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.

grow after subjection to this process; and secondly, if so, whether the lifespan was increased. This paper reports the survival and growth of orchid seeds when vacuum dried after freezing at low temperatures.

A miniature, "lyophile" apparatus[2] described by Flosdorf and Mudd[3] was used, and with slight modifications the procedures outlined by these authors were applied to orchid seeds. The seeds used were the results of two primary crosses, *Cattleya Loddigesii* crossed upon *Cattleya Schroederiana* and *Laelia anceps* crossed upon *Cattleya Trianaei*. These species are natives of either Brazil, Colombia or Mexico and grow in climates where freezing temperatures are unknown or unusual.

In comparison with seeds of most flowering plants, those of orchids are very small. The ones used in this experiment measured on an average 527.7 μ by 73.5 μ. They had been stored for 7 months in a glass jar in a refrigerator. Their moisture content was not determined before they were "lyophiled" nor after, at the time of planting. The percentage of moisture in seeds of another cross stored in the refrigerator under similar conditions for 4 months has been found to be 1.5 per cent. The residual moisture which Flosdorf and Mudd found remaining in bacterial preparations after subjection to the "lyophile" process was 0.5 per cent.[3]

Two sets of tubes were prepared for desiccation. In one, sterile blood serum (commonly utilized in the preservation of bacteria by this method) was used as the suspending fluid and in the other, autoclaved coconut liquid. A mass of seeds which would approximate two drops of water in volume was placed in each tube. The tubes were plugged with sterile cotton and thoroughly agitated so that the seeds became completely coated with the liquids. This required some manipulation, for, due to the structure of orchid seeds, the outer alar cells resist wetting. The tubes were plunged into the dry ice bath of the main condenser of the apparatus at a temperature of −78° C. for about three minutes until thoroughly frozen. They were then attached to the vacuum manifold, being kept immersed at the same time in a dry ice bath maintained at −5° to −10° C. The vacuum pump was started and dehydration allowed to proceed for three to four hours. At the end of the second hour, the tubes were removed from the cold bath and held at room temperature. They were sealed off while under vacuum with an oxygen flame. These procedures (using the same volume of suspending fluid, degree of vacuum and time of desiccation) had given consistently satisfactory results with this apparatus in the preservation of bacteria.

[2] Made available through the courtesy of the Department of Bacteriology at the University of Washington, Seattle, Wash.

[3] E. W. Flosdorf and S. Mudd, *Jour. Immunol.*, 29: 389–425, 1935.

Half of the tubes were stored for future tests of viability. The other half were broken open and the contents planted. Following Knudson's technique[4] for growing orchid seeds non-symbiotically, they were planted aseptically on a nutrient agar medium using a slightly modified Knudson's Solution B[4] with sucrose as the sugar and the pH 6.3. Some of the contents of the tubes were planted directly from the tubes while others were first sterilized with hypochlorite solution after Wilson's method.[5] No immediate contamination resulted from either of these procedures.

None of the seeds which had been immersed in blood serum germinated. All but one tube of those suspended in coconut liquid not only germinated but, with the exception of a few flasks which later became contaminated, grew satisfactorily. One flask containing seeds of *Cattleya Loddigesii* crossed upon *Cattleya Schroederiana* germinated within the short period of seven days, which was four days before the controls showed signs of germination. After four months, most of the seedlings had two leaves and one or two roots and were sufficiently large to remove from the containers and plant into community pots.

Although various kinds of seeds have survived exposure to low temperatures after varying degrees of drying,[6] to our knowledge this is the first time that seeds have been subjected to the "lyophile" process and have grown. Seeds of the tuberous begonia and snapdragon, which are roughly 2 to 10 times the size of orchid seeds, were similarly treated but failed to survive and grow.

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[4] L. Knudson, *Bot. Gaz.*, 73: 1–25, 1922.

[5] J. K. Wilson, *Amer. Jour. Bot.*, 2: 420–427, 1915.

[6] B. J. Luyet and P. M. Gehenio, *Biodynamica*, Normandy, Missouri, 1940.

[7] Formerly with the University of Washington.

BOOKS RECEIVED

COLEMAN, LAURENCE VAIL. *Company Museums.* Illustrated. Pp. viii + 173. American Association of Museums. $2.50.

FREEMAN, FRANK N. and M. A. WENGER. *The Chicago Mental Growth Battery.* Illustrated. Pp. v + 58. The University of Chicago Press. $1.00.

JOHNSON, WILLIAM H. and LOUIS V. NEWKIRK. *Fundamentals of Electricity.* Illustrated. Pp. x + 212. Macmillan. $2.00. *Fundamentals of Shopwork.* Illustrated. Pp. viii + 200. Macmillan. $2.00.

LUCK, JAMES MURRAY. *Annual Review of Physiology.* Pp. vii + 613. Annual Reviews, Inc. $5.00.

SARGENT, PORTER. *War and Education.* Pp. 506. Porter Sargent. $4.00.

TULEEN, LAWRENCE F., GEORGE S. PORTER and ARTHUR HOUSTON. *Prepare Yourself.* Illustrated. Pp. vi + 298. Scott, Foresman and Company. $0.96.