

only in the egg. It is the sterile symbiotic organ which shows the three forms illustrated by Buchner at their best; I hold them to be tissue debris. Perhaps the real bacterium has been mistaken for cell-granules and overlooked as such. However, this bacterium produces a red pigment and, moreover, fills the culture plate with a mass of delicate crystals of phosphates. When the cultures become old, the red pigment turns burnt sienna in colour. The observations on the pigments formed by these cultures further enable me to identify Buchner's illustration as belonging to *A. alni*.

Buchner² also illustrates the symbiotes of *A. salicis* in Fig. 4, p. 103, which are supposed to be two, one resembling a piece of protoplasm, while the other, the genuine symbiote, is a long thin bacterium. Its culture forms a pale ochre-coloured pigment which, in time, slightly darkens to give the colour of the adult insect. When *A. alni* and *A. salicis* are compared, along with the cultures of their bacteria, there is no doubt that the symbiotes produce the pigments of their hosts.

Buchner² likewise illustrates the symbiotic microflora of *Fulgora europea* (p. 175, Fig. 30, a-d) with bright yellow pigment granules. The bacteriome from which these illustrations have been derived is also yellow in colour. The adult insect is greenish, but in alcohol its colour changes immediately to yellow, the green colour being soluble in it. A culture from the above insect gave a colony of bacteria producing a yellow pigment. Other insects like *Philemus* species and *Aphrodes bicinctus* also owe their pigments to their symbiotic bacteria.

On account of the high prices of bacteriological materials, nearly all my cultures had to be sacrificed, but those of *Cicadella viridis* are still available, and have been sent to the National Type Culture, Lister Institute, London.

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¹"Symbiose" (Sammlung Götschen, 1939).

²*Biol. Zentralb.*, 53, 199 (1933).

³*Verhandl. d. Deutschen Zool. Ges.*, 420 (1939).

⁴*Deccan Medical Journal* (Hyderabad, 1941).

⁵"Tiere u. Pflanzen in intrazellulärer Symbiose", 238, Fig. 67 (1921).

⁶*Z. f. Morph. u. Ökol.*, 25, 657, 659 (1932).

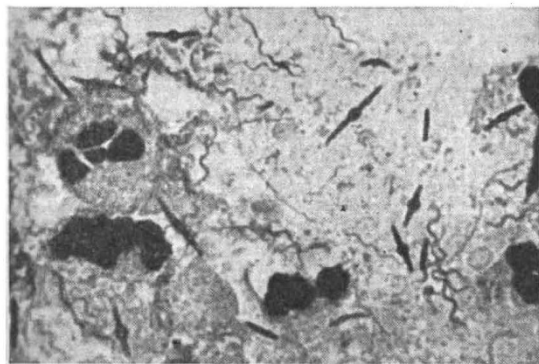
⁷*Archiv. f. Protis.*, 68, 613 (1929).

⁸*Archiv. f. Protis.*, 26, 115 (1912).

⁹*Z. f. Morph. u. Ökol.*, 4, 103, 113, 175 (1925).

Changes Occurring in *Bacillus fusiformis* During the Application of Penicillin

WHILE investigating microscopically smears of 210 cases of ulcerative gingivitis we found the usual crowd of typical spirochaetes and fusiform bacilli. Routine treatment with penicillin produced clinical and bacteriological clearance. In 41 cases the fusiform bacilli showed after treatment with penicillin a peculiar change in shape. Central round or spindle-shaped swellings (see accompanying illustration) of



LARGE SWELLINGS IN FUSIFORM BACILLI AFTER PENICILLIN TREATMENT. STAINED WITH CRYSTAL VIOLET. $\times c. 900$

intensively stained plasma were found in varying numbers. These large swellings are obviously an expression of the influence on these micro-organisms by penicillin. These bodies were never observed in untreated cases, or in any other treatment of this condition. A study of this appearance is in preparation.

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Polarographic Determinations in the Presence of Triethanolamine

IN a communication in *Nature*, Mr. H. Wolfson¹ reported that iron can be determined satisfactorily on the polarograph if the solution contains triethanolamine. This method seemed promising for the measurement of iron in small quantities, which has hitherto been practically impossible. It would be particularly suitable for use on biological materials and foodstuffs, especially if lead and copper could be measured in the same solution. In Mr. Wolfson's solutions, con-

taining ammonia, copper gives two steps, the second one interfering with that of ferric iron. He stated, however, that the properties of the solution can be considerably modified by the presence of a strong alkali. When this suggestion was followed up, it was found that the steps for copper, lead and iron can be separated and that the three metals can be determined simultaneously under suitable conditions.

