separately by cutting each centrifuge tube (Beckman, cellulose nitrate) into four sections with a specially designed cutter. Aliquots of all fractions were taken for biochemical estimations. For electron microscopy some of the fractions containing particulate material were recentrifuged to form pellets which were first fixed in a triple aldehyde mixture (2·5% w/v glutaraldehyde—4% paraformaldehyde—2% acrolein) and then fixed in 1% osmium tetroxide. For histochemistry the pellets were fixed only in the aldehydes.

The secretory granules separated between 1·7 M and 1·85 M sucrose on the second density-gradient showed peak kallikrein activity. These organelles were almost completely free of other subcellular particles as determined by electron microscopy and by subcellular enzymes (succinate-neotetrazolium reductase for mitochondria and ß-glucuronidase and acid phosphatase for lysosomes). The isolated secretory granules reacted strongly with toluidine blue and periodic acid-Schiff reagents. The ultrastructure of the granules containing kallikrein resembled the electron-dense secretory (zymogen) granules observed in the intact guinea-pig submaxillary gland (Heap & Bhoola, 1969).

K.D.B. thanks the Royal Society for a grant.

REFERENCES

BHOOLA, K. D. (1968). Intracellular distribution of submaxillary kallikrein. *J. Physiol., Lond.*, **196**, 431–445.

BHOOLA, K. D. & OGLE, C. W. (1966). The subcellular localisation of kallikrein, amylase and acetylcholine in the submaxillary gland of the guinea-pig. *J. Physiol., Lond.*, **184**, 663–672.

HEAP, P. F. & BHOOLA, K. D. (1969). Ultrastructure of granules in the submaxillary gland of the guinea-pig. *J. Anat.*, in the Press.

Oxidative phosphorylation in heart mitochondria isolated from chlorpromazine-treated animals.

A. S. F. ASH* and H. T. TOH, *Department of Pharmacology, Royal Free Hospital School of Medicine, London, W.C.*1.

Guinea-pigs, rats and cats were treated orally with either chlorpromazine (10, 15 and 20 mg/kg per day), saline, or placebo tablets for periods of 3 to 7 months. While under treatment the animals appeared healthy: their growth rate was normal and their body temperature remained at normal levels.

At the conclusion of treatment, mitochondria were isolated from ventricular myocardium by an adaptation (Zaimis, Papadaki, Ash, Larbi, Kakari, Matthew & Paradelis, 1969) of the method of Safer & Schwartz (1967). Respiration of the mitochondria was measured at 37°C with an oxygen electrode in the presence (state 3) and absence (state 4) of adenosine diphosphate (ADP) (Chance & Williams, 1956). The ratio of these two oxidation rates (state 3/state 4) gives an index (RCI) of respiratory control. The ADP:oxygen ratio, a measure of the efficiency of adenosine triphosphate (ATP) synthesis, was obtained by measuring the oxygen consumed in the conversion of a given quantity of ADP to ATP.

In each experiment the respiration of the mitochondria was studied (*a*) immediately after their isolation; and (*b*) after they had been stored at 0°C for 4 hr.

When freshly isolated, mitochondria from chlorpromazine-treated animals oxidized succinate at rates similar to those of the controls. However, mitochondria from the treated guinea-pigs and rats showed some loosening of respiratory control with glutamate and pyruvate, substrates linked to nicotinamide adenine dinucleotide (NAD^+). An increase in the basal (state 4) rate of mitochondrial respiration was accompanied by a corresponding decrease in RCI. Rates of oxidation of the same substrates by cat heart mitochondria were unaffected by chlorpromazine treatment. In all three species, ADP:oxygen ratios remained unchanged.

Storage of isolated mitochondria results in loss of respiratory control (Slater & Hülsmann, 1959); oxidative phosphorylation is subsequently uncoupled; and finally respiratory activity fails. In the present experiments, mitochondria isolated from the control animals showed this characteristic loss of respiratory control when kept for 4 hr at 0°C. In contrast, respiratory control of the stored mitochondria isolated from chlorpromazine-treated animals was maintained or even increased and basal rates of NAD^+-linked substrate oxidation reduced.

The chlorpromazine-induced changes which were observed with freshly isolated mitochondria suggest an effect of the drug on the first phosphorylation complex of the electron transport chain, while the experiments with stored mitochondria indicate a stabilization of the mitochondrial membrane.

REFERENCES

CHANCE, B. & WILLIAMS, G. R. (1956). The respiratory chain and oxidative phosphorylation. *Adv. Enzymol.*, **17**, 65–134.

SAFER, B. & SCHWARTZ, A. (1967). Active transport of potassium ion in heart mitochondria. *Circulation Res.*, **21**, 25–31.

SLATER, E. C. & HÜLSMANN, W. C. (1959). Control of rate of intracellular respiration. In Ciba Foundation Symposium on *The Regulation of Cell Metabolism*, ed. Wolstenholme, G. E. W. and O'Connor, C. M., pp. 58–83. London: J. & A. Churchill.

ZAIMIS, E., PAPADAKI, L., ASH, A. S. F., LARBI, E., KAKARI, S., MATTHEW, M. & PARADELIS, A. (1969). Cardiovascular effects of thyroxine. *Cardiovascular Res.*, **3**, 118–133.

Evidence that 1-methylimidazole-5-acetic acid is not a metabolite of histamine.

A. S. KELVIN (introduced by P. B. MARSHALL), *Department of Pharmacology, University of Dundee.*

The major metabolite of histamine in man is 1-methylimidazole-4-acetic acid (1-MeIm4-AA). Both this compound and its isomer 1-MeIm5-AA are normally present in human urine (Tham, 1966a; Granerus, 1968). The origin of the 1,5-isomer is uncertain, but it is probably not a histamine metabolite (Tham, 1966b). Although small doses of histamine are not metabolized to 1-MeIm5-AA in mammals (Schayer, 1959), mice metabolize large doses of histamine to both 1-MeIm4-AA and 1-MeIm-5-AA (Karjala & Turnquest, 1955). We have measured these compounds in urine by gas chromatography (Kelvin, 1970). Our results indicate that 1-MeIm5-AA is not a catabolite of histamine.

The 24 hr excretions of 1-MeIm4-AA and 1-MeIm5-AA by a healthy adult male volunteer were 2·99 mg and 1·92 mg respectively. After histamine (8 mg/kg orally), which resulted in moderately severe hypotension, flushing and headache, the values were 56·9 mg and 2·04 mg. Thus in this man histamine was not metabolized to 1-MeIm5-AA.