the tropolone dicarboxylic acid decarboxylase described here. Unless fixation of carbon dioxide is involved in formation of the carboxyl groups, and the previous isotope data do not support this possibility¹, it is clear that the precursors of stipitonic and puberulonic acids must at least be C₉ compounds.

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Action of Ribonuclease on Nerve Axoplasm as Demonstrated by Silver Staining

It was observed that crystalline ribonuclease selectively prevented demonstration of nerve axoplasm by a simple silver method reported elsewhere¹. The tissue was treated with 0.01 per cent crystalline ribonuclease in distilled water at 60° C. for 1 hour. Ribonuclease was also observed to prevent axon staining as well as to alter the staining capacity of the Nissl substance and nucleoli of the spinal cord when the Bodian and toluidine blue methods were used.

Two lots of crystalline ribonuclease were used. The results were similar in the two instances. Both lots of enzyme had given negative reactions to standard tests for proteolytic activity. In addition, the enzyme was treated with ammonium sulphate heated at 100° C. after the procedure suggested by Swift², without change in the results obtained.

A series of inhibitors of the activity of ribonuclease was utilized. Sodium chloride, magnesium chloride, copper sulphate, phenylisocyanate and periodate, in concentrations published as inhibiting agents for ribonuclease, allowed silver staining of the axoplasm to occur. Control sections treated at 60° C. for an hour with ribonuclease alone showed no staining.

Extraction of formalin-fixed nerve tissue with 10 per cent perchloric acid for periods up to 24 hours did not change the stain. Extraction of Bouin-fixed material with trichloroacetic acid at 90° C. for 30–60 minutes did remove the staining capacity of axoplasm.

The nature of the substance that permits silver staining of nerve tissue is not known. The action of ribonuclease points towards a nucleoprotein basis for staining. Purine and pyrimidine bases may bind silver as seen in the methods for urates. Silver purine com-

pounds are used in the isolation and analysis of nucleic acid. The relation of formalin fixation to ribonuclease activity and other related problems are under investigation.

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ANIMAL PHYSIOLOGY

Active Transport of Uric Acid Through the Human Erythrocyte Membrane

Active Transport of Uric Acid Through the Human Erythrocyte Membrane

IN connexion with determinations of the uric acid pool in man by means of labelled uric acid, it has been repeatedly observed that it takes several hours before the injected uric acid reaches equilibrium in the total volume of distribution. This indicates that the cell membranes have a limited permeability to uric acid. It is known^{1,2} that uric acid is able to penetrate the erythrocyte membrane of man. The ratio between uric acid/l. of red blood cells and uric acid/l. of plasma³ is 0.55. The extra- and intracellular concentrations of uric acid in the water phase are nearly the same when correction is made for the Gibbs-Donnan effect.

We have now been able to demonstrate, that a part of the transport of uric acid into human erythrocytes is inhibited by hypoxanthine. These results, together with results from experiments employing variations in temperature, indicate that the system responsible for the transport of uric acid through the human erythrocyte membrane has two components. Furthermore, experiments carried out at different pH values, suggest that the uric acid crosses the erythrocyte membrane in its undissociated form.

Fresh heparinized blood was washed three times with isotonic phosphate buffer (0.11 M), pH 7.0 (unless otherwise indicated). The buffer contained 1 gm. of glucose per litre, and the same uric acid concentration as the original plasma to avoid fluctuations in the intracellular concentration of uric acid. After washing, the red blood cells were suspended in the buffer, with or without addition of hypoxanthine, to give a final haematocrit of 50–60 per cent. After 15 min. equilibration at 22° C. (unless otherwise stated) and shaking, 1 μ c. of uric acid-8-¹⁴C (2.4 μ c./ μ M) was added at zero time. The flasks contained a total volume of 24 ml. each. At different time-intervals, samples were taken, and the cells removed by centrifugation in the cold at 9,000g. The supernatant was measured for radioactivity, and from the decline in specific activity with time, the velocity of uric acid exchange was calculated.

Fig. 1 shows uric acid exchange as a function of hypoxanthine concentration. It can be seen that by increasing hypoxanthine concentrations, the uric