must be present in the diet in order to maintain nitrogen equilibrium and that this amino acid may be regarded as a human dietary essential.

Weight curves and nitrogen balances on 3 subjects on a cystine-deficient diet are given in Figs. 3 and 4. It will be noted that the female subject weighing 55 kg maintained her weight and remained in nitrogen equilibrium throughout the experiment. The 2 male subjects, both of whom weighed 75 kg at the outset, lost weight throughout the deficiency period. One of them showed a definite negative nitrogen balance during the deficiency period with a return to nitrogen balance in the post period when the cystine containing diet was substituted. The other subject showed only a minimal negative N balance during the deficiency with some indication of a rise in N retention in the post-period. We do not feel justified in concluding from these experiments that cystine is a human dietary essential. Although the evidence is somewhat suggestive that this may be the case, it would seem desirable to obtain confirmatory data from further experiments in which the energy quotient is constant for all subjects and protein sparing effects are eliminated.

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Enumeration and Differentiation of Leukocytes in the Counting Chamber with Propylene Glycol-Aqueous Stains.*

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The hemolytic and solvent properties of propylene glycol suggest its use as a white blood cell diluting fluid and staining base.

Noting the occurrence of hematuria in animals following the administration of various glycols,[1,2] Von Oettingen and Jirouch[3] demonstrated that the glycols exert a hemolytic action *in vitro*. Lehman and Newman[4] in 1937 mixed propylene glycol with water, thereby discovering that all concentrations produced immediate hemolysis of rabbit blood. Similar observations with human blood were made independently by Weatherby and Haig.[5] The effect of these mixtures on the white blood cells was not mentioned.

The following observations were made by the author. Freshly drawn human blood was mixed one part in 20 parts with equal proportions of propylene glycol and water and with a concentration of 55% propylene glycol and 45% distilled water. Within 5 min the red blood cells became almost invisible, a few remained in the background but in no way interfered with the counting of the white blood cells. Leukocyte counts remained within the limits of normal variation for a period of 60 hr. (Table I.)

The mixture of propylene glycol and water in these proportions possessed many desirable features as a white blood cell diluting fluid. Its increased viscosity compared with aqueous solutions was a distinct advantage; in filling the pipette the end point was reached more accurately, there was less settling and clumping after standing in the pipette and there was less tendency to over-

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1 Bachem, C., *Med. Klinik.*, 1917, **13**, 7.

2 Page, I. H., Coryllos, P., *J. Pharm. and Exp. Therap.*, 1926, **27**, 189.

3 Von Oettingen, W. F., and Jirouch, E. A., *J. Pharm. and Exp. Therap.*, 1931, **42**, 355.

4 Lehman, A. J., and Newman, H. W., *J. Pharm. and Exp. Therap.*, 1937, **60**, 312.

5 Weatherby, J. H., and Haig, H. B., *J. Am. Pharmaceut. Assn.*, 1938, **27**, 466.

TABLE I.

Concentration of propylene glycol	Concentration of distilled water	30 min	2 hr	12 hr	36 hr	60 hr
50%	50%	7450	6450	6500	7200	7150
55%	45%	7250	7200	7050	7000	6850

flow the counting chamber. The background of the chamber remained clear in contrast to the debris present with acetic acid diluting fluid. The slower rate of evaporation from the chamber allowed at least 30 min for the enumeration and study of cells. When dealing with normal blood the accuracy of the total leukocyte count was not altered by standing in the pipette or by the degree of shaking.

When 0.05% eosin was dissolved in solutions containing 50 or 55% propylene glycol in distilled water the eosinophils stained brilliant red allowing their identification in the counting chamber. With 0.05% eosin and 0.05% methylene blue in the same proportions of propylene glycol and water the nuclei of all cells stained blue but the cytoplasm remained clear. The granules of the eosinophil stained rose color but allowed the blue staining nucleus to be seen through them. With low magnification (16 mm objective) the eosinophil was differentiated from other leukocytes by its color and slightly larger size. The contrast in color was intensified by increasing the light. With high magnification (4 mm objective) polymorphonuclear cells were differentiated from mononuclear cells, including lymphocytes, with sufficient clarity to allow their enumeration.

The most satisfactory results were obtained when methylene blue was dissolved in propylene glycol and later mixed with equal parts of aqueous eosin. The 2 stock solutions are made up as follows:

Solution I.	
Methylene blue	0.1 g
Propylene glycol	100.0 cc
Solution II.	
Eosin B†	0.1 g
Distilled water	100.0 cc

† Eosin Y was also used and found satisfactory although it stained the eosinophil granules more lightly than eosin B. If staining is not satisfactory the eosin content may be increased.

Mix equal parts of solutions I and II and use as the diluting fluid in the standard white blood cell pipette. The total and differential leukocyte counts are made in the standard counting chamber. A period of 3 min from the time the counting chamber is filled is required for the red blood cells to fade and for the leukocytes to settle sufficiently to count. If the chamber is filled immediately after diluting and mixing, a period of 15 min is recommended for the maximum differential staining of the white cells. A more satisfactory procedure is to allow the mixed blood to remain in the pipette for 15 min prior to filling the chamber.

It sometimes becomes necessary to filter Solution II as the eosin may precipitate on standing. The presence of a fine precipitate in the counting chamber indicates that the eosin solution requires filtering.

When made up for immediate use, filtering of the mixed stain is not essential. After standing 24 hr the mixed stain is less effective in that the outlines of the nuclei are less sharp and precipitation may occur. However, the staining of eosinophils remains unchanged.

