a substance from ergot having chemical and physiological properties almost identical with those of β-imidazolethylamine.

A further point of interest is that Barger and Dale(3) have isolated this substance from the mucous membrane of ox small intestine. They were led to make this investigation by the similarity in physiological action between β-imidazolethylamine and the hypothetical substance called by Popielski(4) "vaso-dilatin." This substance was supposed to be accountable for the marked depressant effect produced by extracts of tissues, particularly extracts of the intestinal mucosa, when injected into animals. Such extracts also produce a characteristic contraction of the uterus. In consequence of the isolation of the base from the intestinal wall by Barger and Dale, it may be assumed that this substance is largely responsible for the physiological properties of extracts of the mucous membrane of the alimentary canal.

Barger and Dale do not account for the appearance of β-imidazolethylamine in the intestinal mucous membrane and point to the probability that it is a normal product of the mucosa. On the other hand the possibility that it is present as a result of bacterial decomposition has always been prominent, particularly because Ackermann(5) has been able to obtain large quantities of the base by allowing fluids containing histidine to undergo putrefaction.

A short time ago we published work(6) describing methods for isolating creatin-destroying micro-organisms from the alimentary canal. We have extended the general principle of one of these methods to the present research. The general principle is as follows: in the first place the conditions are determined under which a mixture of intestinal bacilli will carry out the definite chemical change required. When the bacilli producing the change are at their maximum growth relative to the remaining micro-organisms, sub-cultures are made on fresh media so constituted as to eliminate as far as possible the unsuitable micro-organisms and at the same time raise the percentage of the active bacilli. The mixed culture is if necessary carried through a series of media, some of which are simplified, while others contain additional substances. The chemical compound to be split up is present in all the media, for not only does it give the active micro-organisms a better chance to grow and survive, but without its presence in the medium it is impossible to know whether or not any micro-organisms producing the change are present. When, after repeated sub-culturing, a mixture is obtained which contains only a few varieties of micro-organisms and the desired bacillus in sufficient numbers, plate cultivations are made and the bacteria capable of effecting the specific chemical action obtained in pure colonies.

The details of the process that we used will now be described. In order to test the media for the presence of the histidine base we used the method of Kehrer(7) which consists of testing its action on the isolated uterus suspended in oxygenated Ringer's solution. We used the uterus of a virgin guinea-pig as recommended by Dale and Laidlaw(8). As pointed out by these workers the action of β-I. on the isolated uterus is most delicate, for a solution containing 1 in 5,000,000, as a rule, has a marked pressor effect. It is possible also by using such a physiological method to obtain a good idea of the amount of base present in the solution, by comparing its action with one of known strength.

If a minute quantity of fæces be inoculated into tubes of medium

consisting of ordinary peptone broth containing 1 % of histidine and incubated at 37° C. either ærobically or anærobically, the histidine base can be demonstrated to have been produced. From one such tube grown anærobically for seven days we sub-cultured into fresh tubes of the same medium, and grew anærobically at 37° C. and 45° C.

The tube grown at 37° C. contained β-I. after four days, but in that grown at 45° C. none of the base was produced. The chief interest of this observation is that it almost negatives the idea which we had held possible, that the bacillus we had already isolated from the intestine capable of destroying creatin, or an allied micro-organism, was also responsible for the production of β-imidazolethylamine from histidine. The creatin-destroying bacillus belonged to the large gram positive group of anærobes and carried out its action very effectively at 45° C. In the histidine tube grown at 45° C. similar large bacilli were present in sufficient numbers to carry out any chemical change they might be capable of producing, and since none of the histidine base was produced even in fresh tubes containing meat to encourage growth of the anærobes we concluded that probably quite a different type was responsible for the production of the histidine base.

The tube grown at 37° C. which contained the base was sub-cultured after five days' incubation on to fresh tubes of the same medium and as before grown anærobically at 37° C. This was the third generation of cultures and β-I. was produced in three days. We now prepared a simple salt medium, using one of Naegeli's formulæ with the addition of histidine, and having the subjoined constitution:

Water	100 grm.
Ammonium Tartrate	1 ,,
Di-potassium Phosphate	0·1 ,,
Crystallised Magnesium Sulphate ...	0·02 ,,
Calcium Chloride	0·01 ,,
Histidine	1 ,,

