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Sustained complete autonomic blockade

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1. A 3-hr period of complete autonomic blockade was induced in anaesthetized sheep and dogs using a continuous intravenous infusion of trimetaphan camphorsulphonate and atropine. Circulatory, respiratory and metabolic parameters were studied over 24 hr or until the animal died, whichever came first.

2. After termination of the blockade all animals remained hypotensive and within a few hours all died in irreversible shock which was not accompanied by any of the otherwise characteristic respiratory or metabolic consequences of circulatory failure.

3. Administration of trimetaphan alone for a similar period was innocuous.

4. When during complete autonomic blockade a continuous infusion of dopamine or hypertensin was additionally administered mortality rate was halved but all survivors remained hypotensive.

Both nicotinic and muscarinic receptors are involved in ganglionic transmission (Trendelenburg, 1967) and this explains why the combination of a so-called ganglionic blocking agent and atropine is more effective than the former substance used alone. The condition resulting from the combined use of these two agents was termed by Steinberg & Hilton (1967) as "complete drug sympathectomy," but because the postganglionic response to acetylcholine is also blocked, we rather refer to it as "complete autonomic blockade."

Complete autonomic blockade could well represent a valuable technique for assessing the role of the autonomous nervous system in various conditions provided it could be proved innocuous if administered over prolonged periods. Only short-term studies have previously been performed, so we decided to subject animals to a 3 hr period of blockade. In order to terminate the blockade at the predetermined time we used atropine and a short acting ganglionic blocking agent by the intravenous route.

We found that after termination of the infusion systemic arterial pressure remained low and did not respond to pressor agents, fluid infusions or administration of cortisone and within a few hours all animals died in shock. Originally these observations were made in sheep and to exclude species-specificity they were repeated in dogs, whose response was found identical.

Hypotension, irrespective of its aetiology, may become irreversible by being sustained over a certain period of time. We wondered if irreversible shock in our

blocked animals could be prevented by maintaining systemic arterial pressure during the blockade at near-normal level. Because infusion of any of the pressor catecholamines over a period of 3 hr itself produces irreversible shock (Halmagyi, Irving, Gillett & Varga, 1967), these could not be used. In spinal cats dopamine (3,4-dihydroxyphenyl-aethylamine) acts as a pressor agent (Burn & Rand, 1958) and its prolonged use is innocuous (MacCannel, McNay, Meyer & Goldberg, 1966). Similarly, synthetic angiotensin can also be used over 3 hr without risking the onset of irreparable hypotension. We have used these agents to raise systemic arterial pressure during complete autonomic blockade.

Circulatory failure in shock is accompanied by hyperventilation, metabolic acidosis and hyperglycaemia. These are generally considered as consequences of tissue hypoxia, but we previously related them to increased catecholamine secretion consequent on shock-induced sympathetic stimulation (Halmagyi, Gillett & Irving, 1967; Halmagyi, Irving & Varga, 1968). We wondered how these parameters would be affected if shock occurs in animals subjected to autonomic blockade.

Methods

Twenty-one sheep weighing 32–45 kg and ten dogs weighing 23–31 kg were used.

Anaesthesia

The fasting supine animals were given an intravenous dose of thiopentone 15 mg/kg followed by a continuous intravenous infusion of thiopentone 0.25 mg/kg per min. The resulting anaesthesia was light and corneal reflex was preserved. About 40–50 min later the dose of thiopentone could be reduced first to 0.125 mg/kg per min and later to 0.06 mg/kg per min. In some instances anaesthesia could be discontinued in the last hour of the experiment.

Procedures

A cuffed Magill tube was inserted into the trachea, a gastric tube through the oesophagus (in sheep only) and a small Malecot catheter into the intrapleural space through the fourth interspace. Both femoral arteries and veins and both foreleg veins were exposed. Cardiac catheters were passed through the femoral veins under fluoroscopic guidance: a double-lumen catheter was introduced into the pulmonary artery with its distal opening in the wedged position and the other catheter was introduced into the right atrium. Polythene tubes were inserted into the femoral arteries and in the foreleg veins and hourly doses of heparin 2 mg/kg were administered.

Measurements

Oxygen saturation of the arterial and mixed venous blood was measured with a Haemoreflector (Kipp & Zonen, Delft, Holland). The haemoglobin content of the arterial blood was measured spectrophotometrically and duplicate samples were spun in capillary tubes for 5 min in a microhaematocrit centrifuge.

