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Study of postmortem blood circulation

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Summary. The objective of this work was to study the postmortem redistribution of blood volumes and its influence of the distribution of substances of different molecular weight (194.2 and 150,000 Da). Lipiodol Ultrafluide, Omnitrast, or a radioisotopic mixture of aminohippuric acid and human IgG were injected into the left ventricle of a total of 20 rabbits divided into three groups. Our results demonstrate the importance of related factors, including left ventricular postmortem contraction, the arterial vascular bed, diffusion processes connected to the physicochemical characteristics of the substances, and the anatomical distribution of the vessels. Postmortem diffusion of aminohippuric acid to the urine was observed.

Key words: Postmortem heart contraction – Postmortem blood circulation

Zusammenfassung. Der Zweck unserer Arbeit war das Studium der Erscheinungen der Blutvolumenverteilung und des Einflusses der Verteilung der Substanzen, die verschiedene Molekulargewichte (194.2 und 150,000 Da) haben, nach dem Tode. Man hat 20 Kaninchen benutzt, die in drei Gruppen eingeteilt wurden. In den linken Ventrikel wurde der einen Gruppe Lipiodol ultrafluide, der anderen Omnitrast und und der letzten eine Mischung Radioisotop aus Aminohippursäure und menschlichem Gammaglobulin gespritzt. Unsere Ergebnisse beweisen die Wichtigkeit der Erscheinungen in Verbindung mit der Kontraktion postmortem des linken Ventrikels, der arteriellen Gefäßlage, der Diffusionsvorgänge in Verbindung mit den physikalisch-chemischen Merkmalen der Substanzen und der anatomischen Gefäßaufteilung. Bei den Versuchskaninchen kommt es sogar im Urin zum Vorhandensein einer Diffusion Aminohippursäure post mortem.

Schlüsselwörter: Postmortale Blutverteilung – Herzkontraktionen, postmortem

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Introduction

The close relationship between postmortem changes in blood volume and contraction of the muscle fibers of the arterial vessels and heart is well known. After death, contraction of the heart muscle and arterial vessels can produce postmortem circulation with a potentially significant redistribution of blood volumes to other organs. Several articles describe the evolution of rigor mortis and the influence of individual and environmental factors [1–4]. We have found only two references dealing with the chronology and distribution of postmortem general circulation [5, 6]. Our study, based on animal experimentation, was designed to analyze these phenomena using two complementary techniques: angiography with contrast medium and labelled isotopes.

Material and methods

A total of 20 New Zealand rabbits aged 3 months and weighing approximately 3 kg each were used. The animals were killed by traumatic decerebration following light anesthesia with ketamine hydrochloride (50 mg/kg i.m.). The thorax was opened medially and the ascending aorta was clamped. After draining all blood from the left ventricle, 5 ml of radiologic contrast fluid was injected.

The animals were divided into three groups. In the first, 12 animals were injected with Lipiodol Ultrafluide (Guerbet), a liposoluble contrast fluid. Another group of three rabbits was studied with Omnitrast 300 (Schering), a hydrosoluble medium. Finally, five animals were injected with a mixture of 0.25 ml (2 mCi) ^{14}C-labelled *P*-[glycyl-1-aminohippuric acid (194.2 Da molecular weight; Dupont) and 0.5 ml (100 μCi/ml) ^{125}I-labelled human IgG (150,000 Da molecular weight; Dupont). Premortem microangioradiographic studies were performed in five animals not included in the experiments to visualize the arterial tree; this information was used as the basis for analyzing the intravascular movement of contrast fluid in the three groups mentioned above.

The animals were immobilized in a supine position throughout the experiment to avoid movement of the blood resulting from postural changes of the corpses, a precaution first recommended by Reinhardt and Zink [6]. Upon unclamping the ascending aorta, a series of X-ray films were taken at 0, 1, 3, 6, 12, 24, 48, and 72 h postmortem. The radiologic studies were performed with a General Electric TX apparatus set at 40 kV, 10 mA, 0.4 s. Agfa Curix Special Frame and Agfa Curix PR2 film was used. Animals from group 3 were autopsied after 24 h, removing samples of vitreous humor (both eyes), brain, lung, heart, liver, spleen, pancreas, both kidneys, skeletal muscle (lower left limb), urine, external ear, and skin. The gamma radiation emitted by ^{125}I was counted with an MR 48 Automatic Gamma Counter System (Kontron) while beta radiation emitted by ^{14}C was counted with an SL 20 Liquid Scintillation Spectrometre (Intertechnique); for the latter, all samples were pretreated with tissue solvent (Amersham) and scintillation fluid.