A tube of this medium was inoculated from our mixed culture of the third generation and the tube incubated anærobically at 37° C. This tube of medium was markedly alkaline after five days and minute quantities caused tonic contraction of a guinea-pig's uterus, thus showing the presence of the base. Films from the culture showed only gram negative bacilli and streptococci, all the gram positive bacilli had been completely eliminated, and the fact that the histidine base was formed finally disproved the possibility that the large creatin-destroying bacillus or any allied micro-organism was responsible for the production of the

histidine base. On plating out from this tube on ordinary agar we obtained colonies of two types of colon bacilli and a few colonies of streptococci. A number of colonies of each were tested on tubes of Naegeli histidine medium and in all the cultures taken from one type of colon bacillus the histidine was changed to β-imidazolethylamine. The bacillus proved to belong to the typhoid-coli group and shows the following characters: it is a small bacillus with rounded ends, non-motile and gram negative. It will grow ærobically or anærobically on the ordinary laboratory media. The optimum temperature is about 37° C. The growth on gelatine, agar and broth is similar to that of bacillus coli. Milk is clotted, and no liquefaction of gelatine takes place. Acid and gas are produced in media containing glucose, lactose, or dulcite.

The bacterial method of preparing β-imidazolethylamine from histidine.

The histidine base has such marked ecbolic properties that it seemed worth while to define the conditions under which the best yield could be produced by the bacillus.

From the first we never had any difficulty in causing a complete production of the base so long as we worked in test-tube quantities. When, however, we inoculated large volumes of the salt medium containing histidine, the destruction of the histidine was only partial. The explanation of this is that the salt medium can afford only a minimum of nutriment to the bacilli which are growing in it. Consequently the bacilli can only divide a limited number of times, and, as soon as they have used up any reserve material they may contain, multiplication and activity cease. In order, therefore, to get a large quantity of histidine base formed it is necessary

(1) To inoculate the solution with a most vigorously growing culture of bacilli.

(2) To inoculate a corresponding large number of bacilli when large quantities of medium are used.

As regards the first point, we made experiments to ascertain the best nutrient material on which to grow the bacilli, before inoculating into the salt-histidine medium, so as to allow of a better and more rapid growth in the latter. We found that the best medium was ordinary glycerine-agar and that the culture should not be more than 24 hours old.

Another point to be mentioned is that the bacillus at first grows

well on ordinary Ringer's solution. Even in redistilled water to which histidine has been added the bacillus grows well for a time, and large quantities of β-I. are produced. The growth is not so good in distilled water as in Ringer's solution.

The production of the base is certainly greater if the histidine solution be more dilute. For instance we got larger yields of the base when 0·1 % or ·05 % solutions of the histidine were inoculated with the bacillus than when we used a 1 % solution of histidine in Ringer. Besides estimating the quantity of base present in a solution after bacterial action by its action on the isolated uterus, we prepared the dipicrate of the base by the ordinary method. The bacilli are filtered off through kieselgühr and the solution neutralised and evaporated to dryness. This residue is extracted with methyl alcohol which is in turn evaporated off. The base is then crystallised out from a watery solution as the dipicrate.

For a good yield of β-I. from histidine, it is well then

(1) To use a 0·1 % solution of histidine in Ringer's solution. The solution must not be acid before inoculation.

(2) To inoculate with the special bacillus taken from a glycerine-agar medium of not more than 24 hours' growth. The growth is washed off the surface of the medium with a little normal saline and inoculated into the histidine medium with a sterile pipette. By this means a small amount of nutriment from the agar is also inoculated into the medium. The growth from two agar tubes should be used for every litre of 0·1 % histidine solution.

(3) To allow a week's incubation.

The effect of acidity on the decomposition of histidine.

Further experiments showed that, although the bacillus will grow quite well in an acid medium yet, under these circumstances, no β-imidazolethylamine is produced from histidine. This can be easily proved either by inoculating a slightly acid medium containing histidine or by adding glucose to the medium. In the latter case the acid produced by the action of the bacillus on the sugar also prevents the production of the base. When using a salt solution as a medium, this is especially important, as there is not in this case the same production of alkaline bodies as in the case of peptone broth.

