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Isolation of a Non-dialysable Active Pressor Principle from Pig Serum

A NON-DIALYSABLE hypertensive substance stable to heat is found in rat serum after incubation at 37° C (refs. 1 and 2). The same activity can be demonstrated in the serum and plasma of human beings, pigs and rabbits, but not of frogs[3]. The blood pressure response to this active pressor principle (APP) was demonstrated in rats during an infusion of hypotonic glucose and compared with the hypertensive effect of synthetic angiotensin[1]. Because APP seems to be different from known hypertensive polypeptides and renin[1], further purification of it is of interest.

Blood was withdrawn by carotid puncture without the use of an anticoagulant from pigs which had been stunned by electric shock. It was then centrifuged at 4° C and the unhaemolysed serum was incubated at 37° C for 15 h (fraction *A*) after addition of toluene or merthiolate. (The precautions taken against bacterial contaminations have already been described elsewhere[3].) Solid ammonium sulphate was added to 2,420 ml. of incubated serum at 0° C to a saturation of 55 per cent. After vigorous stirring for 2 h the precipitated proteins were removed by centrifugation and washed with saturated aqueous solution of 55 per cent ammonium sulphate. The supernatants were combined and the active pressor principle precipitated at a saturation of 65 per cent ammonium sulphate at 0° C. This precipitate was dissolved in distilled water and dialysed overnight against running tap water (fraction *B*). The resulting solution was acidified with 1 normal hydrochloric acid to a *p*H of 4·0 and treated with two volumes of 96 per cent ethanol at 0° C. The precipitated proteins were removed by centrifugation and discarded. The supernatant was freed from ethanol below 15° C by evaporation under reduced pressure and then dialysed against running tap water for 30 h (fraction *C*). 0·1 normal sodium hydroxide was added until a *p*H of 7·3 was attained and the solution brought to a concentration of 0·9 per cent sodium chloride. Zinc sulphate was then added to a final concentration of 20 mmoles Zn^{++}/l. After stirring at 0° C for 2 h the mixture was allowed to stand for a further 2 h at 4°. The clear supernatant obtained by centrifugation at 5,000 r.p.m. was discarded and the precipitate redissolved in 600 ml. of 0·03 molar phosphate buffer (*p*H 7·35). Insoluble material was removed by centrifugation at room temperature. The resulting solution (fraction *D*) contained 12·5 per cent of the APP contained by fraction *A*. Ultrafiltration across a cellulose membrane revealed that APP was also non-dialysable in fraction *D*.

The purification obtained by this method is shown in Table 1. The specific pressor activity of the single fractions is determined in arbitrarily chosen units as the increase in blood pressure ($\Delta p = +25$ mm mercury)/mg nitrogen.

Table 1. PURIFICATION OF APP FROM PIG SERUM

Fraction	Total activity ($n \times \Delta p25$)	Yield (per cent)	Specific activity	Purification
A	9,680	—	0·29	—
B	7,744	80	1·47	5
C	2,400	25	7·71	27
D	1,200	12·5	166·66	575

To obtain information on the homogeneity of the isolated substance, fraction *D* was subjected to starch gel electrophoresis. Although the solution was concentrated to a tenth of its volume only a weakly coloured single band was present in the albumin region after staining with amido black 10 *B*. We therefore assume that APP, or its possible carrier protein, is a macromolecule similar in character to albumin. Assuming a molecular weight of 70,000, $5{\cdot}8 \times 10^{-10}$ moles of APP would cause a rise of blood pressure of 25 mm mercury. The same effect can be obtained by 0·05 μg, that is, $4{\cdot}8 \times 10^{-11}$ moles of angiotensin. Thus, compared on a molecular basis, APP has about a tenth of the activity of angiotensin.

The active pressor principle liberated by the procedure described here proved to be non-dialysable even after further purification and is therefore different from the known hypertensive polypeptides, such as angiotensin, anephrotensin and pepsitensin[1,4]. It is also different from renin because it does not disappear from serum after nephrectomy and induces an immediate rise of blood pressure after intravenous application[1]. The observation that concentration of APP increased during pregnancy, especially in eclamptic and hypertonic cases, and diminished in patients with liver cirrhosis[6] suggests that this substance is important both physiologically and pathologically.

Further investigations are required to ascertain whether APP interferes with the methods commonly used to determine renin–angiotensin effects. Such a hypertensive effect should be considered as a source of error when determinations are made of dialysable hypertensive substances in serum or plasma if the dialysability of the pressor material investigated has not been proved.

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Continuous Automatic Fluorometric Evaluation of Total Blood Histamine

AMONG the numerous methods of estimating blood histamine, the fluorometric method seems to be one of the most sensitive and specific[1], but it is not very easy to carry out[2]. We have recently adapted and modified this method for use with the 'Technicon' auto-analyser. An extension of this method makes possible the continuous determination of blood histamine and is here used to investigate the activity of some histamine releasers.

The flow diagram (Fig. 1) shows the successive steps: haemolysis, dialysis, extraction with alkaline butanol and condensation of the alcoholic solution with *o*-phthalaldehyde (OPT) to yield a fluorescent component.