

ance of hair. The dietary essential which prevented or cured the pathological manifestations was termed the mouse anti-alopecia factor. In its absence from the diet, growth ceased and extensive alopecia developed, followed by severe dermatitis. Liver was found to be a good source of the protective factor, and concentrates were prepared from this organ.

Many of the properties of the active substance suggested that a phosphoric ester of inositol was involved. For example, our best concentrates contained organically-combined phosphorus. The active substance was precipitated by barium hydroxide and was insoluble in alcohol. Furthermore, it was found that cereal grains, known to be good sources of the phosphoric ester of inositol, were also good sources of the anti-alopecia factor. All these facts seemed to justify a test of the potency of phytin, the salt of inositol phosphoric acid. While the assays were in progress, a crystalline substance was isolated from active liver concentrates which proved to be inositol. Phytin caused restoration of hair and resumption of growth in the depleted animals. Activity was not limited to the phosphoric ester, for inositol itself was subsequently found to be potent.

Assays were performed as described previously, and were always based on curative rather than pre-

ventative technique. For both phytin and inositol the level fed was 100 mg per 100 gm of ration. Cures have also been obtained with 10 mg of inositol per 100 gm of ration. Gain in weight, as well as restoration of hair, followed administration of these substances.

Inositol was isolated from the non-dialyzable, alcohol-insoluble portion of aqueous liver extract. This fraction had been autoclaved with alkali in order to render the active substance dialyzable. The crystals which were obtained melted at 214–216° and had a carbon content of 39.8 per cent. Inositol in the same bath melted at 215–216°.

The above facts indicate that the mouse anti-alopecia factor is inositol or its derivatives. They suggest that inositol exists in liver in alkali-labile combination with a large molecule which renders the former non-dialyzable. While it has been reported² that inositol stimulates the growth of certain strains of yeast, its place in the nutrition of higher animals has not previously been observed. Details of the work will be published elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW TYPE OF SHIELDED GLASS ELECTRODE¹

THE high resistance of glass electrodes and their consequent sensitivity to stray currents require careful shielding in addition to an adequate insulation. The shielding is important when the electrode is to be employed at distances from the measuring apparatus that require long leads. This shielding is ordinarily accomplished by the use of a more or less flexible metal sleeve, thus providing an equipotential layer around the lead. The sleeve is conveniently connected to the equipotential shield about the measuring apparatus. Commercially manufactured shielded wires are frequently employed.

The special feature of the shielded glass electrode now to be described is an arrangement by means of which the KCl-solution which ends in a fluid junction surrounds completely the stem of the electrode and the entire lead up to the terminal of the measuring apparatus. In this manner the KCl-solution that is a part of the low resistant half of the circuit acts as a shield against external stray currents. With our equipment best results were obtained when the termi-

nal of the reference half cell was connected to the equipotential shield around the potentiometer or directly to the ground.

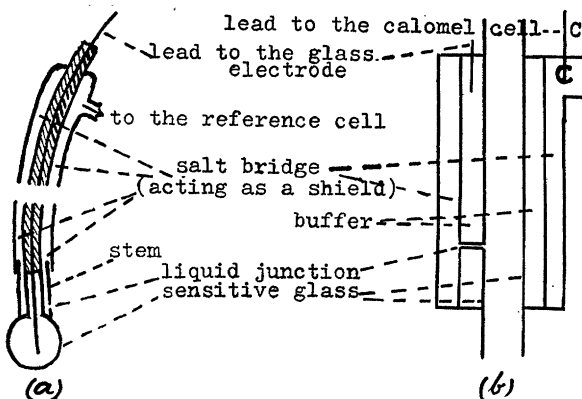


FIG. 1. Diagrams of glass electrodes (a) bulb type, (b) condenser type with the use of the salt bridge as a shield.

Fig. 1 shows two types of glass electrodes, the bulb type (a), and the condenser type (b), also used as a continuous flow electrode with the use of the KCl-solution as a shield.

The arrangement calls for a very careful insulation

² E. V. Eastcott, *Jour. Phys. Chem.*, 32: 1094, 1928.

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of the leading wire against the surrounding fluid. Its advantages are twofold: (1) With a metal shield there is always some space between the shield and the conductor, thus producing a condenser with the resulting disturbances usually seen on bending the metal shielded wire. In the type described, the KCl-salt bridge fills the space between the insulated wire and the outer cover entirely and follows uniformly any movement of the flexible lead. Merely a film of liquid or semi-liquid, as used in agar-KCl or similar bridges, is sufficient for the shielding. BaSO₄ or another x-ray-opaque material may be added when location of the electrode by fluoroscopy is desired. (2) The combination of the glass electrode and the reference half cell in one piece saves space and is easy to handle. Even a calomel or another reference half cell may be incorporated to form one piece with the electrode as seen in Fig. 1 b. The use of this type of shielded electrode is of particular advantage in the measurement of pH in body cavities.

A more detailed description of a measuring device, in which this type of shielded glass electrode is used in the determination of intestinal pH, with a report of clinical findings will be presented later.

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A DIRECT METHOD OF DETERMINING THE ERYTHROCYTE, LEUCOCYTE AND THROMBOCYTE COUNT OF FOWL BLOOD

A MODIFICATION of the Blain method of staining the leucocytes in bird blood yields a rapid, reliable means of enumerating the cellular elements of the blood in the counting chamber. Two solutions are employed, the first containing the stain and the second the preservative for the cells. Because granulocytes have an affinity for brilliant cresyl blue and lymphocytes for pyronin, a stock solution of these is prepared consisting of 1 cc of 1 per cent. aqueous brilliant cresyl blue and 0.25 cc of saturated aqueous solution of pyronin (1 gram in 15 cc of water).

The first solution for staining used in the method described herein consists of 0.2 cc of the stock dye mixture in 25 cc of normal saline. This is filtered once through neutral paper. The second solution is that employed by Blain—12 per cent. of formalin in Locke's solution.

Blood is procured from the wing vein, and immediately after puncture it is drawn up to the 1 mark in the red-cell counting pipette. The pipette is then half filled with the first solution, gently rotated for about five seconds, and then filled to the 101 mark with the second solution and shaken for half a minute to mix. Counts may be made at once, although better differentiation is obtained if the mixture is allowed to stand

15 minutes or more. Thin covers, not more than 0.5 mm in thickness, are used, and the preparation is studied under a 4 mm objective.

The erythrocytes appear as pale oval discs with the nuclei sometimes barely perceptible. The thrombocytes vary from pale ovals slightly smaller than the erythrocytes and with a single polar granule, to small lance-shaped, faintly lilac-colored bodies. The latter type tends to occur in clusters and is more numerous in specimens made just before clotting begins, *i.e.*, after one or more pipettes have been filled in succession from the same puncture. The heterophiles take varying quantities of the blue dye and stand out sharply as blue-black bodies slightly smaller than the erythrocytes. The lymphocytes are uniformly round, faintly pink bodies, clearly distinguishable from thrombocytes, which in their colorless state appear to belong more to the erythroblast series. Monocytes are indistinguishable from the large lymphocytes, but since this is not primarily a differential count, that is not important.

Checked against Shaw's method over a series of blood counts on the bobwhite quail, this method gives approximately the same total leucocyte count and has the added advantage that the solution is more stable than Shaw's. The solution containing the dye will keep for about a week. The stock solution is less stable, but the dyes kept separately in solution are good indefinitely. The formalin solution must be made fresh each day, but adjustment of the pH and temperature are unnecessary.

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