Arterial blood was taken anaerobically and analysed immediately after its collection at 38° C for oxygen and carbon dioxide tensions and pH with Radiometer electrodes operating through a model 22 pH meter equipped with an expanded scale (Radiometer, Copenhagen).

Expired air was collected for 1.5–2.5 min in Douglas bags. Their contents were measured in a model E-40 wet test gas meter (Parkinson Cowan, London) and analysed for CO_2 concentration in a Beckman model LB-1 medical gas analyser and for O_2 concentration in a Beckman model E-2 paramagnetic O_2 analyser.

Needle electrodes were connected to the limbs and chest wall for electrocardiography.

Femoral and pulmonary arterial, intrapleural, right atrial and pulmonary arterial wedge pressures were measured with Sanborn transducers and recorded on a multichannel direct writing Sanborn oscillograph.

A type VX-1 intracardiac applicator was introduced into the inferior vena cava and temperature was constantly monitored on an electric thermometer (Type TE3, Electrolaboratoriet, Copenhagen).

Blood glucose was measured by using the Gluco-Pak reagent set (Unitech Co., Panorama City, Calif.) based on the *o*-toluidine reaction. A filtrate prepared immediately after collection of the blood sample was stored at 0° C for periods not exceeding 26 hr. Blood samples for lactic (Olson, 1962) and pyruvic (Segal, Blair & Wyngaarden, 1956) acid measurements were collected into ice-cold trichloracetic acid. Free fatty acids were also determined in the plasma (Henry, 1964). Arterial blood samples were used for all these determinations.

In seven sheep plasma adrenaline was isolated by thin-layer chromatography using cellulose layers and 160 ml. of methanol + 40 ml. of distilled water + quinoline as the developing solvent (Mattock, 1964). Adrenaline was eluted with 0.2 N acetic acid and assayed by the trihydroindoxyl technique using a Turner model 110 fluorometer (G. K. Turner Ass., Palo Alto, Calif.) with a blue ultraviolet lamp and a high sensitivity conversion kit. Details of this technique are described elsewhere (Pullin, 1967).

Each measurement was preceded by three inflations of the lung using a pressure of 30 mm Hg to prevent positional atelectasis of the lung.

Calculations

The Fick principle was used to calculate cardiac output. Vascular resistances were obtained by standard formulae and expressed in dynes/sec cm^{-5}. Arterial pH was converted into H^+ activity and expressed in μ-equiv/l. Gas tensions and H^+ were corrected for blood temperature using the blood gas calculator (Severinghaus, 1966) (Radiometer, Copenhagen). Arterial plasma bicarbonate was calculated by the Henderson equation. Total and alveolar ventilation, O_2 uptake, CO_2 output were calculated by standard formulae. All volumes, flows and resistances were expressed on the basis of 1 m^2 body surface area using the appropriate formulae for sheep and dogs (Dittmer & Grebe, 1958). Standard statistical methods were used for the analysis of the results (Snedecor, 1956).

Groups ; protocol ; agents used

Atropine sulphate and trimetaphan camphorsulphonate (Arfonad, Roche) (Randall, Peterson & Lehman, 1949) were used to produce complete autonomic blockade.

In five sheep control measurements (zero time) were followed by a continuous intravenous infusion of trimetaphan 200 μg/kg per min using an electrically driven slow infusion pump. Twenty minutes later as systemic arterial pressure stabilized at a new lower level, rate of infusion was halved and this rate was maintained over the next 170 min. Measurements were taken at 60, 120 and 180 min and, again, at 235 and 350 min (45 and 160 min after termination of the infusion). The endotracheal tube and the gastric tube were then removed, anaesthesia (if still given) was discontinued, the animal was placed on its side and restrained with wide canvas belts. A continuous infusion of 0.8 ml./min of physiological saline solution or Darrow solution (NaCl, KCl) was started and pressures were continuously recorded at slow paper speed.

In five sheep control measurements (zero time) were followed by a continuous intravenous infusion of trimetaphan 200 μg/kg per min and atropine 14 μg/kg per min. Twenty minutes later as systemic arterial pressure stabilized at a new lower level and the pupils became fully dilated the rate of infusion was halved and this rate was maintained over the next 191 min. Measurements were taken at 40, 150 and 211 min and again at 261 min (50 min after termination of the blockade). The following procedures were similar as in the previous group.

