

in the contractile response in direct relationship to the length of gestation of the cardiac tissue. Contractile responses to carbamyl choline and isoprenaline develop before any alteration in the electrophysiological record is observed.

A study of the foetal myocardium offers normal cardiac tissue with a human electrophysiological response. The frequent finding of automaticity makes the foetal myocardium particularly useful for the investigation of the action of anti-arrhythmic agents. The insensitivity of the receptor to autonomic agents allows a study of the direct effect of drugs on the membrane action potential without the influence of nervous factors.

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### Cardio-active amines found in ox spleen extracts

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The presence of a cardio-active principle in freeze-dried extracts of ox spleen has been reported (Cobbin & Thorp, 1957), and later studies (Cobbin & Thorp, 1959, 1960; Temple, Thorp & Gillespie, 1966) suggested that its activity on cat papillary muscle was attributable to a low molecular weight base, but not to histamine, choline, acetylcholine, catecholamines, 5-hydroxytryptamine, dopamine, tyramine or a number of other cardio-active amino-acids. More recently the substance was shown to be widely distributed in mammalian tissues (Jackson & Temple, 1969) and claimed to be  $\beta$ -phenylethylamine (Jackson & Temple, 1970).

We have looked for the cardio-active principle in spleen extracts and have found  $\beta$ -phenylethylamine and several other amines which have positive inotropic actions.

Guinea-pig isolated left atria suspended in Krebs-Henseleit solution at 32°C and stimulated supramaximally at 1 Hz were used to monitor the extraction. Some extracts were also tested on isolated guinea-pig ileum in Krebs-Henseleit solution at 32°C.

Ox spleen, which was frozen immediately after its removal from the animal, was freeze-dried, defatted with chloroform and extracted with acetone. The concentrate was partitioned between methyl isobutyl ketone and water, the aqueous phase freeze-dried and extracted with ethanol and the alcoholic solution evaporated. This fraction (0.1% w/w wet tissue) caused a positive inotropic effect at concentrations between 10 and 100  $\mu$ g/ml. A similar concentration caused a contraction and initiated spontaneity in the ileum; these effects were blocked fully by 100 ng/ml of triprolidine.

Separation of this alcohol-soluble extract by gradient elution on IRA 50 resin gave acidic, neutral and basic fractions, of which only the basic fraction was cardio-active. This was subjected to chromatography on Dowex 50 resin using a pyridine acetate buffer at pH 5.0 and fifteen discrete fractions isolated. Only fractions 6-9 showed positive inotropic activity and all, except 6, caused triprolidine-sensitive contractions of the guinea-pig ileum.

The following amines were identified in the fractions indicated within brackets, ethanalamine (3-5), isoamylamine (5 & 6), tyramine (5 & 6),  $\beta$ -phenylethylamine (7-9), histamine (7-9), putrescine (7 & 8) and cadaverine (8).

Atrial contractions were increased by 50% with 0.1–0.3 µg/ml histamine, 0.3–1.0 µg/ml β-phenylethylamine, 3–10 µg/ml tyramine, 10–30 µg/ml isoamylamine and 300–1,000 µg/ml ethanolamine but were slightly reduced by 100–300 µg/ml cadaverine and by 100–300 µg/ml putrescine.

We conclude that histamine, which was found in the alcohol-soluble extract at a concentration of 1 µg/mg of extract, accounts for much of the activity observed on the atrial and ileal preparations. The contributions of the other amines cannot be assessed without knowing their concentrations in the spleen extract and this may vary.

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#### Effect of dihydroergotamine (DHG) on the capacitance, resistance and precapillary sphincter vessels of denervated cat skeletal muscle

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Mellander & Nordenfelt (1970) have reported that, in the vascular bed of both human and cat skeletal muscle, the constrictor effect of DHE is mainly confined to the capacitance vessels. These authors used one dose of DHE in each species (10 and 15 µg/kg for human and cat studies respectively). We have now confirmed and extended their observations using the denervated vascular bed in calf muscles of the cat.

Forty male cats were anaesthetized with chloralose (40 mg/kg) and urethane (500 mg/kg) intramuscularly and prepared according to the method of Mellander (1966). The experimental findings were interpreted following the principles described by Mellander (1960).

DHG methanesulphonate was administered by close intra-arterial injection to the calf muscles over the following dose range (base): 1.67–405 µg/kg of muscle; one dose only/animal. The constrictor effect of DHG on the capacitance and resistance vessels is shown in Table 1. The threshold dose for constriction of the capacitance vessels was about 5 µg/kg and a dose-response relationship existed up to a dose of 405 µg/kg. Maximal capacitance responses were of similar magnitude to the capacitance response during supramaximal sympathetic nerve stimulation. On the arterial side no significant constriction was observed in doses of less than 45 µg/kg and even at the highest dose the mean constriction never exceeded 9% of the response during supramaximal sympathetic nerve stimulation. In none of the experiments was DHG found to change the capillary filtration coefficient. DHG does not, therefore, influence the tone of the precapillary sphincters.