Results

Displacement of the contrast medium was evident 1 h postmortem, revealing medium-sized arteries such as the liennalis, the hepatic, and the intercostalis, as well as the origin of the pancreaticoduodenal artery. The column of contrast fluid was seen to move up through the abdominal aorta, at times reaching as far as the 2nd or 3rd sacral vertebrae. The images obtained with lipiodol were brighter and more radioopaque than those produced by Omnitrast.

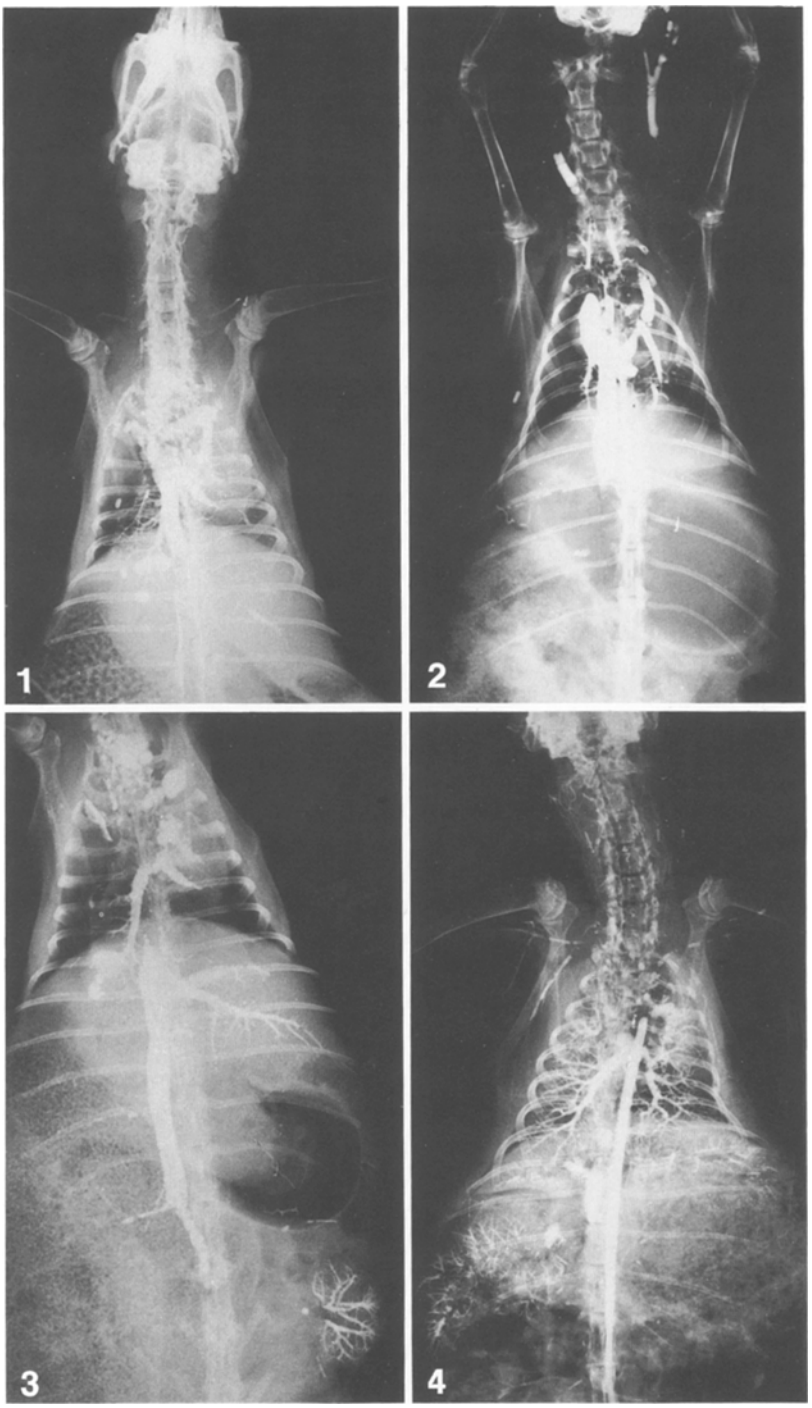

Fig. 1–4. Angiographic results at different postmortem intervals: **1** 1 h; **2** 6 h; **3** 12 h; **4** 72 h

Table 1. Results of isotope studies

	Mean values (β) cpm/g · tissue	Mean values (γ) cpm/g · tissue
Right vitreous (cpm/ml)	900	109
Left vitreous (cpm/ml)	900	100
Brain	270	60
Lung	1,160	6,942
Heart	31,760	1,803
Liver	500	54
Spleen	290	65
Pancreas	290	54
Right kidney	310	55
Left kidney	510	117
Muscle	280	54
Urine (cpm/ml)	710	49
Right ear	280	217
Left ear	280	455
Skin	190	48
Basal counts	60	40

After 3 h, contrast was observed in distal locations, and fragmentation of the medium showed individual images of easily identifiable rectilinear spherical or intraluminal spaces. Filling of the left renal arterial system (left renal, interlobular renal, and arcuate arteries), but not of the right system, was occasionally seen owing to the different anatomical location of the two renal arteries in the rabbit.