A somewhat different protocol was followed in five dogs. After the control measurements (zero time) atropine 1 mg/kg was administered intravenously and this dose was repeated 2 hr later. Trimetaphan was started (zero time) at a rate of 12.5 μg/kg per min in order to avoid a precipitous fall in systemic arterial pressure and respiratory arrest to which atropinized dogs were found to be particularly prone. This dose was then gradually raised over the next 40–50 min until the rate of 100 μg/kg per min was reached. We found that at this stage the rate of trimetaphan infusion could be doubled or trebled without causing further fall in blood pressure. Measurements were taken at 68, 135 and 196 min. Trimetaphan infusion was then discontinued and further measurements were taken 60 and 177 min later.

In some animals (not included in this series) whose hypoglycaemia was found to be excessive the overnight infusion fluid was changed to 5% glucose in saline and 1 or 2 mg of glucagon (Lilly) was given intravenously.

In five sheep a continuous infusion of val^5-hypertensin-11-asp-β-amide (Hypertensin, Ciba) 10–70 μg/kg per min was administered during the blockade. Measurements were taken before blockade (zero time) and at 55, 120 and 188 min during the blockade ; both blockade and hypertensin infusion were then terminated and repeated measurements were taken 55 and 120 min later.

In five sheep and in five dogs a continuous infusion of dopamine (3-hydroxytryptamine HCl, Calif. Corp. Biochem. Research, Los Angeles, Calif.) 7.5 μg/kg per min was started. Thirty minutes later (zero time) trimetaphan and atropine infusion was started as described before for sheep and dogs while dopamine infusion was continued. After 195 min the blockade was terminated and dopamine infusion was continued for further 40 min. Measurements were taken before dopamine (control), 29 min after the start of dopamine infusion (zero time), 73, 131 and 196 min after commencement of the blockade, at 235 min (39 min after termination of the blockade) and, finally, at 282 min (47 min after dopamine had been discontinued).

In both hypertensin and dopamine-infused blocked animals procedures following the last measurements were identical to those already described for the first (trimetaphan only) group.

Next morning the survivors were again intubated, anaesthetized and further measurements were taken.

In five dogs and three sheep termination of the blockade was followed by 0.4 mg of intravenous crystalline lanatoside C (Cedilanid, Sandoz). Measurements were taken before and 40 min after the injection.

Postmortem examinations were not carried out.

Results

As already demonstrated (Halmagyi & Gillett, 1966) in non-medicated animals subjected to these procedures circulation and respiration remains stable over 24 hr: these control studies were not repeated for this series.

Effects of trimetaphan infusion in sheep
(Figs. 1 and 2)

One hour of trimetaphan infusion significantly depressed systemic arterial resistance ($P<0.02$) and heart rate ($P<0.05$); systemic arterial pressure ($P<0.02$), alveolar ventilation ($P<0.01$) and blood temperature ($P<0.02$) fell significantly in the second hour while cardiac output, O_2 uptake and arterial P_{CO_2} remained virtually unaffected. There was a progressive decrease in lactic acid, NEFA and glucose plasma levels.

One hour after termination of the infusion systemic arterial pressure returned to normal; heart rate returned to normal 2 hr after termination of the infusion. Blood glucose, plasma lactate and free fatty acid levels continued to decline and were statistically significantly reduced 1 hr after termination of the infusion ($P<0.02$).

Next morning cardiac output, O_2 uptake and systemic arterial pressure were somewhat depressed but not significantly different from the control values. All other measured variables were normal.

Right atrial, pulmonary arterial, pulmonary arterial wedge and intrapleural pressures and arterial P_{O_2} remained normal in all animals throughout the entire experiment.

Effects of trimetaphan + atropine infusion in sheep and dogs
(Figs. 1 and 2)

In dogs, heart rate ($P<0.02$), cardiac output ($P<0.01$), systemic arterial pressure ($P<0.01$) were significantly reduced after 1 hr of infusion, while fall in blood temperature ($P<0.02$) and in O_2 uptake ($P<0.05$) was significant in the third hour only. Changes in ventilation and in systemic arterial resistance were not statistically significant.

In sheep, systemic arterial pressure ($P<0.001$), alveolar ventilation ($P<0.05$), heart rate ($P<0.01$) and systemic arterial resistance ($P<0.01$) were significantly reduced after 1 hr of infusion and fall in blood temperature reached statistical significance ($P<0.02$) in the second hour of the infusion. The fall in cardiac out-

put ($P<0.05$) and O_2 uptake ($P<0.05$) was statistically significant only after completion of the infusion.

