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Discussion

We infer that hyperexcitability of the nervous system can be associated with hypomagnesaemia without concomitant hypocalcaemia. This observation accords with the findings of Vallee et al. (1960) but not with those of other workers (Hanna et al. 1960, Zimmet et al. 1968) who have claimed that a low serum-calcium arising from the hypomagnesaemia is necessary to produce the signs seen in the hypomagnesaemic syndrome.

One should not conclude from this association that the hypomagnesaemia caused the tremors or convulsions, although this seems likely in view of the fact that magnesium levels returned to normal on recovery. Furthermore, the dose of magnesium in six of the cases was not large enough to cause drowsiness yet the tremors were quickly controlled.

The cause of the low serum-magnesium is unknown. Since some of the cases recovered spontaneously within a day or two, it is possible, at least in some of the patients, that the hypomagnesaemia was due to a transient shift of the mineral from the extracellular to the intracellular compartment. The feasibility of such shifts has been mentioned by Martin et al. (1952), and Hasselman and Van Kampen (1958). It is possible that because of metabolic alterations in the neonatal period and in children who are ill, the magnesium homeostatic mechanism might not be functioning efficiently.

A decreased serum-magnesium could, in turn, stimulate the parathyroids to release parathormone in an attempt to conserve magnesium (MacIntyre et al. 1963). At the same time the excess parathormone would raise blood-calcium, thus accounting for the elevated ultrafiltrable calcium level.

The combination of hypomagnesaemia and hypercalcaemia is not incompatible with the production of hyperexcitability of the nervous system. In magnesium-deprivation studies in rats, the animals demonstrate hyperirritability, hypomagnesaemia, and hypercalcaemia (Wang and Welt 1963). Furthermore, there is evidence to suggest that calcium specifically facilitates the release of acetylcholine by a nerve impulse (Fatt 1959) and that the action of excess magnesium is to cause a block in neuromuscular transmission by decreasing the amount of transmitter substance released at nerve terminals (del Castillo and Engbaek 1954). Hence a combination of hypomagnesaemia and an elevated calcium concentration may act synergistically in facilitating acetylcholine release, thus accounting for the neuromuscular irritability seen in our patients. This antagonistic action of magnesium and calcium at nerve-endings contrasts with their common action in raising the threshold depolarisation for the initiation of an action potential in a nerve or muscle fibre (Fatt 1959).

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AN ADVERSE EFFECT OF POLYVINYLCHLORIDE TUBING USED IN EXTRACORPOREAL CIRCULATION

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Summary The pulmonary vascular resistance is increased by ventilation hypoxia both in isolated perfused lungs from cats and in anaesthetised cats under conditions of circulatory control. This effect of hypoxia also occurs in man and dog. The pulmonary blood-vessels of isolated perfused cats' lungs do not respond to hypoxia when the perfusion circuit is partly made from polyvinylchloride (P.V.C.) tubing. Normal pulmonary vascular responses to noradrenaline, 5-hydroxytryptamine, and prostaglandin $F_{2\alpha}$ are still obtainable when the response to hypoxia is abolished. It is suggested that a new British Standard should be devised to cover the acute effects produced by injection into the body of fluids which have been in contact with P.V.C. tubing.

Introduction

ADVERSE effects have been shown to follow the use of polyvinylchloride (P.V.C.) tubing in normal laboratory procedures. Stewart and Sturridge (1959) found, for instance, that some types of tubing caused haemolysis of the blood perfused through them. Meigler and Durrer (1959) and Meyler et al. (1960) found that isolated rats' hearts were killed within 15 minutes when perfused with fluid delivered through some types of P.V.C. tubing, whereas when the same fluid traversed glass tubing the isolated heart preparations survived many hours; they ascribed the toxicity to the presence of organic tin compounds in certain kinds of P.V.C. tubing. Haberman et al. (1968) described harmful effects of plastics and their component materials on reactions involving human and rabbit antibodies, guineapig complement, and serum-proteins. Few, if any, of the commonly used components of plastic tubing were without effect on their test systems. Bowery and Lewis (1968) found that substances were leached out of P.V.C. tubing which contracted guineapig isolated ileum and inhibited the contractions of the rat uterus induced by many substances. Considerable amounts of activity were added to plasma after 10 minutes' contact and these reached a maximum after 60 minutes. The leaching process occurred again and again each time fresh plasma was added. The amount of activity leached out was directly dependent on the protein concentration in the plasma, and this dependence was also demonstrated with purified bovine albumin solutions.

