IMPROVED STAINING TECHNIQUES FOR THE DEMONSTRATION OF NON-ACID-FAST TUBERCLE BACILLI AND GRANULES

THE two staining methods here described are improved counterstain techniques for the demonstration of non-acid-fast tubercle bacilli and granules. Both methods gave more effective color contrasts between acid-fast and non-acid-fast bacilli than the counterstains employed in the Ziehl-Neelsen,[1] Gram,[2] Cooper,[3] Kühne,[4] Fontes[5] and Dreyer[6] methods. The improved methods consist in adding alkali (NaOH, Na_2CO_3, or $NaHCO_3$) of an experimentally determined optimum strength and amount.

The organisms employed in this study were ten-day cultures of an avian strain of tubercle bacilli obtained from Bellevue Medical College. They were grown on a glycerine potato agar medium which contained, in the case of some slants, 10 per cent. normal horse serum, and with other slants 10 per cent. immune rabbit serum. After the usual 5-minute staining period with Ziehl-Neelsen carbol-fuchsin according to the Ziehl-Neelsen method for staining tubercle bacilli, destaining with 3 per cent. HCl alcohol, and washing with tap water, the slides were counterstained according to the techniques described below.

COUNTERSTAIN METHOD I

Flood the smear with 8 drops of Löffler's methylene blue. Add immediately 6–8 drops of 0.05 per cent. NaOH from a medicine dropper. Move the slide gently from side to side to mix, and let stand two to three minutes. Wash with tap water, dry and examine under the oil immersion lens. The contrast between the red acid-fast tubercle bacilli and the blue non-acid fast tubercle bacilli is striking.

COUNTERSTAIN METHOD II

Flood the smear with 8 drops of 1 per cent. aqueous crystal violet solution. Add immediately 6–8 drops of 5 per cent. $NaHCO_3$. Move the slide gently from side to side to mix, and let stand not more than 2 minutes. Wash in tap water, apply Gram's iodine for 2 minutes, wash, and decolorize 20–30 seconds with a mixture of equal parts of acetone and 95 per cent. alcohol; wash, dry, and examine under the oil immersion lens. The non-acid-fast bacilli appear violet. In addition the "granules" in the red acid-fast bacilli and in the violet non-acid-fast bacilli stand out prominently as violet-black bodies.

With Löffler's methylene blue solution, it was found that 0.05 per cent. NaOH, 0.1 per cent. NaOH, 1 per cent. Na_2CO_3, and 10 per cent. $NaHCO_3$ gave about equally satisfactory results. Both higher and lower concentrations of these solutions, for example, 1 per cent. and 0.01 per cent. NaOH, 10 per cent. and 0.5 per cent. Na_2CO_3, 5 per cent., 1 per cent., and a highly supersaturated solution of $NaHCO_3$, gave less effective results. Control slides using Löffler's methylene blue without additional alkali, also gave less effective results.

With the 1 per cent. aqueous crystal violet solution, 5 per cent. $NaHCO_3$, 0.5 per cent. Na_2CO_3 and 0.05 per cent. NaOH gave the most satisfactory results.

All the chemical solutions used were freshly prepared in clean sterile bottles.

The above results suggested that there might be an optimum pH zone within which the above counterstains can most effectively penetrate and stain the non-acid-fast tubercle bacillus. The pH's of the alkali solutions tried out were determined colorimetrically with the following results:

Indicator	Alkali	Dilution Per cent.	pH
Trapaeolin O (range 11.1–12.8)	NaOH	0.01	11.4
		0.05	11.9
		0.10	12.2
	Na_2CO_3	0.50	11.4
		1.00	11.7
		5.00	11.9
Thymol Blue (range 8.0–9.6)	$NaHCO_3$	1.00	8.6
		5.00	8.4
		10.00	8.3

The relatively low pH shown colorimetrically by the $NaHCO_3$ indicates that the hydroxyl-ion concentrations of the above solutions do not constitute the sole factor in determining the permeability of the bacterial surface to the dye solution, and the penetrability and fixation of the dye. The ionic activities[7] of these solutions, the degree of dispersion of the dye, and other factors[8] must be considered.

[1] Park and Williams, "Pathogenic Microorganisms," p. 85, 1929.
[2] *Ibid.*, p. 82.
[3] *Ibid.*, p. 86, also *Arch. Path. and Lab. Med.*, 2, 382, 1926.
[4] Calmette, "Tubercle Bacillus Infection and Tuberculosis in Man and Animals," p. 16, 1923.
[5] *Ibid.*, p. 21, *Centralbl. f. Bakt.*, 49, 317, 1909; also Kieffer, *Am. Rev. Tuberc.* 5, 662, 1921.
[6] G. Dreyer and R. L. Vollum, *Lancet* 220, 1015, 1931.
[7] Lewis and Randall, "Thermodynamics," p. 35, 1923.
[8] J. W. Churchman, in "The Newer Knowledge of Bacteriology and Immunology," (Jordan and Falk), pp. 19–37, 1928.

