treated with 1 cc of Wilson's solubilized liver suspension for each of 10 successive days. The data for these animals are given in Table II, and as noted all showed some response at the end of five days; near normal levels were observed after 10 days of treatment. The one anemic animal not included in the table had an initial hemoglobin value of 6.5 gms per 100 cc. After 10 days of treatment with another fraction, it had fallen to a level of 2.3 grams per 100 cc, the hematocrit remaining unaltered at 18 cc per 100 cc. Treatment with the suspension of Wilson's solubilized liver was then given for 12 days. The hemoglobin level rose to 11.5 grams per 100 cc and the hematocrit to 56 cc per 100 cc. All the 14 animals treated with liver fractions and the three treated with brewers' yeast showed a resumption of growth and a marked clinical improvement.

TABLE II

BLOOD VALUES SHOWING A RESPONSE OF SEVERELY GRANULOCYTOPENIC OR ANEMIC RATS TO TREATMENT WITH LIVER FRACTIONS

	Number of experimental rats	Before treatment		Number of days of treatment	After treatment		Normal values cited in literature	
		mean	range		mean	range	mean	range
Total polymorphonuclear granulocytes per cu mm	10	29	0–103	4	3,203	1,550–5,760	3,465	1,200–6,825
Percentage of polymorphonuclear granulocytes	10	0.8	0–3	4	30.1	21–41	29.9*	15–45.5*
Total white blood cells per cu mm	10	3,808	550–7,320	4	10,550	4,950–16,950	11,590*	8,000–15,000*
Hemoglobin in gms per 100 cc	4‡	7.0	6.4–7.4	5	9.1	8.2–9.7	15.6*	14.0–17.2*
				10	13.6	13.0–14.1		
Red blood cell volume (Hematocrit) in cc per 100 cc	4‡	24	21–27	5	35	33–39	50†	.....
				10	45	41–47		

* R. A. Scarborough, *Yale Jour. Biol. and Med.*, 3: 267, 1931.
† A. J. Creskoff, T. Fitz-Hugh, Jr., and E. J. Farris, in "The Rat in Laboratory Investigation," edited by J. G. Griffith and E. J. Farris, p. 351. Philadelphia: J. B. Lippincott Co., 1942.
‡ One additional animal is discussed in the text.

The mechanism of action of the sulfonamide drugs in producing these blood dyscrasias as well as other toxic manifestations is obscure. It has been suggested[1] that there may be an indirect toxicity such as an interference with tissue enzyme systems. Also, the possibility was considered that such an indirect toxicity, a direct toxicity and a reduction of intestinal synthesis of essential factors might all be involved.

SUMMARY

(1) A severe granulocytopenia or anemia, or both, have been produced in rats fed sulfathiazole, sulfadiazine or sulfanilamide at a 1 per cent. level in purified diets.

(2) Treatment with certain liver fractions orally has succeeded in correcting the granulocytopenia in four days and the anemia in about 10 days in spite of continued ingestion by these animals of the sulfonamide-containing diet.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DETERMINATION OF CELL VOLUME AND HEMOGLOBIN ON THE SAME DROP OF BLOOD

AN adequate clinical description of the erythrocytes in blood requires the determination of the cell volume, the hemoglobin content and the cell count.

A method for determination of cell volume, called the cell opacity method, has been described recently.[1] It involves the measurement in a photoelectric colorimeter of the transmission of light at a wave length of about 660 mμ through a suspension of blood in citrate solution. The cell opacity thus recorded is proportional to the cell volume, which can be read from a line or table showing this relationship. This method requires only 5–25 cmm of blood.

A method for the determination of hemoglobin by the measurement in a photoelectric colorimeter of the transmission of light of about 540 mμ has been described by Evelyn.[2] This determination requires a similar amount of blood.[3]

[1] A. T. Shohl, *Jour. Lab. and Clin. Med.*, 25: 1325, 1940.

[2] K. A. Evelyn, *Jour. Biol. Chem.*, 115: 63, 1936; also K. A. Evelyn and H. T. Malloy, *ibid.*, 126: 655, 1938.

