after injection of potassium.[10] Muscular weakness has long been known to be associated with high blood potassium, and since this applies also to cardiac and smooth muscle it accounts for the circulatory disturbances. Dehydration of the blood, often reported in adrenal insufficiency, may be considered a consequence of diuresis and stasis. Finally, the lesions found in the kidney in potassium poisoning are of the same type as those in adrenal insufficiency. The renal lesions, low filtration pressure and diminished circulatory volume would also account for the uremic symptoms of late adrenal insufficiency.

Considerable variations in the survival time may be the result of the application of other mechanisms which may be brought into play, such as selection or avoidance of food and muscular activity. The latter has been shown to lower muscle potassium[11] and increase muscle sodium, thus bringing about the reverse changes in the blood. An adrenal insufficient animal as a rule does not tend to move unless disturbed and any excessive activity thus produced shortens its survival period. This would agree with the observations that muscles from adrenalectomized animals fatigue readily and that normal animals can be protected against fatigue by injections of cortin. It has been found that patients with Addison's disease have a greatly increased capacity for doing work after cortin administration.[12] Preexisting lesions of the kidneys would also lead to a more rapid course of the syndrome. Where the survival time is long, the results of individual sensitivity of different organs will complicate the picture, and death may often result from secondary causes. For these reasons it is not claimed that the terminal stages of the syndrome are in every case identical with those of potassium poisoning, but it is felt that the latter is the basal cause.

Preliminary experiments performed on mice, rats and guinea pigs have afforded some evidence that cortin has a protective action against potassium. Work is now in progress on the application of this finding to the assay of cortin.

Feeding insufficient animals with high potassium diets was found to aggravate the condition in cats, and in rats during the first few days following adrenalectomy. Similar results with dogs have been reported recently.[13]

In the therapy of Addison's disease it might be of greater importance to eliminate potassium from the diet than to administer high doses of sodium chloride, and probably the best results could be expected if both of these measures were used. Finally, cortin administration would appear to be indicated in many other conditions involving a rise in blood potassium, such as nephritis, traumatic shock and certain acute febrile diseases.

[10] E. Kylin and A. Engel, *Klin. Wochnschr.*, 4: 653, 1925.

[11] W. O. Fenn, *Am. Jour. Physiol.*, 116: 47, 1936.

[12] F. A. Hartman, G. W. Thorn, L. M. Lockie, C. W. Greene and B. D. Bowen, *Jour. Am. Med. Assoc.*, 98: 788, 1932.

[13] E. C. Kendall, H. L. Mason, C. S. Myers and W. D. Allers, *Jour. Biol. Chem., Proc.*, XXX: lvii, 1936.

Conclusion

It is believed on the basis of the above findings that an important function of the adrenal cortex is the regulation of potassium metabolism and that the various known symptoms of corticoadrenal insufficiency may be explained in terms of a disturbance of corticoadrenal-potassium interrelations. It is further considered that the beneficial action of cortin in certain other pathologic conditions would suggest that the same mechanism is involved.

R. L. Zwemer
Richard Truszkowski
College of Physicians and Surgeons
Columbia University

DIETARY PRODUCTION AND PREVENTION OF ANEMIA IN LARVAL AMBLYSTOMA

Amblystoma larvae growing rapidly on a diet of raw beef liver developed long and pointed gill filaments with an abundance of red blood cells flowing through the capillary network. In contrast, larvae fed on raw beef muscle ate less, grew more slowly and had shorter gill filaments with a relatively smaller quantity of blood. This growth rate was raised when the beef muscle was powdered and formed 60 per cent. of a synthetic ration containing carbohydrate, fat, cod-liver oil and yeast; there was also an abundance of blood with deeply colored red cells and its rapid flow through the marginal vessels rounded the ends of the gill filaments. On a diet with the same protein: carbohydrate: fat ratio and the same vitamin supplement, but deriving the protein from a highly purified casein and from powdered milk, larvae grew poorly and had short curling gills with pointed filaments. By use of a low-powered microscope it was seen that the gill circulation of these larvae carried few and pale erythrocytes as compared with that of larvae fed on the synthetic beef muscle diet, and these observations were confirmed by standard blood tests (red cell counts and Newcomer readings of hemoglobin). It was possible to alter the severity of the disease and to vary the length of time before death by quantitative regulation of the diet, but development was rarely carried through the first visible changes preliminary to metamorphosis.

Supplementation of the anemia-producing diet with iron and copper, separately and combined at three different levels, had no effect upon the retarded growth nor upon the anemia. The disease was less severe

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.

ESTHER M. PATCH
THE UNIVERSITY OF WISCONSIN

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.