There was no linear relationship between the fall in heart rate and blood temperature. During the first hour of the combined infusion mean heart rate fell by 33

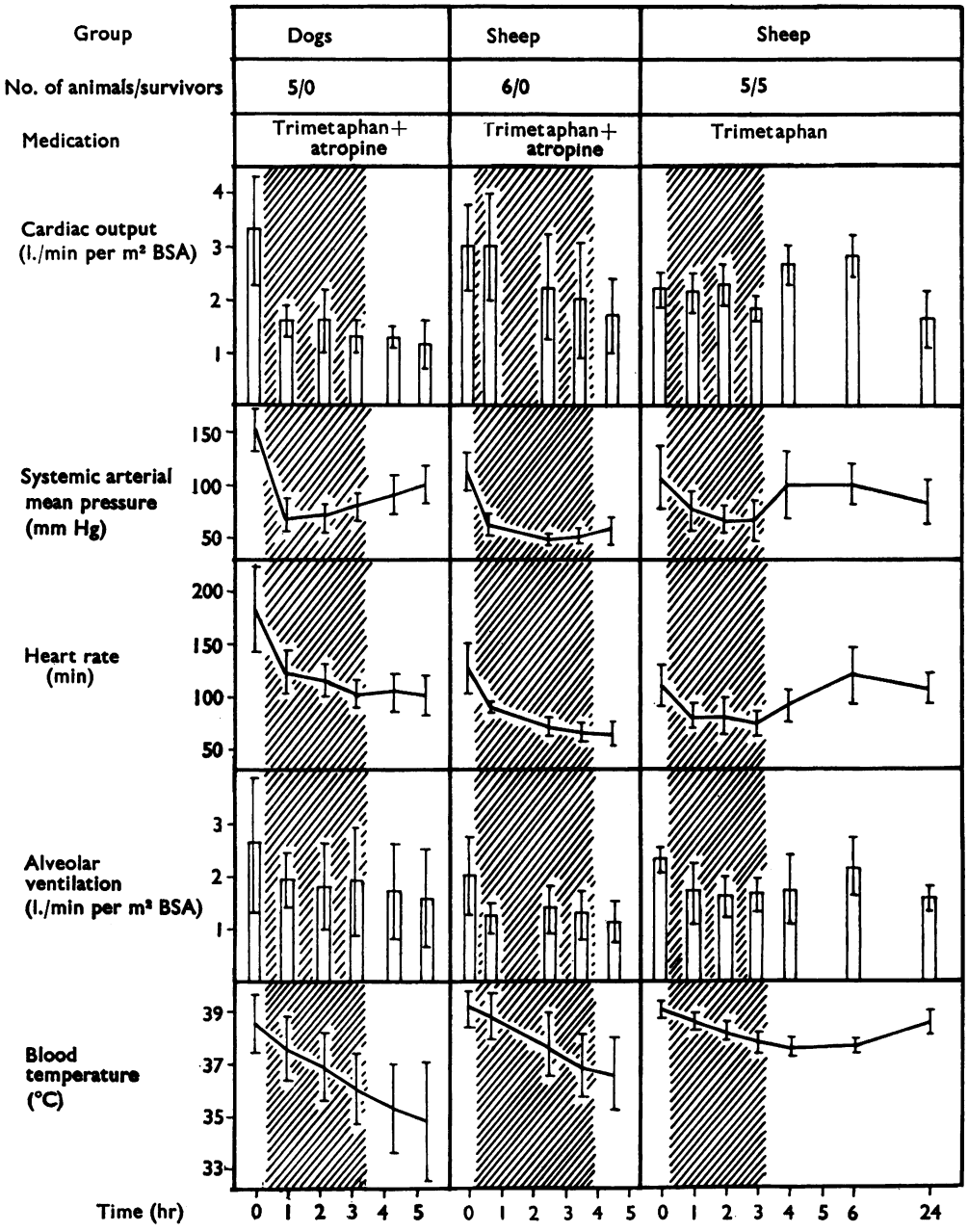

FIG. 1. Complete autonomic blockade: cardiorespiratory effects. Note failure of different functions to return to normal after termination of the trimetaphan and atropine infusion as opposed to their return after trimetaphan infusion. Also note absence of hyperventilation during hypotension. Crosshatched area: duration of infusion; vertical lines: standard deviations.

