THE PARA-DIMETHYL-AMIDO-BENZALDEHYDE TEST FOR INDOLE[1].

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(Three Charts.)

This indole reaction, first described by Ehrlich, and used by him as a urinary test, has been applied by Böhme in determining the presence of indole in bacterial cultures. Böhme claims for this test that it is more delicate and more exact than the usual nitrite and sulphuric acid test, and it has given such good and constant results in this laboratory, that the following facts and figures may be given in confirmation of Böhme's claim.

The test, as recommended by Böhme, consists of two solutions made up as follows :—

Solution 1.	Para-dimethyl-amido-benzaldehyde ...	4 parts.
	Absolute Alcohol	380 „
	Concentrated Hydrochloric Acid ...	80 „

Solution 2. Potassium persulphate in saturated watery solution. To about 10 c.c. of the broth culture of the organism add 5 c.c. of solution 1, and then 5 c.c. of solution 2, shake the mixture and the presence of indole is indicated by the appearance, in a very short time, of a red colour, which gradually becomes darker on standing

This test can not only be used qualitatively for detecting the presence of indole, but it can also be used quantitatively for estimating the amount of indole produced by the micro-organism.

As a Qualitative test. I think most observers will agree that the nitrite and sulphuric acid test is not always satisfactory, and there is often great doubt as to whether a slight reaction really means the

[1] The usual spelling "indol" is here altered to "indole" in accordance with a rule laid down by the Chemical Society whereby the termination "ol" is in future to be used to indicate substances containing an OH group.—Ed.

production of indole by the micro-organism. The formation of indole, however, can be accurately determined by distillation and this fact was used as a confirmation in comparing the two tests, which, for the sake of brevity, we will now call the "old" (nitrite and sulphuric acid) and the "new" (para-dimethyl-amido-benzaldehyde).

Fifteen different micro-organisms were inoculated into peptone beef broth, and, at various intervals of time, were tested for indole, both tests being used. The results obtained are shown in the following table:

Micro-organism	24 hours' culture		3 days' culture		5 days' culture		12 days' culture	
	New test	Old test	New test	Old test	New test	Old test	New test	Old test
B. enteritidis (Gaertner)	0	0	0	+	0	+	0	trace
B. typhi murium	0	trace	0	+	0	trace	0	+
B. psittacosis	0	trace	0	+	0	+	0	trace
B. Hanstedt (Fisher)	0	0	0	+	0	+	0	+
B. cloacae	0	+	0	+	0	+	0	+
B. of epidemic jaundice	+	+	+	+	+	+	+	+
B. acidi lactici	+	+	+	+	+	+	+	+
B. Hog cholera	0	trace	0	+	0	+	0	trace
B. Abel	0	+	0	+	0	+	0	+
B. coli communis	+	+	+	+	+	+	+	+
B. dysenteriae (Flexner-Gray)	+	trace	+	+	+	+	+	+
B. pyogenes fetidus	+	trace	+	+	+	+	+	+
B. typhosus	0	trace	0	trace	0	+	0	+
B. paratyphoid A	0	trace	0	+	0	+	0	+
B. paratyphoid B	0	trace	0	+	0	+	0	+

According to the new test only five micro-organisms in the above series produced indole, namely, *B. of epidemic jaundice, B. acidi lactici, B. coli, B. dysenteriae* (Flexner-Gray) and *B. pyogenes fetidus.* Accordingly, large quantities of peptone beef broth, 50 c.c. to 100 c.c., were inoculated with these micro-organisms, incubated at 37° C. for some days, and then distilled. The distillate was tested for indole as before.

Micro-organism	Amount of culture	Time grown at 37° C.	Distillate	
			New test	Old test
B. coli communis	100 c.c.	9 days	+	+
B. dysenteriae (Flexner-Gray)	100	8	+	+
B. pyogenes fetidus	50	9	+	+
B. acidi lactici	50	9	+	+
B. of epidemic jaundice	100	5	+	+

This proved that these five micro-organisms, which always gave a constant reaction with the new test, were real indole producers, and the question next arose as to whether the positive reaction given with the old test by the other micro-organisms denoted the presence of indole. The following were therefore also distilled and the distillate tested.

