TABLE I.—*B. Typhosus*, 24 Hours' Broth Culture at 37° C. (Room Temperature, 15 to 18° C.)

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Sanitas	1 in 1	—	—	—	—	—	—	48 hours	37° C.
"	1 in 2	×	×	×	—	—	—	"	"
"	1 in 3	×	×	×	×	—	—	"	"
"	1 in 4	×	×	×	×	×	—	"	"
Phenol	1 in 100	×	×	×	—	—	—	"	"

Carbolic Acid Coefficient, $\frac{1}{100} = 0.02$.TABLE II.—*B. Typhosus*, 24 Hours' Broth Culture at 37° C. (Room Temperature, 15 to 18° C.)

Sample.	Dilution.	Time culture exposed to action of disinfectant—minutes.						Sub-cultures.	
		2½	5	7½	10	12½	15	Period of Incubation.	Temperature.
Formalin (40 per cent.) ...	1 in 10	—	—	—	—	—	—	48 hours.	37° C.
"	1 in 20	—	—	—	—	—	—	"	"
"	1 in 30	×	×	—	—	—	—	"	"
"	1 in 40	×	×	×	×	—	—	"	"
Phenol	1 in 100	×	×	×	×	—	—	"	"

Carbolic Acid Coefficient, $\frac{1}{100} = 0.4$.

etc., used in making dilutions were previously rigorously sterilized.) Into four sterile test-tubes were put dilutions of sanitas of the several strengths of 1 in 1, 1 in 2, 1 in 3, 1 in 4, and in a fifth sterile test-tube a solution of phenol of the strength of 1 in 100. In each case 5 c.cm. were used.

Now into each of these five disinfectant solutions of 5 c.cm. content five drops of a 24 hours' bouillon culture of *B. typhosus* are allowed to fall at intervals of exactly 30 seconds, and the mixture in each case well shaken. Continuing the 30 seconds' interval, a loopful is removed in serial order from each of the medicated cultures and sown in a bouillon tube, care being taken that the platinum loop is thoroughly sterilized in the flame after each inoculation. This manipulation is continued until the last of the thirty tubes is inoculated. The tubes are held during inoculation, withdrawal and replacement of plugs, etc., in such an oblique position that any extraneous organisms floating in the atmosphere are excluded.

Table I shows that an undiluted sample of sanitas prevented the growth of the bacillus when the time of contact with the culture was 2½ minutes. A dilution of 1 in 2, however, was not able to prevent the growth of the bacillus until a contact of 10 minutes' duration had been effected. The same result exactly was attained by phenol diluted to 1 in 100. Thus it is seen that 1 in 100 pure phenol solution performs the same amount of bactericidal work as a solution of sanitas of the strength of 1 in 2. The carbolic acid coefficient of sanitas is therefore $\frac{1}{100} = 0.02$ (See Table I.)

The bouillon tubes after inoculation, each with a loopful of the serial mixtures of antiseptics, were incubated for 48 hours at 37° C. The temperature of medication was 15 to 18° C. At the end of 48 hours each tube was individually examined for the presence of bacterial life. No adventitious organisms were at any time found.

Table II gives the results of experiments made in the same manner, and under exactly similar conditions with formalin (40 per cent. formic aldehyde in H₂O). (See Table II.)

By this it is seen that 1 in 100 phenol performs the same bactericidal work as 1 in 40 formalin.

The carbolic acid coefficient for formalin is therefore $\frac{1}{100} = 0.4$.

A friend informs me that in working, by the same method, with formalin of strength 39.2 per cent., he obtained a carbolic acid coefficient of 0.3. These figures strikingly demonstrate at once the accuracy and delicacy of this bacteriological test.

SCIENTIFIC GRANTS COMMITTEE

OF THE

British Medical Association.

REPORT LXXXVII.

THE EFFECT OF CERTAIN DYES UPON THE CULTURAL CHARACTERS OF THE BACILLUS TYPHOSUS AND SOME OTHER MICRO-ORGANISMS.*

BY

E. W. AINLEY WALKER, and W. MURRAY,
M.A., D.M. Oxon. M.B. Edin., D.P.H. Lond.

Fellow of University College, Oxford.

(From the Gordon Laboratory, Guy's Hospital, and the Pathological Department of the University of Oxford.)

