TABLE I.

I. SENSITIZED TO EGG ALBUMIN.

(a) Shocked with Egg Albumin.

No.	Weight	Dose	Effect
	gm.	cc.	
1	340	1	Gasping, convulsions, in 2 min. Collapse in 4 min. Dead—butterfly lungs.
2	295	0.5	After 2 min. dyspnea, 4 min. side position, 7 min. slight convulsions—recovered.
3	230	1	Immediate gaspings, convulsions, in 1 min. dead —butterfly lungs.
		(b) Shocked with Egg Albumin and Anayodin.	
4	260	1	After 4 min. dyspnea, 5 min. scratching—survived.
5	250	1	Rapid respiration, 28 min. side position, after 42 min. dead—very slight emphysema.
6	240	1	1 min. convulsions, after 5 min. dead—butterfly lungs.
		II. SENSITIZED TO EGG ALBUMIN AND ANAYODIN.	
		(a) Shocked with Egg Albumin.	
7	250	1	Immediate dyspnea, convulsions, side position. dead after 5 min.—butterfly lungs.
8	220	1	Cough, dyspnea—recovery.
9	315	1	Gasping, convulsions, after 4 min. dead—butterfly lungs.
10	215	1	Marked dyspnea, gasping, nervous, recovery.
		(b) Shocked with Egg Albumin and Anayodin.	
11	335	1	Dyspnea, convulsions, in 3 min. dead—butterfly lungs.
12	245	1	Rapid respiration, 4 min. highly dyspneic, 5 min. nervous—survived.
13	234	1	Gasping, convulsions, in 2 min. dead—butterfly lungs.

group of 13 animals Anayodin (Yatren) has no clearly demonstrable influence in preventing either sensitization or shock.

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An Improved Direct Method for Obtaining the Total White Cell Count in Avian Blood.

BRUCE K. WISEMAN. (Introduced by Charles A. Doan.)

From the Department of Medical and Surgical Research, Ohio State University.

Because of the normal presence of nucleated erythrocytes and thrombocytes in avian blood, it has always been difficult to count

accurately all of the white blood cells. For this reason, most direct methods have been based upon some technique providing for the recognition of specific types of cells in the counting chamber and then, from the percentage of these types found in the differential count made upon the stained smear, the total number of white cells per cu. mm. of blood have been calculated. To distinguish between the various types of white blood-cells, previous investigators have used a solution of vital neutral red alone,[1] vital neutral red with formalin in a separate solution,[2] or neutral red with a separate solution containing formalin plus crystal violet.[3] Neutral red, however, stains both granulocytes and monocytes, and it is often difficult to distinguish the one from the other under the powers of magnification that can be used in conjunction with the counting chamber. On the other hand, if one attempts to include both of these cell types in the count, the wide variation in staining intensity of the monocytes frequently occasions inaccuracies through the missing of individual cells. This is especially a source of error when the counting of blood cells is assigned to technicians. Moreover, it was found that the diluting fluids which do not contain a fixative frequently fail to preserve the red blood cells intact, the resulting hemolysis making a further complication in arriving at accurate counts of both the red and white blood cells.

The fact that the predominating granulocyte in avian blood contains eosin-staining granules suggested the use of the dye given below, which, when combined with a fixative to preserve the erythrocytes, stains these granular cells so as to make them stand out sharply and distinctly in the counting chamber. The solution now being used in this laboratory has the following formula: Phloxine 50 mgm., Formalin 5 cc., Ringer's Solution 95 cc.

This solution does not deteriorate and preserves the red blood cells indefinitely. Used as a diluting fluid, it stains the eosinophilic granules a brilliant red, imparting to these cells a distinguishing characteristic that is in marked contrast to the remainder of the cells seen in the counting chamber. All other cells take the stain much less brilliantly and are not to be confused with the eosinophilic leucocytes. The concentration of phloxine as given is such as to cause maximum staining within one hour. More dilute solutions (3 mg. dye in 100 cc. solution) stain these cells almost specifically, but the time interval required is at least 3 hours. Where

[1] Forkner, C. E., *J. Exp. Med.*, 1929, **50**, 121.

[2] Blain, D., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 594.

