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Vital Staining Characteristics of the Epithelioid Cell in Experimental Tuberculosis.

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Twenty young rabbits, weighing from 600 to 800 grams, were each inoculated intravenously with 1/2 cc. of saline emulsion of a three weeks culture of virulent bovine tubercle bacilli. The emulsion contained about 35 bacilli to each oil immersion field. After 10 days, animals from this series were studied at bi-weekly intervals, to 6 weeks after infection, in the following manner:

Ten to 15 cc. of a 1 per cent normal saline solution of neutral red* was slowly injected into the ear vein. After 10 minutes the rabbit was quickly killed by air embolism. Thin slices of the lungs, which in most cases were well stained with neutral red, were fixed in Zenker-formol for 12 to 24 hours. Thin blocks of tissue (2 mm.) were quickly dehydrated, without washing, in acetone, cleared in benzene or xylol, and rapidly imbedded in paraffin.

Thin sections are then made and stained with carbol fuchsin and methylene blue. In the staining process both alcohol and water should be avoided as much as possible. The paraffin is removed by xylol, and the section transferred to acetone. It may then be passed rapidly through water, and washed quickly in Gram's iodine, or Lugol's solution, to remove most of the mercurial precipitate. After blotting dry, the staining with carbol fuchsin was performed on a warm plate in the usual manner. This process should not exceed more than one minute. This allows for rapid decolorization in acid alcohol which is essential, if the color of the neutral red in the section is to be preserved. The acid alcohol at the same time removes the iodine from the tissues. After quickly rinsing in water, the section is blotted dry, stained with Loeffler's methylene blue, decolorized slightly in 95 per cent alcohol, dehydrated further in acetone, cleared in xylol and mounted in balsam. In properly stained sections the neutral red taken up by cells in the lung tissue does not vary appreciably in color and amount from that in fresh, unfixed films from the cut surface of the lung, or unstained sections of fixed tissue. One experiences no difficulty in identifying the tubercle bacilli which are stained brilliant scarlet; the neutral red is of rather salmon-red color.

* National Aniline and Chemical Co.

In such preparations neutral red is seen in alveolar epithelium, in large phagocytic cells of two varieties, and in the epithelioid cells of the tubercles. Very little of the dye is taken up by the alveolar epithelium, which may be readily distinguished. The first and less numerous group of vitally stained, mononuclear phagocytes consists of large, irregularly-shaped cells with small nuclei, which are often obscured by masses of dye or cellular débris within the abundant cytoplasm. Such cells are found mainly in the alveolar walls, interlobular septa, and at times within the alveolar spaces. They seem in no way directly or specifically related to the tuberculous lesions, and have never been seen to contain tubercle bacilli.

The other group of vitally stained phagocytes consists of large round or oval cells, with abundant cytoplasm, and large, sharply defarcated nuclei. The nuclei, in sections, vary in shape with the direction in which the cell is cut, being round, oval, or indented, and have a light violet color when stained with methylene blue. The cytoplasm is just perceptibly stained pale blue, and a large zone by the side of the nucleus is not stained at all. In this clear zone the neutral red is taken up in coarse granules of about the same size, which are arranged about a small clear space in the form of a rosette. These cells are found in small groups within the alveolar walls, septa and alveoli. Moreover, they prove to be the large mononuclear cells with which the alveoli are filled in patches of tuberculous pneumonia. They frequently contain tubercle bacilli.

The epithelioid cells of the young tubercles have identically the same staining characteristics as this latter group of mononuclear cells. Due to their more compact arrangement, the shape of the epithelioid cells is often more elongated or irregular, but otherwise these two groups of cells show no fundamental differences. The epithelioid cells contain many tubercle bacilli, and in each cell, cut through or near the nucleus, one sees the characteristic rosette of neutral red. One frequently sees epithelioid cells with two or more nuclei grouped about a common rosette of neutral red. In several instances giant cells with 10 to 15 nuclei surrounding a large, single rosette were found.

It will be recognized at once that this characteristic staining with neutral red of the epithelioid cells of tuberculosis, and the mononuclear cells of tuberculous pneumonia, is the same as that which has been described for the large mononuclears or monocytes of the peripheral blood.¹ The following table summarizes the results of differential blood counts, by the supravital technique, upon 10 of the rabbits of this series. It confirms the numerous other observations

of an increase in the monocytes of the blood during tuberculous infection.