Methods

Isolated lungs of cats anaesthetised with chloralose (80 mg. per kg. intraperitoneally), were perfused by the method of Duke (1951). A Dale-Shuster pump was used to perfuse the lungs through the pulmonary artery (fig. 1). The perfusion was maintained at constant minute volume (10 ml.) with the animal's own heparinised blood (10 I.U. heparin per ml.) at a temperature of 37–39°C. The volume of blood in the apparatus was approximately 120 ml. The venous outflow from the lungs was led through a left-atrial cannula into a venous reservoir which

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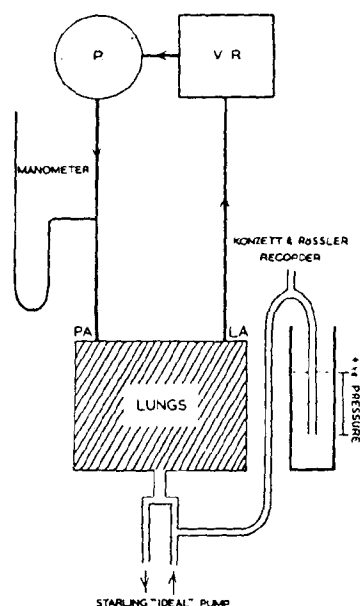


Fig. 1—Perfusion circuit for isolated lungs.

P: Dale-Shuster pump. V.R.: venous reservoir. Single lines: tubing containing blood. Double lines: tubing containing air. Arrows show direction of air or blood flow. P.A.: pulmonary artery. L.A.: left atrium.

fed the pump, thus completing the external circuit. Pulmonary arterial pressure (PAP) was measured with a manometer and a tambour filled with 0.9% sodium-chloride solution attached to a sidearm on the pulmonary-arterial inflow tubing. The lungs were ventilated with a Starling 'Ideal' pump (6 cycles per minute), and an overflow valve ensured that the peak positive pressure remained constant at a predetermined value of between 6 and 12 cm. H₂O in each experiment. The resistance of the lungs to inflation was measured by the method of Konzett and Rössler (1940). The time taken to set up the preparation was 4–8 minutes, but ventilation of the lungs was continued throughout collection of the blood from the carotid artery and cannulation of the pulmonary artery and left atrium. The pulmonary arterial pressure was 20–25 cm. H₂O at the start of perfusion; this was achieved at a minute-inflow rate of 65 ml. or more per kg. body-weight. The glass parts of the apparatus were cleaned by boiling in chromic-acid solution, rinsed thoroughly, and dried in an oven. The rest of the extracorporeal circuit consisted of approximately 160 cm. of flexible tubing. When red-rubber or silicone-rubber tubing was used it was boiled in 1–2% sodium-bicarbonate solution before being thoroughly washed and oven dried.

Results

Since the lungs were perfused at a constant minute rate of blood-flow and the left atrial and peak ventilating pressures were both fixed, the recorded changes in PAP were due to changes of pulmonary vascular resistance. The results of the experiments now reported confirmed others (see Duke and Lee 1963) in that ventilation of the lungs with nitrogen instead of air produced an increase of PAP. The PAP rapidly returned to normal when the lungs were ventilated with air. This increase of vascular resistance occurred in all 12 experiments in which red-rubber or silicone-rubber tubing was used in the circuit. The pressor response could be elicited at intervals of 10–15 minutes for 5 or more hours.

When P.V.C. tubing (10 or 12 H.E., Portex Limited) was

used as part of the perfusion circuit instead of rubber tubing, no pressor response to anoxia could be demonstrated (12 experiments). For these experiments new P.V.C. tubing, previously rinsed in 0.9% sodium-chloride solution, was used. Washing the P.V.C. tubing in a strong detergent ('Pyronex', Diversey U.K. Ltd.) followed by thorough washing in distilled water and 0.9% sodium-chloride solution did not enable anoxic pressor responses from the lungs to be shown. Pulmonary pressor responses to anoxia could only be regularly obtained when the P.V.C. tubing had been boiled 2–3 times in chromic acid solution and thoroughly rinsed and dried.