Trials of these counterstaining techniques have been made on several bovine and human strains with satisfactory results. The concentrations of the alkali solutions required to stain effectively the non-acid-fast bacilli and granules of these strains, were found to be higher than those required for staining the avian bacilli. Best results for the bovine and human strains stained according to Method I, were obtained with 1 to 5 per cent. NaOH and 3 to 10 per cent. NaOH respectively, whereas the highest optimum NaOH concentration found for any of the avian cultures studied was 0.5 per cent. With Method II, both bovine and human bacilli gave effective results with saturated $NaHCO_3$. With Na_2CO_3, best results for bovine bacilli were obtained with a 5 per cent. solution, and for human bacilli with a 10 per cent solution.

Further study is being carried on to determine whether or not these staining techniques may be of use in differentiating avian, bovine, and human types of tubercle bacilli.

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SPECIAL ARTICLES

POLIOMYELITIS AS AN ESSENTIAL NERVE SYSTEM DISEASE THROUGHOUT ITS COURSE

IN 1912, Peabody, Draper and Dochez[1] in their monograph on acute poliomyelitis postulated a preliminary period of generalized systemic infection preceding invasion of the nervous tissues. Since the publication of their hypothesis and its reassertion by Draper[2] in 1917, the conception of poliomyelitis as essentially a general infectious disease with secondary and incidental invasion of the nervous system has come to be widely accepted and has largely governed medical thought in the fields of epidemiology, clinical interpretation of the early symptoms and serum treatment. That it has stood at variance with much of the experimental work—such as the great difficulty of producing the disease in apes by intravascular injection of virus, as contrasted with the great ease of producing the disease by direct applications to nervous tissue—and with certain clinical aspects of the disease in man, such as the peculiarly scattered and asymmetrical distribution of the lesions and paralyses, must be evident to any unbiased student of poliomyelitis.

An analysis of the 115 case histories given in the Rockefeller Monograph and in Draper's book with special reference to the onset symptoms shows no necessity for assuming an early phase of systemic, extranervous infection, but on the contrary that all the most frequent and characteristic onset symptoms can be explained as manifestations of infection of the central nervous system. In order of frequency, the commonest onset symptoms in the 115 cases were found to be: fever; vomiting; drowsiness, restlessness and irritability; headaches; vague symptoms, usually subjective, of discomfort or awareness of bodily disturbance; pain or hyperesthesia, and constipation. Each of these, and none of the other symptoms, occurred in more than 10 per cent. of the cases. To them should be added a peculiar psychic change which Draper regards as specific and as characteristic of the earliest hours of the disease, but is not shown in the actual protocols. Considered as a group, the symptoms are abviously of a nervous order. Moreover, these same symptoms, together with others indicating an extension in nervous involvement, almost equally characterize the later stages of the disease. It is notable that in the "dromedary" cases in which an interval of symptomatic silence separates the initial symptoms from those of the later, indubitably nervous phases of the disease, the predominant symptoms of the onset recur with significant frequency during the later phases. An analysis of the sites of residual paralysis in the large series reported by Lovett and Lucas[3] shows them to be one-sided in over three quarters of the 628 cases.

If the theory of initial, systemic invasion were correct one would logically expect: (1) a notably different symptomatology in the initial and later periods of the disease; (2) a diffuse or at least a symmetrical distribution of the lesions and paralyses. Differences in the blood supply of the two lateral halves of the bulbospinal axis, such as have been offered in explanation of the asymmetry of the lesions, probably do not exist: if they did, they would fail to explain, in the absence of gross embolism, which is not a feature of the pathology, the prevailing one-sidedness.

The clinical aspects of poliomyelitis harmonize, in my opinion, satisfactorily with the newer conception of axonal transmission of virus proposed in 1930 by Fairbrother and Hurst (which represents a return, with some modifications, to the conception of nerve-

[1] F. W. Peabody, G. Draper and A. R. Dochez, "A Clinical Study of Acute Poliomyelitis." Monographs of the Rockefeller Institute for Medical Research. No. 4, June 24, 1912.

[2] George Draper, "Acute Poliomyelitis." P. Blakiston's Son and Company, Philadelphia, 1917.

[3] R. W. Lovett and W. P. Lucas, "Infantile Paralysis. A Study of 635 Cases from the Children's Hospital, with Especial Reference to Treatment." *J. A. M. A.*, 51, 1677, November 14, 1908.