related to ion and water movements in mitochondria liver slices and other systems. It can be shown that compounds which affect ion and water movements greatly reduced the turnover of phosphoprotein-P. In addition, alteration of conditions which result in loss of sodium extrusion (e.g. low K in medium) reduce the rate of phosphoprotein turnover.

The significance of these results for the pharmacological actions of antihistamine drugs will be discussed with special reference to their protective effects against cellular injury both *in vivo* and *in vitro*.

15 Biochemical and Pharmacological Heterogeneity of Mitochondria isolated from Rat Brain. A. S. MORACZEWSKI (U.S.A.).

On the basis of widely scattered evidence, the hypothesis has been formulated that an understanding of drug action on the brain needs to take into account possible differential effects on biochemical activities of different areas of the brain. To test this hypothesis, several phenothiazines were selected and their effects on cytochrome oxidase activity of mitochondria isolated from four gross areas of the brain were studied. It was observed that the levels of enzyme activity differed in the four areas tested and that the order of activity varied with the particular medium used to test the cytochrome oxidase activity. Since the test system involved the rate at which exogenous reduced cytochrome c was reoxidized, the state of mitochondrial permeability to cytochrome c was an important factor. The final resuspension of mitochondria was done in one of four media: deoxycholate, which solubilizes the cytochrome oxidase system; Triton WR-1339, which fragments the mitochondria; 0·075 M Sucrose, which causes mitochondrial swelling and apparently permits cytochrome c to enter; and 0·25 M Sucrose, which does not favour ready entry of the cytochrome. In addition, the use of these varied media afforded a system which would provide a clue as to whether the drug was exerting an effect on the mitochondrial membrane or intramitochondrially. The data presently in hand suggest that there is some degree of biochemical heterogeneity among brain mitochondria and that there is at least a quantitative difference in the sensitivity to inhibition of cytochrome oxidase activity by phenothiazines.

16 Solubilization and Isolation of Drug-Hydrolysing Enzymes from Microsomes. G. HOLLUNGER and B. NIKLASSON (Sweden).

Twice washed microsomes from rabbit liver were frozen and thawed about 40 times. The clear, slightly yellow supernatant after centrifugation at 150,000 g for 2 hr contained about one-third of the microsomal protein. Further freezing and thawing did not solubilize more protein. All the iminoacylanilid-hydrolysing activity of the microsomes[1] was solubilized by the procedure. Chromatography of the supernatant on calcium phosphate or TEAE-cellulose columns gave 7 distinct peaks. The chromatographical pattern is quite reproducible. The chromatographical distribution of the iminoacylanilid-, acetanilid-, procaine-, cocaine-, atropine- and acetylcholine-hydrolysing enzymes of the microsomes will be demonstrated. By ultrasonic vibration of the microsomes a soluble protein fraction can be obtained which seems identical with that obtained by freezing and thawing.

A highly active non-dialysable inhibitor of the acetanilid-splitting enzyme was also present in the supernatant after ultracentrifugation. The effect of the inhibitor could be completely removed by small amounts of serum albumen. The purification of the inhibitor will be described.

1. HOLLUNGER, G. (1960), *Acta Pharm. Toxicol. Scand.*, **17,** 374.

17 Activation of Phosphofructokinase from the Liver Fluke Fasciola Hepatica by Serotonin and Cyclic 3:5-AMP. T. E. MANSOUR, N. A. LEROUGE and J. M. MANSOUR (U.S.A.).

Stimulation of rhythmical movement of intact liver flukes by serotonin resulted in an increased rate of anaerobic glucose uptake and lactic acid production.[1] The stimulation of glycolysis also occurred in homogenates from flukes which had been preincubated with serotonin when glucose, glucose-6-phosphate or fructose-6-phosphate was used as substrate. However, this effect was markedly reduced when fructose diphosphate was the substrate. This indicated that the activity of phosphofructokinase (a rate limiting enzyme in this organism) is increased. Phosphofructokinase activity in homogenates from control flukes was increased by serotonin and cyclic 3:5-AMP (synthesized chemically or by fluke particles). The activating effect of these agents on the enzyme was dependent on the presence of a particulate fraction ATP and Mg^{++} and was inversely proportional to the substrate concentration (Fructose-6-phosphate). When the fluke homogenates were incubated for 20 min at 30°C with ATP and Mg^{++}, a soluble fraction (105,000 × *g*) was isolated which was still activated by the cyclic nucleotide but not by serotonin. Phosphofructokinase in this fraction was further purified by precipitating the enzyme with ammonium sulphate (40 per cent saturation). The enzyme in this precipitate was still activated when preincubated with ATP, Mg^{++} and cyclic 3:5-AMP. Since the production of cyclic 3:5-AMP is increased by serotonin[2] the stimulation of phosphofructokinase by this hormone is possibly mediated via the effect of cyclic 3:5-AMP on a mechanism controlling the activity of this enzyme.

(Supported by N.I.H. Grants E-1386 and SF-512.)

1. MANSOUR, T. E. (1959), *J. Pharmacol.*, **126,** 212.
2. MANSOUR *et al.* (1960), *J. Biol. Chem.*, **235,** 466.