These experiments showed that the pressor response to anoxia could not be obtained when P.V.C. tubing was used as part of the perfusion circuit. The effect of P.V.C. tubing was also demonstrated in another way. In 12 experiments in which the extracorporeal circuit had been made with silicone or red-rubber tubing, the pulmonary pressor response to anoxia was demonstrated. Different amounts and types of P.V.C. tubing were cut into lengths of approximately 1 cm., washed in saline solution, and added to the venous reservoir. Similar results were obtained in all these experiments, one of which is shown in fig. 2. The pulmonary vasoconstrictor response to 5-hydroxy-tryptamine (5-H.T.), noradrenaline, and anoxia is shown first. The addition of 30 cm. H.E. tubing to the venous reservoir by itself changed neither the PAP, nor the resistance of lungs to air inflow, nor did it affect the response to 5-H.T. or noradrenaline. However, the response to anoxia was completely absent. In other experiments the addition to the venous reservoir of longer lengths (60 cm.) of 10 H.E. P.V.C. tubing caused by itself a rise of PAP and abolished the pulmonary pressor response to anoxia within 5 minutes. The rise in PAP induced by 5-H.T., noradrenaline, and prostaglandin F_{2α} was unchanged when the response to anoxia was abolished. When the tubing was removed from the venous reservoir no recovery of the pressor response to anoxia took place within 30 minutes.

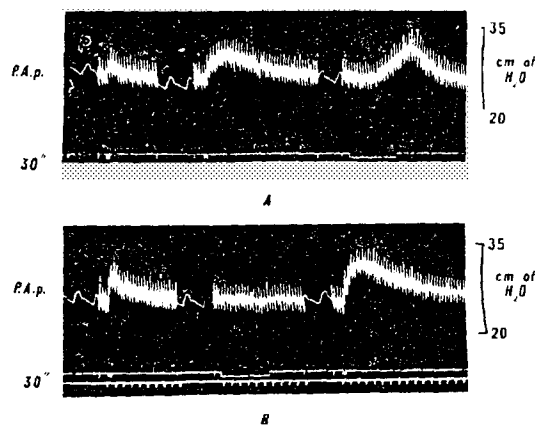


Fig. 2—Experiment in cat weighing 3 kg.

Perfusion started 11.24 A.M. Blood-flow 300 ml. per minute and ventilation with air. Records, from above down: PAP, signal, and time marker (30 seconds). Drugs injected into the pulmonary arterial tubing.

A shows three effects: 1.09 P.M., 20 µg. 5-hydroxytryptamine (5-H.T.); 1.13 P.M., 20 µg. noradrenaline (N.A.); 1.20 P.M., ventilation with nitrogen (N₂) instead of air during signal. At 1.28 P.M., 30 cm. 10 H.E. tubing (previously rinsed with saline solution) was added to venous reservoir.

B shows three effects: 1.47 P.M., 20 µg. 5-H.T.; 1.52 P.M., N₂; 1.57 P.M., 20 µg. N.A.

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The pressor response to anoxia was also abolished when the p.v.c. (10 H.E.) tubing added to the venous reservoir had been previously boiled in 2% sodium-bicarbonate solution.

Some p.v.c. tubing contains different chemical additives from those in 10 H.E. The experiments were therefore repeated using one of these—N.T. 14 SH 80, which is said to be more suitable for perfusion work (Vincent 1968). In one experiment 60 cm. of this tubing abolished the pulmonary pressor response to anoxia, and in another experiment the pressor response was reduced. In both experiments there was a partial recovery of the anoxic pressor response 5 minutes after the tubing was withdrawn from the venous reservoir.

