when crude casein was used in the ration, and a still higher degree of prevention was attained when the purified casein mixture was supplemented with 5 per cent. of powdered yeast. When an equivalent quantity of liver extract was used as a supplement, however, the increase in number and color of red cells was greater, growth was accelerated more, and development was made through successful metamorphosis; this treatment was also found to have a curative effect. Yet, 5 per cent. or 10 per cent. of liver extract added to the casein-milk powder diet did not produce so high a level of growth and erythrocytes as resulted from use of the raw beef liver or the synthetic beef muscle diet; furthermore, metamorphosis was not attained so early.

The rate of growth, red cell formation and development toward metamorphosis was proportional to the amount of a particular liver extract included in the diet, but quantitative relations were not exact between different extracts known to be of the same clinical potency. This led to the question whether the beneficial effect of liver extracts upon these larvae was due to the pernicious anemia factor or to some other constituent. As supplementation with liver extracts did not entirely compensate for the inadequacy of the diet with purified casein, it seemed probable that the deficiency might be due to poor protein constitution or digestion. Accordingly, some of the amino acids which were low in this diet were increased to the levels contained in the synthetic beef muscle mixture. Cystine alone or with alanine produced no significant effect upon growth or erythrocyte level; nor did their combination with glycine, although the three together caused an improvement of general condition. When arginine was added to the casein-milk powder diet with cystine, there resulted a striking and prolonged increase in erythrocytes, hemoglobin and growth; the improvement produced by the arginine was not permanent, however, unless liver extract also was used as supplement.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

DIFFERENTIAL STAINING OF THICK SECTIONS OF TISSUES

WHILE studying human scalps for racial differences it was found that thin sections did not give a clear idea of the relationships of the hair follicle to its appendages and adjacent tissues. As many reconstructions were impracticable, thick free-hand sections were made, after the manner of the early histologists, and cleared in iso-safrol. Later, thick sections from tissues embedded in nitrocellulose were cut and a technique for differential staining developed.

Sections prepared as outlined below lend themselves well to low- and high-power study. The stereoscopic effect obtained with a binocular dissecting microscope is especially adapted to study of the topography of tissues, and with a strong light source the finer details can be studied with high dry and oil immersion objectives. The transparency of the stain depends upon slow, dilute staining and long differentiation as well as upon thorough dehydration before clearing. This technique has been tested on different tissues; the less dense tissues afford good results up to 400 micra. In general, the density of the tissue must be a guide to the thickness. The time given below for the steps in the procedures will vary slightly according to the type of tissue, kind of fixation and the thickness. The time of differentiation, as in the case of thin sections, is determined according to the particular structures to be shown.

Sections 100 to 400 micra were cut by microtome from tissues embedded in low viscosity nitrocellulose. One hundred micra sections may be cut serially in 95 per cent. alcohol and fixed to slides with Mayer's egg albumin by Rubaschkin's clove oil method, but thicker sections must be handled separately. Best results are obtained when the nitrocellulose is removed before staining, although the procedures given below for hematoxylin-eosin and iron hematoxylin may be used when the nitrocellulose is retained to support the tissues.

Tissues should be turned frequently to allow penetration of the reagents from all sides.

I. *Removal of nitrocellulose and preliminary to staining:*
   (1) Absolute alcohol—1 hour.
   (2) Equal parts ether and absolute alcohol—24 to 36 hours; change solution once.
   (3) 75 per cent. alcohol—30 minutes.
   (4) Distilled water—30 minutes.
   For tissues fixed in Zenker's solution:
   (5) Lugol's solution—12 to 24 hours.
   (6) Hypo solution, 5 per cent.—until white—1 to 2 hours.
   (7) Distilled water—8 to 12 hours.

II. *Hematoxylin-eosin:*
   (1) Delafield's hematoxylin, 5 to 8 drops in 50 cc distilled water—8 to 12 hours.
   (2) Tap water or distilled water containing a few drops of lithium carbonate until nuclei are blue—5 hours.
   (3) Eosin solution, pale pink in distilled water—until sections are pale pink.

(4) Absolute alcohol, 2 changes—3 to 5 hours.
(5) Xylol until clear—1 to 2 hours.
(6) Mount in damar, placing small drop of damar on slide beneath sections as well as to cover them.

III. *Hematoxylin-eosin-azure II:*
(Eosin-azure II may be used alone when it is not necessary to stain nuclei well).
(1) Delafield's hematoxylin as for hematoxylin-eosin.
(2) Tap water—3 hours.
(3) Distilled water—30 minutes.
(4) Eosin, 3 cc 0.1 per cent. aqueous solution, add 50 cc distilled water and 5 cc 0.1 per cent. solution azure II—5 to 12 hours.
(5) Differentiate in absolute alcohol 4 to 12 hours or until desired contrast is obtained between epithelial and connective tissues.
(6) Fresh absolute alcohol—10 minutes. Clear and mount as in II.

IV. *Iron hematoxylin:*
(1) Iron alum solution 2.5 per cent.—3 hours.
(2) Tap water several changes—3 hours.
(3) Regaud's iron hematoxylin diluted to pale amber color—until sections are black.
(4) 95 per cent. alcohol—30 minutes.
(5) Differentiate in saturated solution picric acid in 95 per cent. alcohol until only nuclei remain black.
(6) Tap water frequent changes until all picric acid is removed—8 to 12 hours.
(7) Absolute alcohol 2 changes—5 hours. Clear and mount as in II.

V. *Sections containing nitrocellulose:*
Stain as in II or IV. Following staining:
(1) 75 per cent. alcohol—1 hour.
(2) 95 per cent. alcohol—3 to 5 hours.
(3) Creosote solution (1 part creosote, 1 part toluene, 2 parts xylene) until completely clear. Mount as in II.

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A CONVENIENT RESISTANCE FOR DETERMINATION OF REDOX POTENTIALS IN BIOLOGICAL FLUIDS

A DISCOURAGING source of error in the measurement of redox potentials in many biological fluids is the rapid polarization of the cell. The obvious remedy, high resistance in the galvanometer circuit, has been adopted in most laboratories. Many of these devices are inconvenient to manipulate or to assemble. The writer has found that a series of radio grid leaks (or resistors) connected to a multiple point switch allowed a rapid adjustment of the potentiometer with a minimum flow of current from the cell. The device, Fig. 1, was made for $4.00.

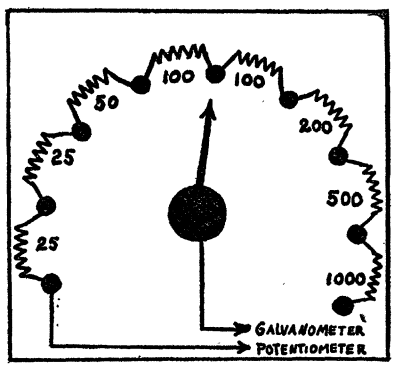

FIG. 1. Variable resistance for measurement of redox potentials of biological fluids. Unit resistance values above are in terms of 10^3 ohms.

The variable resistance is placed in series in the galvanometer circuit. About 500,000 ohms are switched into the circuit. This resistance is cut down step by step until a galvanometer deflection can just be detected. The potentiometer is balanced and the process repeated step by step until the system is balanced with all the resistance cut out. Thus the minimum current necessary to deflect a galvanometer (sens. .018 microamperes) is drawn from the cell and polarization minimized. The values of the unit resistors need not be accurately known, since they are not concerned in the final measurement.

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