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A Direct Method for Making Total White Blood Counts on Avian Blood.

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During the course of a standardization of the blood of fowls, undertaken as a part of a series of studies upon acid fast infections in various species of animals, it was found that no direct method existed for making total white blood counts upon avian blood. The methods commonly employed for mammalian blood, which embody the principle of destruction of the red cells in order that the white cells may thereby be counted without confusion, are not satisfactory for avian blood. This is because the circulating red blood cells in the bird are nucleated and, while acetic acid solutions hemolyze the red cells, they will not destroy their nuclei; the latter are thus liberated and are almost indistinguishable from small lymphocytes.

Because of this difficulty, various indirect methods based upon the ratio of red blood cells to white cells in the same smear were devised for making estimations of the total white cell counts of the bird. The reports in the literature for the blood of normal fowls when such methods were employed are quite divergent. Among the most careful studies that have been reported are those of Schmeisser[1] and of Warthin.[2] The former determined the ratio of red blood cells to white blood cells from smears stained with Wilson's stain. He found the ratio to vary between 1 to 40 and 1 to 150 with an average of 1 to 50. Using these ratios he calculated the total white blood counts and found they varied between 20,000 and 80,000 cells per cmm. Warthin, with similar technique, found the ratios between red cells and white cells to vary between 1 to 102 and 1 to 225, and the total white counts to vary between 12,000 and 29,000 cells per cmm.

In view of such divergent findings from the use of the indirect method, it was felt advisable to develop a direct method for making white blood counts upon chicken's blood. Inasmuch as the nuclei of the red blood cells can not be destroyed without destruction of the white blood cells it seemed indicated to devise a method which

* This work has been assisted by a grant from the Henry Strong Denison Medical Foundation.

[1] Schmeisser, H. C., *Johns Hopkins Hosp. Reports*, 1916, xvii, 551.

[2] Warthin, A. S., *J. Infect. Dis.*, 1907, iv, 369.

would leave the erythrocytes intact, and at the same time would stain the leucocytes without also staining the red cells. The ease with which neutral red is taken up by living white cells and the success of Gardner[3] and of Cash[4] in fixing the neutral staining granules of cells so that they may be studied in fixed material suggested a possible modification of these methods to meet the requisites of total white counts upon the fowl.

For this purpose two diluting fluids were employed. Solution I contained neutral red (1:5000) made up in Lock's solution and adjusted to a pH of 7.4. Solution II contained 12% formalin, also made up in Lock's solution and adjusted to a pH of 7.4. The procedure employed for diluting the blood with these solutions was as follows: The blood was drawn up to the 0.5 mark in a standard red blood pipette. The solution of neutral red (which was kept at a temperature of 39° C.) was then drawn into the pipette until the bulb was about one half full, and the pipette was shaken for 15 seconds. Following this, the pipette was filled to the 101 mark with the formalin solution, and shaking was continued for 2 to 3 minutes after which the counting chamber was filled. It was found that every white cell had taken up sufficient neutral red to make identification from the red cells, which had taken up no neutral red, very easy under a 4 mm. lens.

The accuracy of the method was determined in 2 ways. First, repeated counts were made upon the same chicken at different hours on the same day. Secondly, the method was employed simultaneously with the use of Turk's solution for counts upon human blood. Table I gives these comparison counts.

TABLE I.
Control counts of total white blood cells.

Chicken No. 241		Human Blood	
Hour	Neutral Red and Formalin Sol.	Turk's Sol.	Neutral Red and Formalin Sol.
1:00	26,800	6,000	6,500
1:30	26,000	5,100	4,900
2:00	29,600	5,100	4,950
2:30	28,000	5,650	5,250
3:00	29,600	7,100	6,650
		5,750	5,925
		5,450	5,250
		6,200	6,250
		6,350	6,100
		5,600	5,800

[3] Gardner, L. U., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 646.
[4] Cash, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 193.

It will be seen from the table that the variations in the different counts are not greater than would normally be expected. Counts made at the same time either with different methods or as duplicates with the same method agree within the usual limits. Counts made from hour to hour show variations which are in accord with those which Sabin, Cunningham, Doan and Kindwall[5] have shown to be normal. The greatly divergent variations reported from the use of the indirect methods are, however, not present, and it is permissible to conclude that this method offers increased accuracy over those previously employed in making total white counts of avian blood in which the nuclei of red blood cells are invariably present.

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Mechanism of Allergy in Tuberculosis.

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These experiments are part of an investigation into the mechanism of allergy in tuberculosis and its relation to immunity. This report is concerned with the problem of individual cell sensitiveness in the allergic animal.

The tuberculin reaction and the allergic lesions occurring in the body during the progress of infection are generally regarded as being, most probably, the result of an antigen-antibody reaction, in which bacillary products react with an antibody formed during the course of the infection. Rich and McCordock have been unsuccessful in attempts to demonstrate satisfactorily, *in vivo,* the presence of such an antibody in the plasma of allergic animals, and this has been the experience of others who have recently made similar attempts. The surmise has therefore arisen that if an antibody-antigen reaction is indeed responsible for the allergic inflammation and necrosis, this reaction probably takes place in or on the cells themselves, to which the antibody may be bound.

It has never been proven, however, that the isolated cells of the allergic body are really changed in a manner which renders them more sensitive than normal cells to the effects of the products of the tubercle bacillus. Studies *in vivo* are complicated by the presence

[5] Sabin, F. R., Cunningham, R. S., Doan, C. A., Kindwall, J. A., *Johns Hopkins Hosp. Bull.,* 1925, xxxvii, 14.