It is convenient to make both measurements on the same sample of blood. After the cell opacity has been determined, the blood is hemolyzed and the hemoglobin quantitated with only a slight modification of Evelyn's technique. The blood is hemolyzed by the addition of one drop of saponin solution to the red cell suspension. Merck's saponin has been found to be satisfactory, but different samples have different degrees of activity and deteriorate at different rates. We have used an aqueous solution containing approximately 5 gm of saponin made up to 10 cc. It will retain its efficacy for 3–4 weeks or longer if kept in the refrigerator. The criterion of its adequacy is that one drop should hemolyze the blood and give a clear solution in less than one minute. The oxyhemoglobin thus obtained is made alkaline by the addition of one drop of concentrated ammonia (20 per cent. ammonia gas). Saponin and ammonia are added to the blank containing citrate solution only, and the colorimeter adjusted with a filter transmitting light at or near 540 mμ. The tube containing the sample is then inserted in the colorimeter and the galvanometer reading is interpreted in terms of hemoglobin concentration.[3] Parallel determinations by Evelyn's method and by this variation of it give the same values for hemoglobin.

This method uses such a small amount of material and is so easy to carry out that it may be surmised that accuracy has been sacrificed to convenience. Such is not the case. Each of the methods has been checked, the cell opacity against the hematocrit method and the hemoglobin against the oxygen capacity method. The results obtained show no greater errors than the standards against which they were compared.

To obtain maximum accuracy it is necessary to have carefully calibrated pipettes. However, if the readings of duplicate or multiple determinations made with different pipettes on aliquots of the same blood *in vitro* are not in agreement for the cell opacity, it will be found that the values for the amount of hemoglobin show corresponding variations. Each pair of determinations shows the same relationship because they are made on the same sample, regardless of its size. Thus, even if the pipettes are inaccurate, the relationship between the cell volume and hemoglobin concentration is accurately shown, and for some purposes it is more important to know the relative than the absolute values. Furthermore, two samples of capillary blood taken from the same individual may contain different amounts of plasma, tissue fluids and corpuscles. This source of error in sampling also is eliminated by the combined method.

[3] The full technique for use of the photoelectric colorimeter is described in the manual which is supplied with both the Evelyn and the Klett-Summerson colorimeters.

Attempts have been made to use the transmission of light through a cell suspension for erythrocyte count with the Evelyn colorimeter[4] or the Exton scopometer.[5] These methods have been unsuccessful[6] in so far as they give approximately correct values only when the cells are of constant and normal size. It is primarily the total mass of cells and only secondarily their size which determines the amount of light transmitted.[7] This is shown clearly in the example of rat blood, which gives values similar to those of human blood for cell opacity and has a similar cell volume, but a cell count nearly twice as high.[8] However, if dealing with physiological conditions in which the size of the cells does not vary, the cell opacity gives a correlation with cell count as well as with cell volume.

Most physicians are more familiar with the clinical interpretation of cell count than of cell volume. The erythrocyte count as obtained by the cell opacity method may be used as an approximation for orientation, assuming the cells to be of normal size. For an accurate description of the erythrocytes in blood a cell count should be done in conjunction with the determination of cell volume and hemoglobin content. For the latter two the combined method given above has proved to be simple, accurate and economical of both time and material.[9]

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GROWTH OF ORCHID SEEDS AFTER DEHYDRATION FROM THE FROZEN STATE

CERTAIN orchid seeds have germinated after eight and even fourteen years[1] when stored under optimum conditions, that is, in dry air at 46° F. In general, however, their lifespan is considerably shorter. A method which has proven outstandingly successful in the preservation of such biological materials as bacteria, filterable viruses and serum proteins is that of desiccation from the frozen state. It was thought to be of both practical and theoretical interest to determine, firstly, whether orchid seeds could survive and

[4] R. V. Christie and K. A. Evelyn, *Jour. Clin. Invest.*, 13: 704, 1934.
[5] W. G. Exton, *Am. Jour. Clin. Path.*, 7: 42, 1937.
[6] Unpublished data of Dr. G. M. Guest.
[7] D. Drabkin and R. B. Singer, *Jour. Biol. Chem.*, 129: 739, 1939.
[8] A. T. Shohl, K. D. Blackfan and L. K. Diamond, *Proc. Soc. Exp. Biol. and Med.*, 45: 383, 1940.
[9] Recent notes by F. T. Hunter, *Jour. Clin. Invest.*, 19: 691, 1940, and D. H. Duffie, *Jour. Am. Med. Asn.*, 119: 493, 1942, have shown the possibility of substituting sodium carbonate for ammonia. This procedure recommends itself in a laboratory where nitrogen determinations are being made. At present we add 1 drop of 5 per cent. sodium carbonate instead of ammonia.
[1] L. Knudson, *Amer. Orchid Soc. Bul.*, 9: 36–38, 1940.