Micro-organism	Amount of culture	Time grown at 37° C.	Distillate	
			New test	Old test
B. enteritidis (Gaertner)	100 c.c.	8 days	0	0
B. Abel	100	8	0	0
B. cloacae	100	6	0	0
B. paratyphoid A	100	7	0	0
B. paratyphoid B	100	7	0	0
B. typhosus	100	8	0	0

When, in addition to these results, we find that the para-dimethyl-amido-benzaldehyde gives a definite pink colour with ·0001 milligramme of indole, whereas the nitrite and sulphuric acid test is barely perceptible with ·0005 milligramme of the substance and fails entirely with ·0002 milligramme, we must conclude that the para-dimethyl-amido-benzaldehyde test is both more accurate and more sensitive.

As a Quantitative test. Herter and Foster, and Peckham, have described methods for the quantitative determination of indole, the former observers using B. napthaquinone-sodium-monosulphonate and the latter sulphuric acid and nitrite. I have endeavoured to ascertain whether para-dimethyl-amido-benzaldehyde can also be used colorimetrically in the quantitative estimation of indole, and have found the following method the most satisfactory.

A standard solution of indole is required, and is obtained by dissolving ·05 gramme of indole in 5 c.c. of absolute alcohol and then adding distilled water to 500 c.c. Five c.c. of the distillate to be tested for indole are then taken (having added 1 c.c. of absolute alcohol for every 100 c.c. of the distillate), added to a Nessler's tube and made up to 50 c.c. with distilled water. To this add 2 c.c. of the para-dimethyl-amido-benzaldehyde solution and 2 c.c. of the potassium persulphate solution and thoroughly mix. As before, the presence of indole is indicated by the appearance of a red colour. Varying quantities of the standard solution are then similarly treated in a series of Nessler's tubes until the requisite colour is obtained, when the amount of indole in the distillate can be calculated. The mixtures should be allowed to stand for one hour before matching the colours, as in some tubes the colour sometimes develops more quickly than in others in which there is more indole and which eventually gives a darker colour. A series of Nessler's tubes, containing, say, ·2, ·3, ·4 and ·5 c.c. of the standard solution of indole give quite distinct grades of colour, so that in two solutions, a difference of ·01 milligramme of indole can be accurately determined.

In testing this method the following three series of experiments were done, in order to show the variations in the amount of indole, produced by the same micro-organism, grown for different periods of time.

Seven flasks, each containing 100 c.c. of peptone beef broth were inoculated with 1 loopful of an agar culture of *Bacillus coli*, incubated

at 37° C. and distilled on succeeding days. The distillates, tested for indole, gave the following results:

Distillate after 1 days' growth = 0·86 milligrammes of indole per 100 c.c.

" " 2 " " = 1·20 " " " "
" " 3 " " = 1·80 " " " "
" " 4 " " = 2·00 " " " "
" " 5 " " = 3·80 " " " "
" " 6 " " = 3·60 " " " "
" " 7 " " = 2·40 " " " "

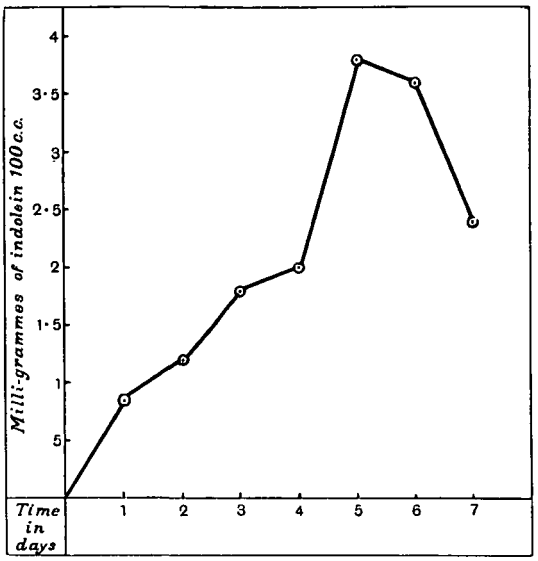

CHART I. Showing the amount of indole present on 7 consecutive days during the cultivation of *Bacillus coli* at 37° C.