[3] Shaw, A. F. B., *J. Path. and Bact.*, 1930, **33**, 833.

time is not a factor of importance, the weaker solutions are recommended as the contrast between eosinophilic leucocytes and all other cells in the counting chamber is then optimal.

The blood, obtained from the wing-vein of the bird, is diluted 1-200 in the red blood cell pipette with this fluid. After standing the required interval, the pipette is then shaken for 2 to 3 minutes, the counting chamber filled in the usual manner, and the count made, using an 8 mm. objective, a 10X ocular, and a strong white light for illumination. The entire ruled area is examined, counting only the brilliantly red-stained eosinophilic leucocytes. This figure is used for calculation of the total white blood cells after the percentage of eosinophiles is obtained from the differential count taken

TABLE I.
Numerical Factors for Use in Obtaining Total White Blood cells per cu. mm. Avian Blood.

Eosinophiles %	0	1	2	3	4	5	6	7	8	9
0	—	22222.2	11111.1	7407.3	5555.5	4444.4	3703.6	3174.5	2777.7	2469.1
10	2222.2	2020.1	1851.8	1709.3	1587.2	1481.4	1388.8	1307.1	1234.5	1169.5
20	1111.1	1058.1	1010.0	966.1	925.9	888.8	854.6	823.0	793.6	766.2
30	740.7	716.8	694.4	673.3	653.5	634.9	617.2	600.5	584.7	569.7
40	555.5	542.0	529.0	516.7	505.0	493.8	483.0	472.8	462.9	453.5
50	444.4	435.7	427.3	419.2	411.5	404.0	396.8	389.8	383.1	376.6
60	370.3	364.2	358.4	352.7	347.2	341.8	336.6	331.6	326.7	322.0
70	317.4	312.9	308.6	304.4	300.2	296.2	292.3	288.5	284.8	281.2
80	277.9	274.3	271.0	267.9	264.5	261.4	258.3	255.4	252.5	249.6
90	246.9	244.1	241.5	238.9	236.4	233.9	231.4	229.0	266.7	224.4
100	222.2	—	—	—	—	—	—	—	—	—

Explanation: The values given in the body of the table are the corresponding factors for the percentage of eosinophiles given at top and side. To obtain the total white blood cells per cu. mm. of avian blood, multiply the number of eosinophiles obtained in the diluting fluid (1-200) in counting the entire ruled area of the counting chamber (0.9 cu. mm.) by the factor in this table corresponding to the percentage of eosinophiles as obtained by examination of the stained smear. Suitable accuracy (less than 100 cells) can be obtained by omitting the decimal from these factors.

at the same time. The red blood cells may be counted from the same preparation in the usual manner.

Derivation of the figure representing the total white blood cells per cu. mm. of blood depends upon calculating the number of eosinophiles in one cu. mm. diluting fluid ($\times$ 10/9) then correcting for the dilution ($\times$ 200) and finally multiplying this result by the fraction $\frac{100}{\text{percentage of eosinophiles}}$.

Since for any particular total cell count these values are all constants with the exception of the percentage of eosinophiles, it is possible to calculate a single factor for use with each percent of eosinophiles. This simplifies the above calculations to one step, namely, the multiplication of the number of eosinophiles counted in 0.9 cu. mm. diluted blood by the factor corresponding to the percentage of eosinophiles found in the differential count. Table I contains 100 factors each corresponding to a particular percentage of eosinophiles.

Two examples will clarify this explanation:

Example 1. *Without Use of Table:*

Number of cells counted in entire ruled area (0.9 cu. mm.) 26
Percentage of eosinophiles in differential count 60
Total white cells $26 \times 10/9 \times 200 \times 100/60 = 9{,}627$

Example 2. *With Use of Table:*

Number of cells counted in entire ruled area (0.9 cu. mm.) 26
Percentage of eosinophiles in differential count 60
Total white blood cells $26 \times 370.3 = 9{,}627$

It is believed that both technical and mathematical errors will be reduced in observations on avian blood by the adoption of the procedures outlined in this presentation.

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Preliminary Observations on Human Blood in Early Syphilis by the Supravital Method.

SAMUEL T. MERCER. (Introduced by Charles A. Doan.)

From the Department of Medical and Surgical Research, Ohio State University.

Cells in the circulating blood frequently reflect the pathological process which is occurring in the tissues. Reports of cellular changes