In the course of a series of experiments upon the *intra vitam* staining of bacteria, which we began about 18 months ago, remarkable changes were observed in the cultural characters of the bacteria employed when they were grown in media to which certain small percentages of various dyes had been added. These changes we have found to occur with constancy, and they appear to possess sufficient interest to be worth recording.

Briefly, the change consists in the conversion of a normal short bacillus into a form which presents itself almost entirely or entirely as long filaments or threads. These may be twenty or more times the length of the ordinary bacillus, and often show no sign of segmentation. They frequently present the appearance of true branching when grown on solid media.

We have worked chiefly with a number of different strains of the bacillus typhosus, but have also obtained similar results with some other organisms, such as the bacillus coli and the cholera vibrio. The effect to be described has been produced in greater or less degree with all the following dyes: Grubler's methyl violet (6B), ordinary gentian violet, fuchsin,

* The expenses of this work were defrayed out of grants received by Dr. Walker from the British Medical Association and from the Scientific Grant Committee of the Royal Society.

methyl-green, and methylene-blue; but the most marked results so far have been obtained with the methyl-violet.

The media used were ordinary agar-agar, bouillon, and gelatine to which a definite quantity of methyl-violet had been added. And after numerous trials with varying strengths of the stain it was found that the best results with typhoid were obtained by the addition of 0.2 per cent. of a saturated alcoholic solution of methyl-violet (or an equivalent amount of an aqueous solution) to the culture medium. The media, which then possessed a strongly-violet colour, were tubed and sterilized in the ordinary way.

Fig. 1.—Film from a methyl-violet broth culture of the bacillus typhosus. $\times 1,000$.

If a broth tube prepared as just described be inoculated from an ordinary typhoid culture, and examined after some five or six hours' incubation at 40°C ., the subculture will be found to consist, not of typhoid bacilli of the usual appearance, but very largely or almost entirely of long filaments, actively

Fig. 2.—Long forms of the bacillus typhosus stained by Van Ermengem's method. $\times 1,000$.

motile with a sinuous or undulating motion, which is well seen in hanging-drop preparations. Permanent preparations, stained preferably with methylene-blue or carbol-thionin-blue, exhibit long threads or filaments which show no evidence

of segmentation in their length. In older cultures (24 to 48 hours old or more) segmented portions may be seen at one extremity or in the middle of a thread. The threads themselves are not infrequently of such a length as to extend beyond the limits of a field of a $\frac{1}{2}$ immersion lens. In other cases, and especially in old cultures, segmentations of the filaments may be present, or a condition of "beading;" while in stronger methyl-violet broth the filaments often assume in cultures some days old what may be spoken of as a coccoid form of the organism, having almost the appearance of a chain of cocci.

Subcultures from the methyl-violet broth into similar tubes of methyl-violet broth give similar appearances; but on transferring the organisms to ordinary unstained media it speedily regains its normal form, though individual long elements appear in the first and even in the second of such subcultures.

As the cultures in methyl-violet broth get older, the motility of the filaments gradually diminishes and is ultimately lost, but growth appears to continue for a considerable period, and what may reasonably be described as giant forms appear. These are of a relatively enormous length, and are much thicker than a normal typhoid organism.

Fig. 3.—Film from young methyl-violet agar culture of the bacillus typhosus. $\times 1,000$.

Figure 1 is taken from a photograph of a film from a 48-hours' old methyl-violet broth culture of an ordinary specimen of typhoid.

Similar effects, though of a less degree, were obtained with the bacillus coli and with cholera. But they were not obtained with every strain of the bacillus coli tested in the particular strengths of stain which we have used.

As regards the naked-eye appearances of the broth cultures; in the case of typhoid the medium usually retains its violet colour almost indefinitely, although a heavy violet-coloured growth sinks to the bottom of the culture tube. But frequently with the bacillus coli most of the colouring matter is precipitated to the foot in about a week, leaving the medium only faintly violet. With cholera a thick scum of growth forms on the surface of the broth, and the pigment becomes concentrated on this layer, leaving an almost unstained layer of medium beneath.

Cultures upon methyl-violet gelatin do not require any separate description, since the results they yield do not differ in any important degree from those which we obtained on coloured agar.

The examination of young methyl-violet agar cultures of the bacillus typhosus reveals the fact that the long forms are

supplied with numerous flagella throughout their length. But it was found almost impossible to obtain successful preparations of very long forms with the flagella stain, owing to their great liability to become broken up in the manipulation necessary to procure a very thin film.