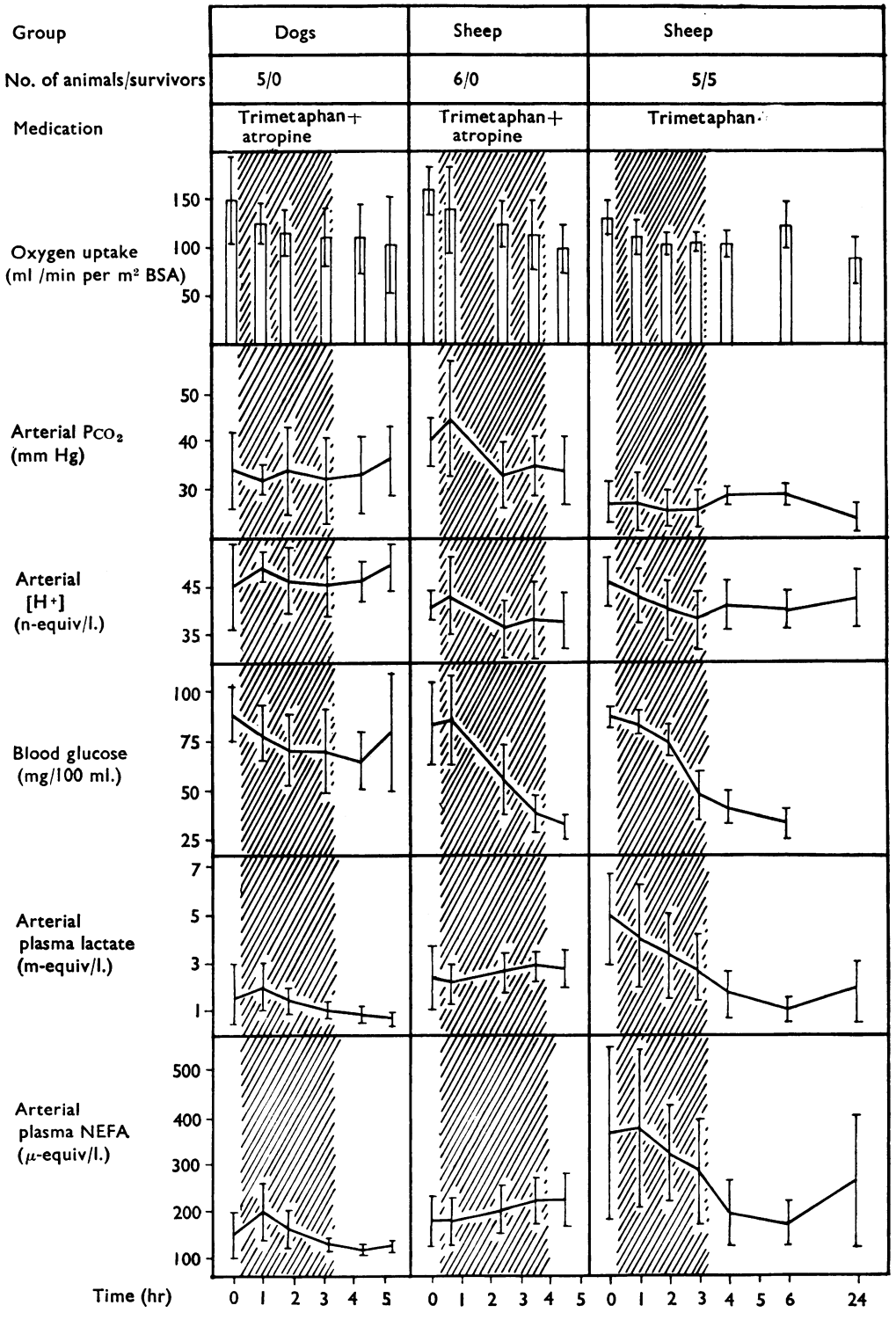

FIG. 2. Complete autonomic blockade: metabolic effects. For explanation see text. Vertical lines: standard deviations; crosshatched area: duration of infusion.

beats/min and mean temperature by 0.4° C in sheep, 60 beats/min and 1.0° C in dogs ; in the next 2 hr the changes were 28 beats/min and 2.0° C, 23 beats/min and 2.8° C in sheep and dogs, respectively.

Despite marked hypotension, hyperventilation was absent, arterial gas tensions, H^+ activity, lactate and free fatty acid levels remained virtually unchanged, blood glucose fell but significant hypoglycaemia was observed only in sheep.

Adrenaline plasma levels in three sheep of this group are shown in Table 1: a gradual fall was observed in all instances.

After termination of the combined infusion systemic arterial pressure remained unchanged in sheep and increased slightly in dogs but remained significantly ($P<0.02$) lower than during the control period. Cardiac output remained low, body temperature and O_2 uptake continued to decline and within 3 to 6 hr all animals died.

During their last hours systemic arterial pressure gradually diminished and they died in cardiac arrest preceded by a sudden rise in right atrial pressure. Arterial lactate levels remained normal even 1 hr before death.

