concentrations between 10^{-4} and 10^{-6} M, spontaneous muscle contractions and spontaneous bursting of motor axons were often recorded (figure 1, E).

Proctolin- or octopamine-induced SR activity was not abolished by 10^{-5} M tetrodotoxin, which blocks axonal conduction in this preparation[4]. Perfusion of calcium-free saline abolished SR activity, which was restored when calcium was added to the saline. Induced SR activity sometimes overshot zero potential (figure 2, A). Induced SR activity was also recorded in the presence of either 10^{-3} M L-glutamate or 10^{-3} M L-aspartate. These agonist concentrations are sufficient to block/desensitize the 'fast' and 'slow' transmitter receptors, respectively[4]. During induced SR activity, neural stimulation would not elicit an excitatory postsynaptic potential (EPSP) (figure 2, C), although recordings from other quiescent fibres in the same 'motor unit'[3] revealed that the nerve was still functioning. Neural stimulation would elicit EPSPs at the start (figure 2, B) and end (figure 2, D and E) of SR activity. Immediately after SR activity, only 'slow' EPSPs could be recorded.

Discussion. SR activity (whether spontaneous or induced) is not caused by nerve stimulation, but bears a marked similarity to cardiac muscle potentials[9] and spontaneous potentials recorded from denervated locust muscle[10]. The insensitivity of SR activity to tetrodotoxin indicates a postsynaptic action which is possibly calcium-dependent. Proctolin and octopamine also have a presynaptic effect at higher concentrations, as indicated by the spontaneous bursting they produce in the motor axons. The postsynaptic action of octopamine and proctolin in producing SR activity is probably mediated by a separate receptor for each substance, as muscles sensitive to one were insensitive to the other. These receptors are probably different from the neurotransmitter receptors, as SR activity could still be recorded when these receptors were blocked.

The low concentrations at which both proctolin and octopamine act, suggest that they might be responsible for spontaneous SR activity. These muscles only receive innervation from 2 motor axons[3], although both axons contain dense-cored vesicles[11,12] which might contain proctolin or octopamine.

The possible function of SR activity is difficult to envisage. During the 3rd instar the innervation changes from a predominantly 'fast' innervation to a predominantly 'slow' innervation[13]. It is interesting, therefore, that following SR activity the 'fast' EPSP is temporarily abolished, leaving only a 'slow' EPSP.

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Total replacement of blood by an emulsion of fluorocarbon in the rat – water extravasation as a cause of failure

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Summary. After exchange transfusion by an emulsion of fluorocarbons (Fluosol 43) in rats, an increase in fluorocarbons and ^{131}I-labelled albumin was observed. These changes suggest a transfer of water from vascular to interstitial space possibly owing to the inability of the emulsion to reproduce the oncotic pressure of normal blood.

Hypovolemia, plasmatic rise in osmolality and viscosity and organ swelling account for the deaths, which are not related to any fluorocarbon-specific toxicity.

Fluorocarbons (FC) can transport important amounts of oxygen and carbon dioxide in solution. Thus FC emulsions have been used as blood substitutes in animals, but few long-term survivals have been achieved[1] in spite of a measured gas transport large enough to sustain life. In a previous work we observed in the rat, after FC total exchange transfusion, an increase in the circulating fluid viscosity and a rise in blood glucose, suggesting hemoconcentration by water extravasation[2].

The aim of this paper is to assess the magnitude of water extravasation and discuss its contribution to the death of animals.

Material and methods. Following a previously reported method[2], 7 rats, weighing from 280 to 350 g, were exchange-transfused with 60 ml of fluosol 43 (Green Cross Corporation) of the following composition: Perfluorotributylamine (FC 43) (20 w/v%); pluronic F68 (2.5 w/v%); NaCl (0.6 w/v%); KCl (0.0034 w/v%); $MgCl_2$ (0.043 w/v%); $CaCl_2$ (0.036 w/v%); $NaHCo_3$(0.21 w/v%); glucose (0.180 w/v%); hydroxyethyl starch (3.0 w/v%); osmolality (280 ± 5 mosm); oncotic pressure (395 mm H_2O); viscosity (3 CP at 37 °C).

In the 7 cases, the exchange-transfusion was achieved in 20 min by the means of indwelling venous and arterial femoral catheters and the hematocrit dropped to less than 3%. The rats were maintained in a 100% oxygen atmosphere. The arterial pressure was monitored by a Statham pressure transducer coupled with a Philips XV1500 monitor. 0.5 ml of ^{131}I-labelled albumin solution (5 µCi/ml) was injected i.v. at time T_0 in 3 treated rats and 2 controls. In the treated group, T_0 corresponded to the end of the exchange transfusion.

1 ml samples of the circulating fluid were taken through the arterial catheter at T_0, 20, 40, 60, 120 and 180 min time. Each sample was replaced by an equal volume of fluosol 43 in the treated animals and by saline in the controls. In 2

treated rats, a 1 ml sample of peritoneal fluid was taken immediately after death.

The concentration of FC 43 in the different samples was measured by gas chromatography and expressed in cg/g of fluid. The radioactivity of the samples was measured, after homogenization by a scintillation counter with a Phillips XL 1100 monocanal spectrometer.

The results are expressed as a percentage of the ^{131}I-labelled albumin volume at T_0+20 min. This time corresponds to a homogeneous diffusion of the tracer in the circulating fluid.