Figure 2 is from a photograph of a preparation stained by Van Ermengem's method.

Fig. 4.—Film from methyl-violet agar culture of the bacillus typhosus, 4 days old. $\times 1,250$.

Streak cultures on sloped methyl-violet agar give a profuse granular pigmented growth of the bacillus, and the colouring matter in the medium is rapidly abstracted from it, and concentrated in the growth, until the former is eventually almost altogether freed from violet. Similar facts are true of cultures of the bacillus coli and of cholera upon this medium.

Fig. 5.—Impression preparation of a 24 hours' old typhoid colony from methyl-violet agar. $\times 200$.

Cover-glass preparations of the typhoid growth show a densely felted network of long forms, which are usually entirely unsegmented and present numerous examples of dichotomy (true branching).

Figures 3 and 4 are photographs from methyl-violet agar cultures of the bacillus typhosus.

A comparison of ordinary agar plates of the bacillus typhosus with plates of methyl-violet agar showed that on the latter medium the colonies were somewhat smaller than on ordinary agar, each colony being deeply stained and possessing a well-marked central nucleus. The edge of such a colony, which has been somewhat spread out by gentle pressure on the cover-glass, is shown in Figure 5, which is taken from an "impression preparation" of a 24-hour-old colony, and exhibits the network of unsegmented wavy filaments.

When tested for the Gruber-Durham reaction in the ordinary way (dilutions of 5 per cent. and 0.5 per cent. serum) the "long forms" of the bacillus typhosus rapidly agglutinated.

One of our strains of typhoid entirely failed to produce "long forms" in the methyl-violet media which we were using at the time. Probably they contained too much stain. Altogether some ten strains of typhoid were tested. Three of them were strains which had been sealed up in agar tubes by one of us in Berne in 1899, and which had travelled to and fro a great deal in the interval through a range of temperature varying from that of the Swiss winters (twice) to that of an Italian summer, but they were found to be living when opened in 1903. Two other strains which had dried up owing to flaws in the tubes were dead.

We are not at present prepared to estimate the importance of the foregoing observations, and therefore restrict ourselves to a brief record of the main results obtained, omitting the description of a large number of minor variations, which we have found occurring with different organisms and with different strengths of methyl-violet. But we conclude that the form in which we ordinarily see the bacillus typhosus and some other bacteria in the laboratory is not the only form in which they may appear. Perhaps it may not even be the form in which by preference they exist as saprophytes.

Further experiments, which are now in progress, suggest the possibility of an unsuspected complexity in the life-history of these micro-organisms. Meanwhile particular attention may be drawn to the undoubted branching and the felted network which we find in our experiments.

The illustrations are from photomicrographs kindly prepared for us by Mr. Coventon in the Pathological Department in the University of Oxford.

MEMORANDA:

MEDICAL, SURGICAL, OBSTETRICAL, THERAPEUTICAL, PATHOLOGICAL, Etc.

PNEUMOCOCCAL APPENDICITIS WITH PNEUMOCOCCAL PERITONITIS.

An unmarried girl, aged 18, was admitted to Guy's Hospital on April 5th, 1904. She came of a very healthy family living at Datchet, and had suffered from no serious illnesses before. When in bed on the night of April 3rd, during a visit to some friends in London, she was suddenly seized with violent pain all over the abdomen, so severe as to "double her up." Early in the morning of April 4th she vomited; between then and her admission she vomited several times; and the very severe abdominal pain continued. There was frequent diarrhoea, but whether this was the result of purgation, or a symptom of the disease, could not be ascertained.

On admission she was too collapsed to allow of any complete examination. Her pulse was 120, almost imperceptible; her respirations 24, thoracic; her temperature 102° F. There was little abdominal tenderness or rigidity. Her condition was clearly one of extreme prostration, resulting from a very acute abdominal lesion. She was too ill to operate upon; she did not rally, and died a few hours after admission.

At the necropsy made by the medical registrar, the lungs, pleurae, pericardium, kidneys, liver, spleen, pancreas, suprarenals, pelvic viscera, brain, and meninges were all perfectly healthy. There was acute general peritonitis, with thick buttery lymph, and about one pint of pale turbid fluid in the peritoneal cavity. The lymph was thickest in the region of the caecum and vermiform appendix. The appendix was swollen, reddened, turgid, and buried in lymph; without adhesions, without visible perforation, and without signs of any local abscess cavity in its neighbourhood. On cutting