Until the premortal stage, right atrial, pulmonary arterial, pulmonary arterial wedge and intrapleural pressures, and arterial P_{O_2} remained normal.

During the combined infusion of the two blocking agents the animals' level of consciousness gradually deteriorated. Although the rate of anaesthetic infusion was progressively reduced or even suspended the animals remained unconscious until they died.

Digitalis failed to improve cardiac output or systemic arterial pressure. Infusion of glucose or of glucagon promptly restored blood glucose to normal, yet the animals still failed to survive. Animals subjected to these unsuccessful therapeutic attempts are not included in Figs. 1 and 2.

Effect of hypertensin on consequences of complete autonomic blockade in sheep (Fig. 3)

In this group cardiac output remained unchanged, heart rate, systemic arterial pressure, O_2 uptake and blood temperature fell less than in the previous group but all other parameters followed a similar pattern. After withdrawal of the infusion

TABLE 1. *Plasma adrenaline concentrations (μg/l.) in sheep subjected to sustained autonomic blockade with and without hypertensin medication*

		Without hypertensin		
No.	A	B	C	Outcome
1	11·3	1·7	3·0	Died
2	21·6	13·8	3·5	Died
3	70·3	0·0	0·0	Died
		With hypertensin		
No.	A	B	C	Outcome
1	8·9	10·0	0·0	Survived
2	33·4	3·7	1·5	Survived
3	28·6	46·2	58·0	Died
4	19·5	0·0	7·5	Died

A, Control period, before blockade or hypertensin. B, End of a 3 hr period of blockade. C, 50 min after termination of blockade and hypertensin infusion.