Results. The rats survived for 20, 140, 200, 220, 420 and 540 min respectively after the end of the exchange-transfusion. The mean survival was 247 ± 125 min. At first, the rats' condition looked quite normal. Then a progressive shock occurred, leading to death. The rats showed progressive swelling of the face with eyes protruding; 2 of them showed foaming at the nostrils, suggesting pulmonary oedema. Autopsy showed pleural and peritoneal effusion in 5 cases. The lungs were heavy, sinking in water, and histological examination disclosed enlargement of the alveolar walls by oedema and macrophage infiltration. All these results were comparable to those we previously observed[3].

The FC 43 concentration in the circulating fluid increased with time until the 120th min as shown in figure 1. The values at T_0 and T_0+60 min were significantly different ($p < 0.001$). The FC 43 concentration at T_0+60 min was 210% of that of fluosol 43.

The ^{131}I-albumin volume (figure 2) was different in the treated animals and the controls. The progressive increase in the controls is caused by the physiological transfer of albumin from plasma to the interstitial fluid. In the treated rats, the albumin volume decreased initially and, at 120 min, reached -21.3% of its value at T_0+20 min.

In the peritoneal fluid, the FC 43 concentration was poor (0.1 and 0.6 cg/g). The specific activity of peritoneal fluid was only 45% of the activity of circulating fluid at T_0+20 min.

Discussion. In the treated rats the increase in circulating fluid FC 43 concentration and the decrease of albumin distribution volume is consistent with water transfer from vascular to interstitial fluid.

The water loss was even more important than indicated by our results, since experimental errors tended to minimize it. Thus FC 43 particle-phagocytosis by hepatic and pulmonary macrophages[4] which starts from the beginning of the exchange-transfusion tends to lower the FC concentration. The replacement of fluid samples preserves the circulating volume, but tends to lwoer the concentrations of FC 43 and ^{131}I-albumin. If we consider that every sample is 1 ml and that the rat blood volume is near 25 ml, this fact may be of some importance. The labelled albumin volume is related to the time T_0+20 min, i.e. 40 min after the onset of the exchange transfusion. At this time, the FC 43 level of 34.4 cg/g already shows an important concentration of fluosol 43.

Water accumulates in the interstitial space as suggested by the swelling of the face, pleural and peritoneal effusion and pathologic findings of interstitial lung oedema. Intravascular fluid osmolality increased to highly dangerous levels. Blood glucose reached the value of 10 g/l in some cases. This increase may be a limiting factor for water loss, explaining the stabilization of the FC 43 concentration curve after the 120th min. Various phenomena have been suggested to account for FC emulsion toxicity. Gas embolism hazards due to the high volatility of some FC components is well documented. This danger can be avoided by the use of other FC compounds of minor volatility.

Our results emphasize the role of peripheral shock with water extravasation. The causative mechanism of shock could be a release of histamine in response to a component of the emulsion. The rat is highly sensitive to histamine shock. Another explanation could be the inability of such emulsions to retain water in the vessels. The addition of pluronic 68 and hydroxyethyl starch would not properly reproduce the oncotic pressure of total blood. This is consistent with the opinion of Hansen[7], who demonstrated that red cells in close contact with the capillary membrane develop a strong osmotic pressure and a direct sucking effect on the interstitial water.

However, some definitive survivals in rats after partial[4] or total[5-8] blood replacement indicate that the preparation of suitable emulsions is possible.

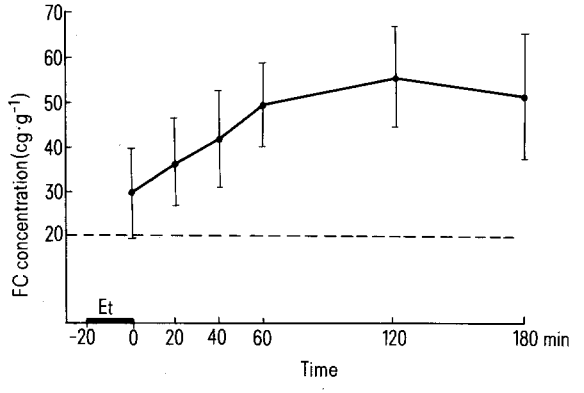

Fig. 1. FC 43 intravascular fluid concentration in 7 rats after exchange-transfusion (ET) with fluosol 43 (mean ± SD). ––– FC 43 concentration in fluosol 43.

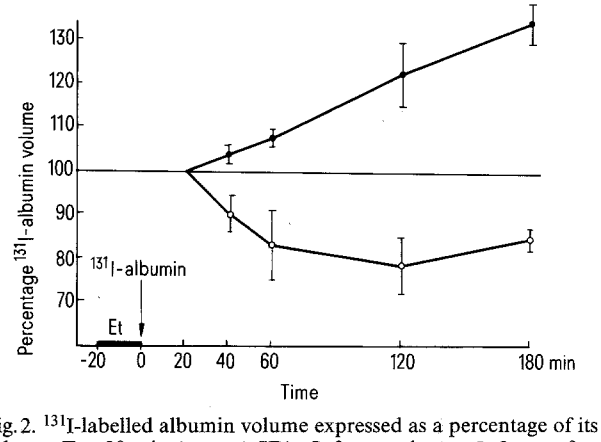

Fig. 2. ^{131}I-labelled albumin volume expressed as a percentage of its value at T_0+20 min (mean ± SD). ● 2 control rats; ○ 3 rats after exchange-transfusion (ET) with fluosol 43.

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