Discussion

Hypoxia causes an almost immediate increase of pulmonary arterial pressure in anaesthetised cats (von Euler and Liljestrand 1946; Logaras 1947) and also in anaesthetised cats with left-lung perfusion (Duke 1957). In isolated lungs from cats perfused with blood at a constant minute volume, hypoxia causes a similar increase of pulmonary vascular resistance with similar time relationships to that in the anaesthetised animal. The pulmonary pressor response to hypoxia is not accompanied by changes in bronchial resistance to airflow or left atrial pressure (Duke 1951, see also Duke and Lee 1963; Daly and Hebb 1966) and is therefore probably due to pulmonary vasoconstriction. In isolated perfused lungs from cats the pressor response to hypoxia of 3–15 minutes' duration is inversely related to the tension of oxygen in the ventilating gas mixture for gas mixtures containing less than 15% oxygen in nitrogen; equivalent effects are produced by ventilation of isolated perfused lungs with neon, hydrogen, or nitrogen (Duke 1951). It appears therefore that the pressor effect of ventilation hypoxia that has been studied in the present series of experiments is a physiological response in the cat, and that the response obtained when the gas mixture is changed from air to nitrogen varies only in degree to the effects produced by changing the ventilating gas mixture from air to 15% or less oxygen in nitrogen. Evidence that pulmonary vasoconstriction to hypoxia is found in man and other species as well as cats was discussed by Duke and Lee (1963) and Daly and Hebb (1966).

The present experiments show that the pulmonary pressor response to hypoxia is abolished by p.v.c. tubing. It is therefore possible that procedures involving the use of plastic tubing in man may be attended by hitherto-unsuspected harmful effects. Such procedures include extracorporeal circulation, renal dialysis, and even blood-transfusion.

In the present experiments two different types of tubes were used, both of which are listed in the current manufacturer's catalogues as of "surgical non-toxic quality". The plasticiser was acetyl-tri-n-butyl citrate in epoxy soya-bean oil. The stabiliser in the H.E. tubing was of an octyltin type, whereas the stabiliser in NT 14 SH 80 was a calcium-zinc compound. The latter type of tubing had a less harmful effect in our preparation.

The manufacturers of the tubing state (Vincent 1968) that acetyl-tri-n-butyl citrate and small amounts of epoxy soya-bean oil are listed under the U.S. Food and Drug regulations as suitable for food packaging, as is also the calcium-zinc stabiliser. Both types of tubing used in the present experiments comply with the requirements of B.S. 2571: 1963 which specifies maximum

permitted weight changes when a plastic disc is immersed in distilled water for 48 hours. The tubing also conforms with the rabbit implantation test laid down in MIL-C-36145A (BuMed.) Oct. 16, 1963. In addition, the NT tubing complies with B.S. 2463 (specification for transfusion equipment) appendix F which uses a toxicity test in mice injected with eluates from tubing heated at 85°C for 1 hour. Mouse fibroblast and 10-day chick-embryo tests are performed by the factory. These tests of biocompatibility are designed to establish the safety of p.v.c. either as a chronic implant or in a simple transfusion apparatus and may be completely unsuitable for testing the potential toxicity of the large amounts of plastic tubing used acutely in an extracorporeal circulation. British Standard 2571: 1963 allows a loss in weight in 48 hours of 20 mg. from a disc weighing about 4.4 g. In our apparatus, which might be regarded as a small-scale cardiac bypass circuit, 60–120 g. of p.v.c. tubing was used; the British Standard would have allowed the leaching out of between 1 and 3 g. of material into the circuit, to reach a concentration of 20 mg. per ml. (2%) in 48 hours. Assuming steady leaching over 48 hours, the allowable concentration of material in the circulating blood could attain at least 0.4 mg. per ml. in 1 hour.

Our results suggest that the toxicity of substances associated with the manufacture of p.v.c. tubing should be further investigated. They also suggest that a new British Standard should be devised to cover the use of such tubing in extracorporeal circulations.

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OXIDIZED GLUTATHIONE LEVELS IN ERYTHROCYTES OF GLUCOSE-6-PHOSPHATE-DEHYDROGENASE-DEFICIENT SUBJECTS

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Summary Oxidised glutathione (G.S.S.G.) was estimated in normal erythrocytes and in erythrocytes deficient in glucose-6-phosphate dehydrogenase (G-6-P.D.) using an improved method in which the oxidation of glutathione was prevented by prior alkylation with N-ethyl-maleimide. The content of G.S.S.G. in the erythrocytes deficient in G-6-P.D. was about 3 times that in normal erythrocytes.

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