This question of acidity may have an important bearing, in view of the attention which is being paid, at the present time, to the question

of the production of harmful substances in the intestine by bacterial action. For if one may judge by the toxic effects following injection into animals, this histidine base might well be placed among the most nocuous constituents of the intestine and it is important to know the conditions under which it is not formed. Dale and Laidlaw(8) describe the following symptoms following its injection into a cat. 10 mgrs. injected intravenously cause immediate vomiting, purging, salivation and laboured respiration with a subsequent period of collapse and slight narcosis. When injected into herbivora, particularly guinea-pigs, the most prominent feature is its effect on the bronchial plain muscle which may result in death by asphyxia. Injected into a pregnant cat, this base caused it to abort but the young were born dead. We injected some of the base into a pregnant rabbit under an anæsthetic and the powerful effect of the drug in producing uterine contractions was very obvious, but these contractions were irregular and did not appear to have much expulsive effect.

It is evident that the toxic symptoms produced by the substance, together with its presence in the alimentary tract, must bring it under consideration as a possible cause of pathological conditions. It is probable that, under normal conditions, the liver can deal adequately with β-imidazolethylamine, as it can with the amines of tyrosine and tryptophane, and render it innocuous, but if this defensive mechanism of the liver breaks down for any reason then many toxic symptoms will, no doubt, follow. For instance, one of us (E. M.) has elsewhere(9) suggested that the condition of cyclic vomiting in children may be due to the excessive accumulation of such substances as β-imidazolethylamine in the intestine, causing, from time to time, an exacerbation of symptoms. In any case a fact which would appear to point to a means of lessening the formation of this substance in the alimentary canal is worth consideration.

This base then is not produced in an acid medium, and this fact is additional support to the medical treatment, as advocated by Metchnikoff, involving the injection of lactic acid producing bacilli. It is necessary, however, to point out that the colon bacillus responsible for the production of the toxic product is not killed by the acidity of a medium, but its energies are only directed along other lines, so that, as soon as an alkaline reaction returns, the production of the histidine base continues.

The distribution of the histidine-destroying bacillus in the intestine.

The serous coats of the alimentary canals of a cat and a guinea-pig immediately after being killed were sterilised by heat, at different positions, and tubes of histidine in Ringer's solution were inoculated with minute quantities of the intestinal contents taken at the various points down the intestines. In the case of the guinea-pig, even above the duodeno-jejunal flexure there was a bacillus capable of producing the histidine base, as well as lower down in the intestine. In the case of the cat no evidence of production of the base was obvious until the ileo-cœcal valve was reached. The positive results in these experiments are of value, but the negative results obtained with the cultures taken high up in the cat's intestine do not prove that the special bacillus investigated was absent at these points. More careful experiments would be required to eliminate the possibility of the histidine-destroying bacillus being over-run and killed by other bacilli.

In the alimentary canal of a guinea-pig, at least, and probably in that of most mammals, the bacillus capable of producing β-I. from histidine is present from the duodenum downwards. It is legitimate, therefore, to assume that the presence of the histidine base, described by Barger and Dale, is due to bacterial decomposition going on in the intestine.

Summary.

The substance β-imidazolethylamine, the base produced by removing carbon dioxide from histidine, and the most active principle in ergot affecting the uterus, seems to owe its presence, in extracts of intestinal mucous membrane, to bacterial action for

(1) By extending a method which we recently described for isolating creatin-destroying bacilli from the intestine, we have been able to isolate a bacillus of the colon group capable of producing β-imidazolethylamine from histidine.

(2) The base is produced by inoculating Ringer's solution containing histidine with the bacillus. Conditions are described for the best production of this substance.

(3) The significance of the effect of acidity on the production of the depressant base from histidine, with regard to its presence in the intestine, is discussed.

(4) In the guinea-pig, at least, the bacillus seems to inhabit the intestine from the duodenum downwards.

REFERENCES.

(1) Barger and Dale. This Journal, XL. Proc. Physiol. Soc. p. xxxviii.
(2) Kutscher. Zntrlb. f. Physiol. XXIV. p. 163.
(3) Barger and Dale. This Journal, XLI. p. 499. 1911.
(4) Popielski. Pflüger's Arch. CXXVIII. p. 191. 1909.
(5) Ackermann. Ztschr. f. physiol. Chem. LXV. p. 504. 1910.
(6) Twort and E. Mellanby. This Journal, XLIV. p. 43. 1912.
(7) Kehrer. Arch. f. exp. Path. u. Pharm. LVIII. p. 366. 1908.
(8) Dale and Laidlaw. This Journal, XLI. p. 318. 1910.
(9) E. Mellanby. The Lancet, II. p. 8. 1911.