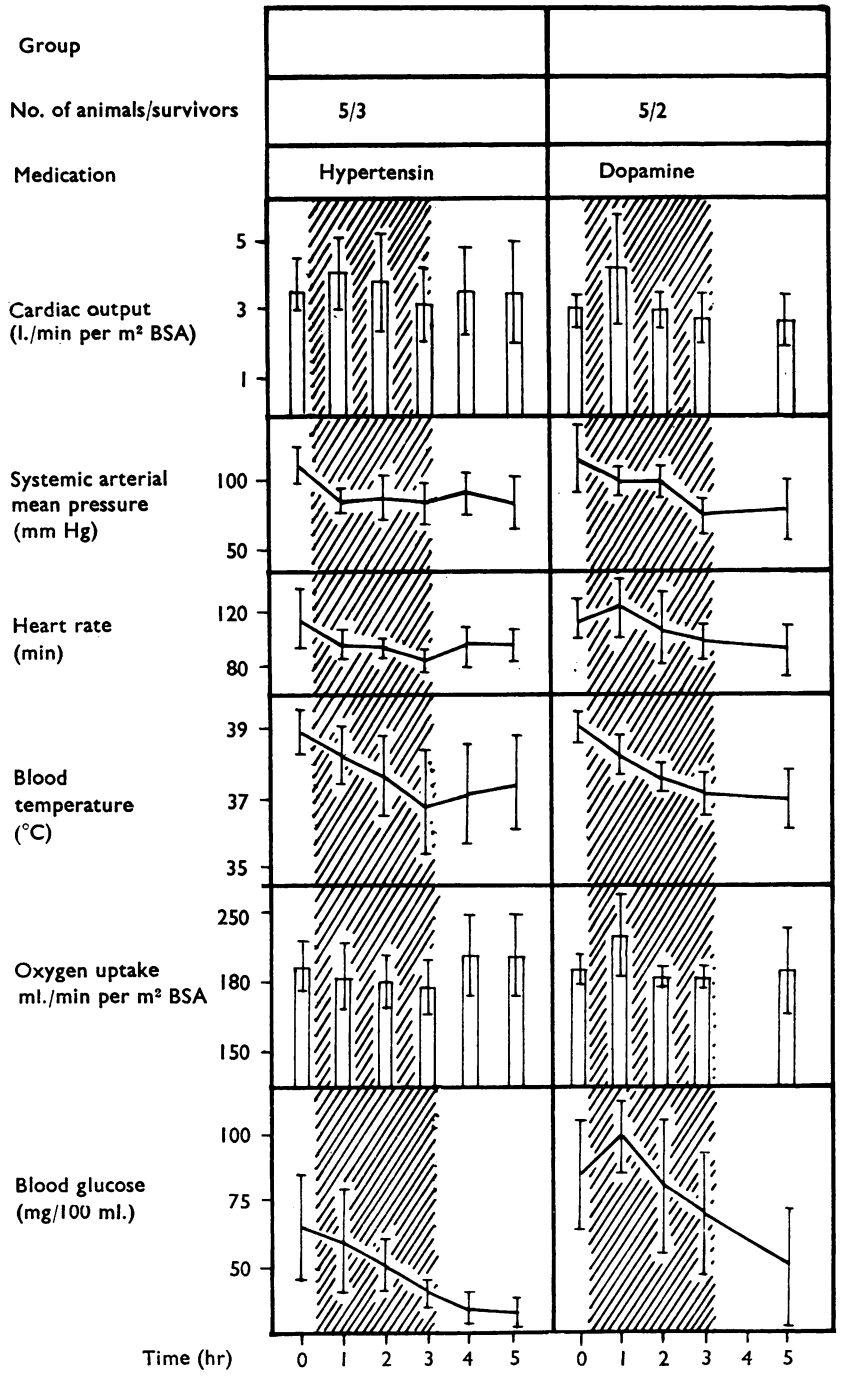

FIG. 3. Complete autonomic blockade: effects of hypertensin and dopamine administration. Note lesser degree of hypotension, absence of fall in cardiac output, less bradycardia and hypothermia as compared to Figure 1. Crosshatched area: duration of infusion (blockade + pressor agent); vertical lines: standard deviations.

systemic arterial pressure remained significantly ($P<0.05$) lower than during the control period. Three out of five animals were alive next morning: this improvement in survival rate barely reached statistical significance ($P=0.06$).

Changes in adrenaline plasma level in four of these animals are shown in Table 1.