Comparative blood studies were made in 20 normal subjects as recorded in Table II. These individuals, members of the hospital personnel, were not known to be suffering from any constitutional or local disease. Four pipettes were filled from the same puncture wound of each person. From these, 2 counts were made with the standard acetic acid diluting fluid, 2 with the stains dissolved in equal proportions of propylene glycol and water. The differential counts of 200 cells from the Wright stained coverslip, and from the direct counting chamber method, are recorded. In general there is good agreement.

Diluents with 50 and 55% propylene glycol are equally effective in determining the total leukocyte count in normal blood. Eosinophils stain well with either diluent.

TABLE II.

Total and Differential Leukocyte Counts by Standard Methods (Acetic Acid Diluent and Wright Stained Films) Compared with Direct Counting Chamber Determinations Using Propylene Glycol-Aqueous Stains.

	Total white cells				Differential white cell counts: Wright stained smear						Differential white cell counts: Propylene glycol stain		
	Acetic acid		Propylene glycol										
Case					Polymorphonuclears	Lymphocytes	Eosinophils	Basophils	Monocytes	Smudges	Polymorphonuclears	Mononuclear cells	Eosinophils
D.F.	5450	4600	5000	5350	60.0	28.5	0.5	2.0	1.0	8.0	65.0	33.5	1.5
E.H.	5200	4900	4650	5550	61.0	36.5	1.5	0.0	0.0	1.0	66.0	33.0	1.0
A.L.	9150	8750	10900	9200	65.5	27.5	2.0	0.5	4.0	0.5	64.5	29.5	1.0
A.H.	6800	6450	6950	7100	59.5	38.0	1.0	0.0	1.0	0.5	55.5	42.5	2.0
M.W.	4050	4350	4100	3750	69.0	29.0	1.0	0.0	0.5	0.5	61.5	37.5	1.0
M.S.	3650	4250	3450	4200	50.0	47.5	0.0	1.0	1.5	0.0	51.5	47.0	1.5
H.S.	4950	5050	5300	6450	57.0	33.0	3.0	2.0	2.0	3.0	61.0	35.0	4.0
S.S.	7100	6800	7850	7000	54.5	40.0	1.0	0.5	1.0	3.0	59.0	40.5	0.5
B.C.	6750	6350	6000	6050	60.0	25.0	2.0	3.0	0.0	6.0	67.0	32.5	0.5
D.J.	6150	6400	5000	5950	63.0	27.5	5.5	0.0	0.0	4.0	65.0	32.5	2.5
E.G.	4500	4750	4750	4550	64.0	32.0	2.0	0.5	0.0	1.5	65.5	31.5	3.0
B.Y.	9450	9000	10500	9900	80.0	19.5	0.5	0.0	0.0	0.0	76.5	22.0	1.5
M.L.	6000	7350	6750	7450	70.5	24.0	1.5	1.0	0.5	2.5	79.0	21.0	0.0
T.R.	6050	5000	5250	5650	72.0	22.5	1.0	1.0	0.5	3.0	71.5	26.0	2.5
R.G.	7700	8900	7750	7000	45.0	42.5	1.0	1.0	3.0	7.5	55.0	42.0	3.0
P.W.	6300	6200	6500	5650	40.5	49.5	7.0	0.5	0.5	2.0	48.5	44.5	7.0
P.T.	9100	9250	9350	9900	68.5	24.0	2.5	2.0	0.0	3.0	74.0	21.5	4.5
J.W.	7750	7250	8050	7100	77.0	22.0	0.0	1.0	0.0	0.0	78.0	20.5	1.5
J.D.	9700	8500	8400	7900	76.5	20.0	2.0	0.5	0.0	1.0	79.5	19.0	1.5
G.P.	5750	6300	7100	7000	68.5	20.0	1.0	0.5	2.0	7.0	72.0	26.5	1.5

With 55% propylene glycol the white cell nuclei stain slightly less rapidly and intensely than with equal proportions of propylene glycol and water. When normal blood is allowed to stand in these 2 dilutions for 12 hr or longer there is a slightly greater tendency for distortion of white blood cells in 50% propylene glycol. From evidence available the higher percentage of propylene glycol may possibly prove more satisfactory in the study of abnormal blood.

The chief technical disadvantages in performing differential white blood counts from the stained film appear to be the inequal distribution of large white cells and identification of smudge cells. The former occurs particularly with eosinophils. Marked variations in the number of eosinophils between the two sides or between the center and margins of a coverslip preparation are common observations. The paucity of our present knowledge regarding the function of the eosinophil may be due in part to the inherent errors in the accepted method of counting these cells.

The routine procedure in reporting differential white blood cell counts is to ignore the unidentified, ruptured cells unless their number is relatively high. This error is also magnified by imperfections in the technic of preparing the film.

The use of the direct counting chamber differential count in conjunction with the stained film differential white cell count may result in additional evidence as to the identity of these smudge forms and may possibly control the errors of distribution resulting from variations in cell size. If this proves to be the case the actual percentage of eosinophil, polymorphonuclear, and mononuclear cells may be computed more accurately. This method is not presented as a substitute for the stained film.

Summary. A diluent of eosin and methylene blue in equal parts propylene glycol and water not only gives accurate total leukocyte counts but stains eosinophils, polymorphonuclear and mononuclear cells differentially and permits their enumeration in the counting chamber.