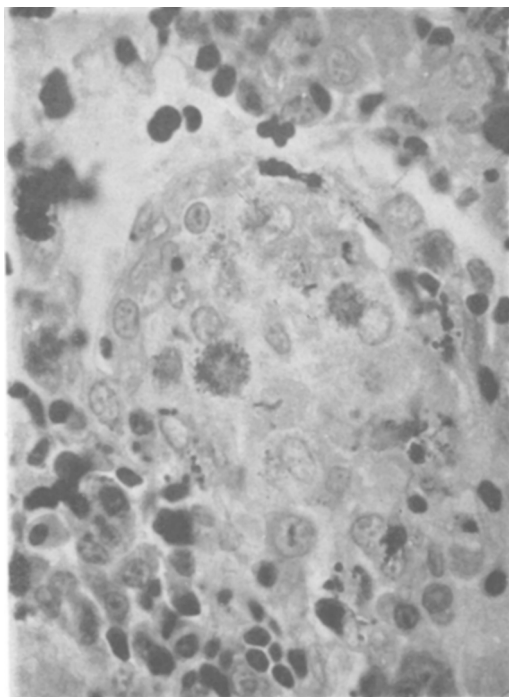


FIG. 1.

Young tubercle with characteristic rosettes of neutral red in epithelioid cells. The short, dark rods are tubercle bacilli stained with carbol fuchsin.

TABLE I. *Peripheral Blood.*

Date 1926	No. of Rabbits	W. B. C.	P. M. N.	P. M. E.	P. M. B.	S. L.	L. L.	Mono.
July 21/26	10	8,670	59.8	2.45	5.8	19.4	3.0	9.8
July 27	10	½ cc. Emulsion Bovine Tb. Bacilli—3 weeks culture—35 to Oil Imm. Field						
July 30 to Aug. 3	9	7,940	56.9	—	—	14.5	1.9	19.3
Aug. 4/11	8	10,500	56.2	0.5	4.6	15.3	3.3	20.5
Aug. 13/21	7	10,650	39.1	1.1	3.7	26.5	3.5	26.1
Aug. 28	3	8,030	40.0	1.3	4.3	24.3	2.3	28.3

Cunningham, Sabin, Sugiyama and Kindwall² have recently concluded, from studies of supravitaly stained films taken from the cut surface of the lungs of tuberculous animals, that the epithelioid cell is a modified monocyte.

Though we have confirmed all of their observations by repetition of their experiments, and in sections of lung tissue have actually

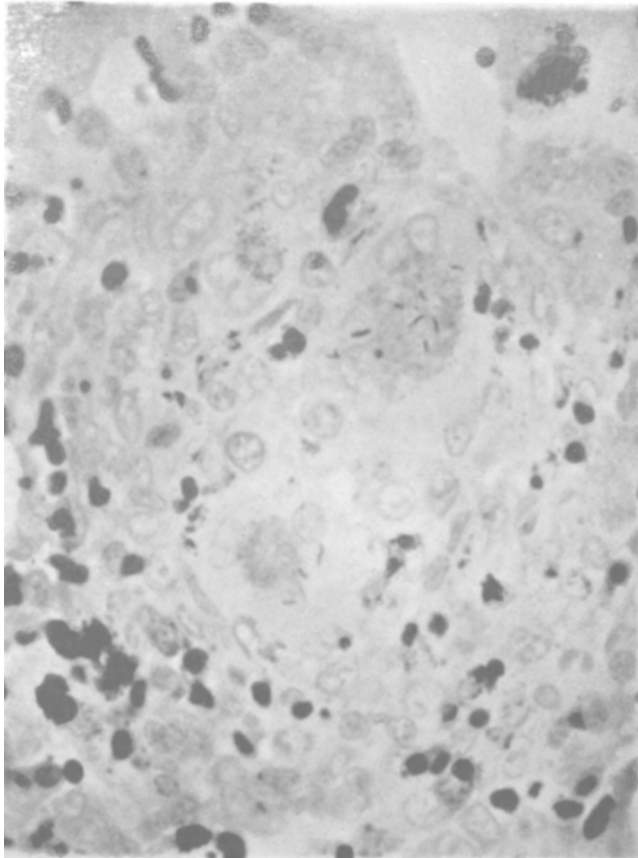


FIG. 2.

Slightly older tubercle showing two multinucleated epithelioid cells (giant cells). In each case the nuclei are arranged at the periphery of a single large neutral red rosette. These two cells contain many tubercle bacilli.

seen that the epithelioid cells are vitally stained with neutral red in the same manner as are the monocytes of the blood, we do not, at present, wish to support their interpretation of their findings, or take any other stand as to the origin of the epithelioid cell. The present report merely describes a method of study, and calls attention to certain striking vital staining characteristics of the epithelioid cells of tubercles, formed in the lungs, which have been demonstrated in sections.

¹ Sabin, Florence R., *Johns Hopkins Hosp. Bull.*, 1923, xxxiv, 277.

² Cunningham, Sabin, Sugiyama and Kindwall, *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 231.