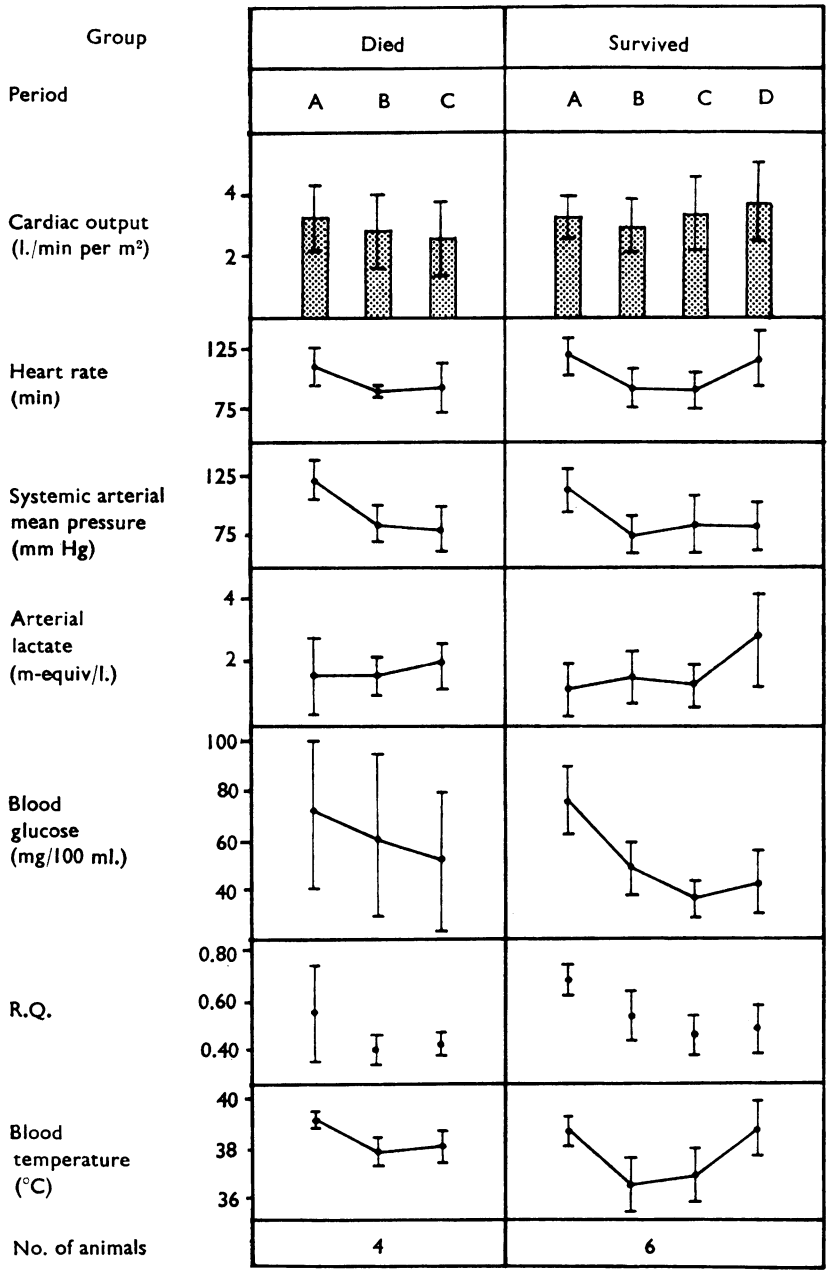

FIG. 4. Complete autonomic blockade with simultaneous administration of hypertensin or dopamine in sheep. Comparison of survivors and non-survivors. Note absence of any significant difference between the two groups at period C. Periods: A, control; B, after 3 hr of blockade + hypertensin or dopamine; C, 1 hr after termination of blockade + pressor agents; D, next morning. Vertical lines: standard deviations.

Effect of dopamine on consequences of complete autonomic blockade in sheep and dogs (Fig. 3)

In this group the small changes in cardiac output, heart rate and O_2 uptake were not statistically significant. Blood temperature fell significantly ($P<0.05$) by the second hour of the infusion and by the third hour systemic arterial pressure was significantly ($P<0.05$) lower than during the control period and remained low after withdrawal of the infusion. Two out of five sheep were alive next morning.

In dogs the blockade-induced changes were similarly modified and are not shown in Fig. 3.

In order to decide if the survival rate in the dopamine-treated blocked animals was significantly improved, the chi-square test was applied to the combined group of five dopamine-treated blocked dogs + five dopamine-treated blocked sheep. The difference was found to be significant ($P<0.05$).

Analysis of parameters determining survival (Fig. 4)

Hypertensin and dopamine-medicated blocked sheep were pooled and divided into survivors and non-survivors in an attempt to identify the changes determining survival. There seemed to be no significant difference between the two groups.

The survivors next morning were all hypotensive and their plasma levels of both lactic acid and free fatty acid (not shown in Fig. 4) were elevated.

Discussion

Bradycardia, hypothermia and the failure to mobilize glucose and free fatty acids in response to hypotension and fall in cardiac output were more marked in animals given trimetaphan and atropine than in those given trimetaphan alone. These changes were consistent with the assumption that interruption of impulse traffic from the central nervous system prevented the release of catecholamines during complete autonomic blockade. Increased lactate production and hyperventilation were also absent. Thus we claim to have succeeded in producing the cardiovascular concomitants of shock without any of the metabolic changes.

In animals subjected to complete autonomic blockade tissue catecholamine content was probably normal. It has been suggested that protection against irreversible shock may be achieved by preventing release of catecholamine from their stores (Zetterstrom, Palmerio & Fine, 1964). Our observations do not seem to support this assumption.

Complete autonomic blockade reduces the contractile force of the heart (Farr & Grupp, 1967), yet no evidence of heart failure was detected in our series: right atrial and pulmonary arterial pressures remained normal and digitalis was ineffective in raising cardiac output. Thus we suggest that circulatory failure in complete autonomic blockade is of extracardiac origin.

The administration of glucose or of glucagon in blocked animals promptly restored blood glucose to normal yet failed to improve survival rate. Hypoglycaemia need not, therefore, be considered as a cause of death.

In sheep, average mean systemic arterial pressure ranged from 62 to 49 mm Hg during infusion of trimetaphan and atropine and 94 to 47 during infusion of trimetaphan: in the former group all animals died, in the latter group all animals survived. In hypertensin or dopamine-medicated blocked animals the blood pressure ranged from 101 to 76 mm Hg and half the animals died. The height of

the blood pressure during the infusion period was virtually identical in survivors and non-survivors (Fig. 4). There seems, therefore, no conclusive evidence in favour of the assumption that prolonged hypotension *per se* was the principal cause of death in complete autonomic blockade and we suggest that the beneficial action of hypertensin and dopamine was at least partly due to factors not revealed in the present study.

Atropine in these experiments was used in a conventional dose and its action, especially in sheep, is of short duration: the vagus-blocking effect of atropine 0.2 mg/kg lasts about 30 min (Colebatch & Halmagyi, 1963). This substance seems to be an unlikely cause of death in the blocked animals.

After termination of the complete autonomic blockade blood pressure in the hypotensive animals no longer responded to the administration of hypertensin, dopamine, noradrenaline, cortisone or dextran infusions, justifying the term "irreversible shock." The reason why simultaneous administration of these two short-acting agents in non-toxic doses resulted in irreversible shock represents a challenging enigma.

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