The following table contains approximate values for the potential of the mid-point of the step (the "half-wave potential") for a number of metals in 0.1 M potassium hydroxide containing triethanolamine. All are negative and are with reference to the saturated calomel electrode. The figures in potassium hydroxide alone, given for comparison, are taken from Kolthoff²; those in 0.4 M triethanolamine were communicated privately by Mr. Wolfson.

Triethanolamine	Cu	Pb	Cd	Fe	Ni	Zn
0	—	0.68	—	—	—	1.42
0.03 M	0.46	0.76	0.77	1.04	1.35	1.45
0.10 M	0.47	—	—	1.02	—	—
0.30 M	0.53	0.88	0.82	1.01	1.40	1.57
0.40 M	0.55	0.91	0.88	1.00	1.45	1.61

In 0.5 M potassium hydroxide, 0.1 M triethanolamine, the figure for copper is -0.53 v. and for iron -1.06 v.

For iron the height of the step can be measured accurately in any of the above solutions, but for copper, lead and cadmium the top of the step tends to be rounded, so that the exact value of the diffusion current is uncertain. This rounding of the step occurs frequently in polarographic curves. Its cause is at present unknown, but it seems to be due to a tendency to form a second complex ion. In 0.03 M triethanolamine, 0.1 M potassium hydroxide, the uncertainty is negligibly small and the steps are of good form for measuring, but as the strength of either reagent is increased the uncertainty becomes progressively greater. The step for zinc, which is good in potassium hydroxide solution, is poor in all the solutions containing triethanolamine. Nickel gives a poor step in all the solutions and its height is reduced by heating. This suggests that the nickel is not in true solution.

The fact that copper, lead and cadmium can be measured accurately only in solutions containing a small concentration of triethanolamine would seem to limit the application of the method. The solubility of ferric hydroxide in the 0.03 M triethanolamine, 0.1 M potassium hydroxide solution is probably not much greater than 100 mgm. iron per litre. The solubility of cadmium in the same solution, judging from the height of the step, is about 30 mgm. per litre. Figures have not been obtained for the other metals.

A few tests have been made using triethanolamine in the presence of ammonia, borax and ammonium sulphate respectively. The following are approximate figures for the mid-point potentials in 50 gm. ammonium chloride, 50 c.c. ammonia per litre.

Triethanol-amine	Cu	Pb	Fe	Cd	Ni	Zn
0	0.19, 0.48	—	—	0.80	1.05	1.31
0.03 M	—	0.53	—	0.78	1.09	1.32
0.30 M	0.21, 0.50	0.56	0.56	0.81	1.18	1.36

The steps for cadmium and nickel are good and are little affected by triethanolamine. The step for zinc becomes less satisfactory as the concentration of triethanolamine is increased. Lead and iron are virtually insoluble in plain ammoniacal solution, but give excellent results in the presence of triethanolamine. Copper gives a double step in all these solutions, but otherwise behaves like zinc.

In 0.1 M borax, 0.3 M triethanolamine, copper gives a single, unsatisfactory step at about -0.3 v.; iron gives a fairly good step at -0.70 v. In 0.25 M ammonium sulphate, 0.3 M triethanolamine, copper gives what appear to be two steps running into each other, with mid-points at about -0.2 v. and -0.4 v. The step for iron depends on the pH of the solution. At pH 4 it is more positive than $+0.3$ v.; at pH 6 it is about -0.2 v. but is poor in shape. The step becomes more negative and improves in shape as the solution is made more alkaline.

It may be noted that no maxima have been observed in any of the solutions containing triethanolamine, consequently a suppressing agent is not needed.

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¹ Wolfson, H., *Nature*, 153, 375 (1944).

² Kolthoff and Lingare, "Polarography" (Interscience Publishers, Inc., 1941).

State of Vitamin A in Human Serum

CHROMATOGRAPHIC separation on alumina was applied to the separate estimation of vitamin A alcohol and its esters in extracts from human serum. It was found in eleven normal subjects that 10-17 per cent of the total vitamin A is present in the form of esters.

Three conditions were studied in which the vitamin A level is known to be temporarily raised. The rises which occurred as a consequence of endogenous mobilization of vitamin A, as after delivery¹ and after drinking alcohol², were found to be due to a rise in the vitamin A alcohol. On the other hand, oral administration of vitamin A, ester or alcohol, resulted in a rise of the ester fraction only.

Experimental details will be published elsewhere.

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¹ Abt, A. F., Aron, H. C. S., Bundesen, H. N., Delaney, M. A., Farmer, C. J., Greenebaum, R. S., Wenger, O. C., and White, J. L., *Quart. Bull. Northw. Univ. Med. School*, 16, 245 (1943).

² Clausen, S. W., Brees, B. B., Baum, W. S., McCoord, A. B., and Ryddeen, J. O., *Science*, 63, 21 (1941).