In this series the indole production gradually rose to a maximum on the fifth day, with a slight falling off on the sixth day, and a still greater falling off on the seventh day. It was considered advisable to repeat this experiment with *Bacillus coli*, but to carry it on for a longer time. Another series of 100 c.c. flasks of peptone beef broth were inoculated with 5 drops, with a sterilized pipette, from a 24 hours' broth culture of *Bacillus coli*, this being considered more accurate than the standard loop. As before these flasks were incubated at 37° C., the first distillation carried out after four days' growth and the last 23 days after inoculation. The distillates gave the following figures:

Distillate after 4 days' growth = 1·20 milligrammes of indole per 100 c.c.

" " 5 " " = 1·30 " " " "
" " 6 " " = 1·80 " " " "
" " 7 " " = 1·60 " " " "
" " 10 " " = 1·80 " " " "
" " 11 " " = 2·00 " " " "
" " 14 " " = 2·90 " " " "
" " 23 " " = 1·80 " " " "

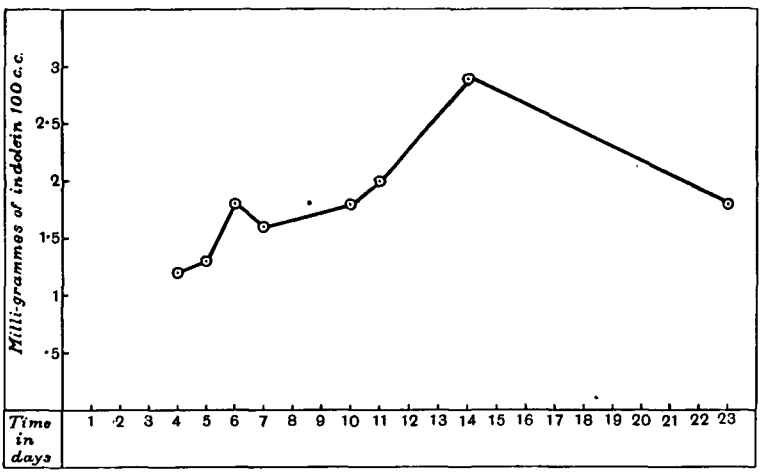

CHART II. Showing the amount of indole present at various stages during the cultivation of *Bacillus coli* for 23 days at 37° C.

In this series the indole production was very much slower, probably on account of the different method of inoculating the flasks. The indole production rose from the fourth to the sixth day, fell a little on the seventh and then rose to a maximum on the fourteenth day.

It was then considered advisable to estimate the indole production by another micro-organism, and cholera was chosen, because it was found on estimation to produce more indole than any other of the indole-producing organisms we had tested. This choice was rather unfortunate, as the nitrite produced by the cholera vibrio was sometimes present in the distillate and it was found that the presence of a nitrite has an inhibitory action on the production of the red colour. Nitrites in strong solutions give a yellow colour on the addition of the reagents. In this series, as in the preceding one, five drops of a bouillon culture were used to inoculate the flasks. The first distillation on this occasion, was done early, seven hours after inoculation, and the last flask had been

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incubating 27 days before it was distilled. The distillates were tested for nitrites.

							Nitrites
Distillate after 7 hours' growth	= 0·0	milligrammes	of	indole	per	100 c.c.	0
,, ,, 24 ,, ,,	= 0·0	,,	,,	,,	,,		+ + +
,, ,, 48 ,, ,,	= 0·0	,,	,,	,,	,,		+ + +
,, ,, 4 days' ,,	= 0·7	,,	,,	,,	,,		+ + +
,, ,, 6 ,, ,,	= 0·8	,,	,,	,,	,,		0
,, ,, 9 ,, ,,	= 2·0	,,	,,	,,	,,		+ +
,, ,, 13 ,, ,,	= 2·8	,,	,,	,,	,,		+ +
,, ,, 18 ,, ,,	= 7·0	,,	,,	,,	,,		0
,, ,, 27 ,, ,,	= 4·8	,,	,,	,,	,,		0

