These experiments clearly show that depletion of myocardial noradrenaline by immunosympathectomy does not reduce the effect of tyramine on the atrial pacemaker. Zaimis (1965) reported that immunosympathectomy produced marked depletion of cardiac noradrenaline but did not inhibit the responses to tyramine and concluded that tyramine has a direct sympathomimetic action. Our results support this conclusion. It is interesting to note that although the chronotropic effect of tyramine is not inhibited by immunosympathectomy, the inotropic effect is significantly reduced. It seems that the inotropic and chronotropic effects are governed by different processes. It is not unlikely that these two effects are produced by activation of different receptors.

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Protamine-induced hypocalcaemia in rats

SIR,—During a clinical trial of the antitumour agent, Prolothan G (an aqueous solution of protamine standardized to contain nitrogen 2·5% w/v with dextrose 40%), some patients developed tetany and almost all became hypocalcaemic (Anderson, Tomlinson & Wright, 1967). The known neutralizing effect of protamine sulphate on heparin was therefore suspected. Heparin enhances the action of parathyroid hormone on bone resorption *in vitro* (Goldhaber, 1965) and may cause osteoporosis in man (Griffith, Nichols & others, 1965). We have now examined the effect of Prolothan G and compared it with clupeine sulphate and thyrocalcitonin in rats.

Male albino Wistar rats, 150 g, were anaesthetized with ether and a polythene cannula was inserted in the right femoral vein. Solutions were infused over an 80 min period at a rate of 0·5 ml/hr. Blood samples were taken from the tail vein (Sandiford, 1965) before, and at 20 min intervals throughout the infusion. The plasma calcium concentration was measured in 0·05 ml of plasma (MacIntyre, 1957) with the Optica CF4 spectrophotometer and flame attachment. The solutions of Prolothan G (Duncan, Flockhart & Evans), clupeine sulphate (B.D.H.) and thyrocalcitonin (M.R.C. calcitonin standard A) were prepared in physiologically normal dextrose saline. The protein estimation of the solutions was by the method of Lowry, Rosebrough & others (1951). Four animals were used for each infusion of Prolothan G, clupeine sulphate, thyrocalcitonin and dextrose saline.

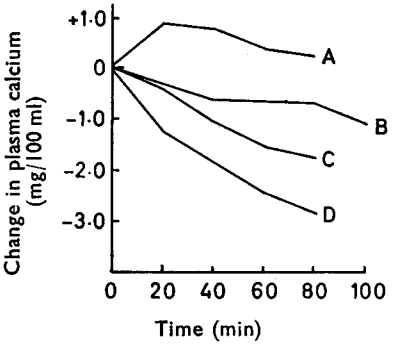

FIG. 1. The effect of intravenous infusion of dextrose-saline (A), Prolothan G (B) (1·4 mg protein), clupeine sulphate (C) (1·8 mg protein) and thyrocalcitonin (D) (0·4 mg protein) on the fall of plasma calcium concentration in young rats.

The infusion of each of the three test solutions produced hypocalcaemia whereas the infusion of dextrose saline occasioned a rise in plasma calcium (Fig. 1).

The hypocalcaemia produced by the protamines (Prolothan G and clupeine sulphate) does not seem to be merely binding of the ionic calcium to the protamine, because incubation of plasma with Prolothan G at 37° for 3 hr *in vitro* did not affect the total plasma calcium concentration on subsequent determination.

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