After 6 h, the water-soluble contrast fluid was lost from the X-ray images because of diffusion through the tissues. In contrast, the liposoluble contrast fluid remained visible until the end of the study with no significant differences in the visualization of phenomena. The contrast became more obviously fragmented as a result of contraction of the nonstriated arterial musculature. This was more evident in small and medium-sized vessels. Extravasation of the contrast was not seen.

After 12 h, fragmented pockets of contrast fluid stood out in the cephalic third of the corpse. Multiple areas of contrast fluid were scattered throughout the afferents of the cranial vessels. Contrast fluid was also present in the arteries of both ears, while in the abdomen contrast fluid was seen in the iliac bifurcation.

After 24 h the contrast fluid was highly fragmented, making newly occupied vessels difficult to identify. At 48 h most of the afferent arterial tree of the pancreaticoduodenal artery was clearly visible. Contrast fluid was also noted in the right and left external jugular veins. No extravasation was seen. After 72 h, pancreatic vascularization could be visualized. Figures 1–4 show a representative sample of the X-ray films taken at different time intervals postmortem.

Isotopic studies yielded a basal count (background noise) of 60 cpm for beta radiation and 40 cpm for gamma radiation. These data are summarized in Table 1.

Discussion

After death, contraction of the left ventricle and arterial vessels is directly proportional to the muscular mass of the vessels involved. This can cause progressive movement of blood and radiographic contrast fluid along natural anatomical trajectories. The left renal artery, a descending vessel, presents clear X-ray images, whereas the oblique ascending position of the right renal artery hinders movement of the fluids. In order to avoid artifacts due to gravity, the animals were kept supine throughout the experiment. The extremities were also immobilized to eliminate any possible influence on blood circulation due to manipulation of the catheters used to inject the contrast medium.

The liposolubility characteristics of the contrast fluid may potentially cause the blood to diffuse out as it passes through the arteries. However, fragmentation patterns observed up to the 3rd h after death suggest that such passive diffusion, if it does occur, is of little importance in the present study.

Radiolabelling with ^{125}I and ^{14}C yielded results in concordance with the radiographic findings. The two radiolabelled substances (human IgG and aminohippuric acid) differ in molecular weight, a factor that may explain the different patterns observed with both. Isotopic aminohippuric acid can diffuse easily to all organs and fluids, including the kidneys. Distribution of isotopic IgG was more restricted, the marker being all but absent in the brain, liver, spleen, pancreas, right kidney, skeletal muscle, urine, and skin.

The reasons for selecting 24-h postmortem as the cut-off point for our study deserve explanation. After death, autolytic changes can break down the IgG molecule which, despite some degree of degradation, remains relatively stable up to 24 h postmortem. During this period the formation of gases due to putrefaction is limited; thus postmortem circulation traceable to this factor would not appear to play a significant role in the present series of experiments. In general, our findings confirm the existence of postmortem circulation influenced not only by processes such as those described in [6], but also by cardiac and arterial bed contraction. These, together with strictly physical (gravitational) phenomena related to cadaver position and the anatomical location of different vessels, appear to be of particular significance in the first 24 h following death.

The interpretation of some of our biological parameters in samples from corpses is problematic in certain aspects. Low molecular weight substances (e.g, urea and creatinine) can be redistributed via postmortem circulation and physicochemical changes in the molecule. This fact is of importance in selecting the fluid to sample and the place where samples are to be drawn from [7]. Our data help to clarify the evolution of some biochemical parameters in corpses and, moreover, facilitate the interpretation fo certain findings.

Damage to the membranes during autolysis favors diffusion by increasing membrane permeability, a phenomenon exemplified by the postmortem hemolysis of red blood cells. In corpses, molecular diffusion follows simple diffusion dynamics, according to Fick's well-known law. A further factor to keep in mind is the affinity of a given molecule with the components of different tissues. Postmortem circulation obviously foments these processes by allowing the transport of substances in the blood, making them more apt to diffuse out to other organs

or fluids. The end result of this process is conditioned by the autolysis occurring throughout different structures in the corpse. Hence the site of blood sampling together with other variables, i.e., the death-to-autopsy interval and the chemical nature of the specific substance to be analyzed, can significantly affect the value of some parameters.

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