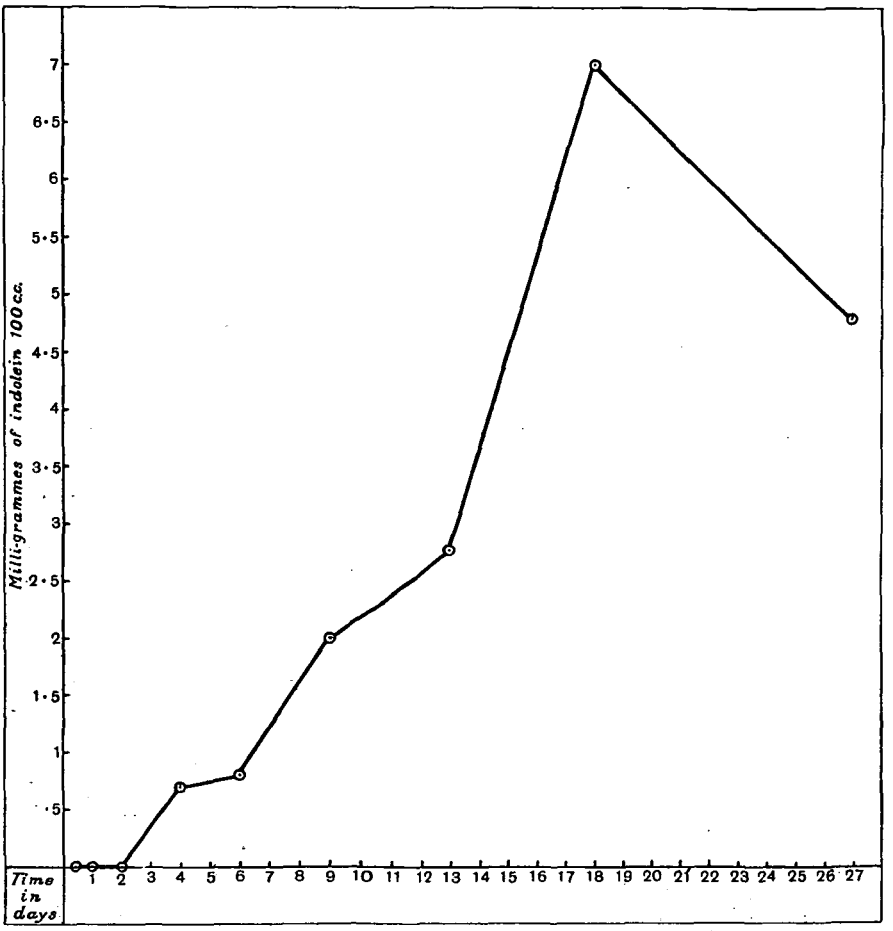

CHART III. Showing the amount of indole present at various stages during the cultivation of the *cholera vibrio* for 27 days at 37° C.

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This completed the series and these three curves of indole production are exceedingly interesting, in that they bear a striking resemblance to curves of toxin production. Probably the metabolic processes concerned in the two functions run parallel and certain circumstances which favour toxin formation also favour indole production. They are sufficient, at any rate, to demonstrate the use of this test in the quantitative estimation of indole.

It can also be demonstrated that the presence of lactose and glucose inhibit the production of indole by bacteria. *Bacillus coli*, grown at 37° C. for five days in 100 c.c. peptone beef broth containing 2 % lactose, and then distilled, failed to show the least trace of indole. In a similar experiment, with 2 % glucose in the broth, only a very small quantity of indole was produced. Here again there is a parallelism between indole production and toxin formation, as the latter, in the case of both the Diphtheria and Tetanus bacillus, is also inhibited by the presence of excess of glucose.

Skatole gives with this reagent a violet colour, which, on standing, becomes deep blue (the appearance of the blue colour is hastened by the addition of the persulphate). Only on one occasion have I found skatole in bacterial cultures, and that was in a broth culture of *Bacillus typhosus*, freshly isolated from the blood.

It may be added in conclusion, that, for laboratory purposes, this test for indole is exceedingly useful. There is no doubt about the result, it shows the presence of very small amounts of indole, and it is easily applied. In the routine examination of organisms isolated from water and from milk, we can say quite definitely in 24–48 hours if indole be present, a great saving of time, as it was customary to wait five days before applying the nitrite and sulphuric acid test.

SUMMARY AND CONCLUSIONS.

1. The high estimate formed by Böhme of the utility of this test has been fully confirmed.

2. Other substances, such as skatole-carboxylic acid, which tended to confuse the results of the older method, do not give an indole-like reaction with this reagent.

3. The test is more delicate, and the time required to ascertain the presence of indole is shortened.

4. The method lends itself to accurate colorimetric quantitative estimations, and the amount of indole present at various stages during the cultivation of certain organisms has been determined.

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