## Distribution of microspheres of $15\pm5\,\mu m$ diameter in dog kidneys

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Summary. The size distribution of plastic non-radioactive microspheres (MS) of  $15\pm5$  µm diameter in the dog kidney was investigated. No diameter-dependent redistribution of the MS was found.

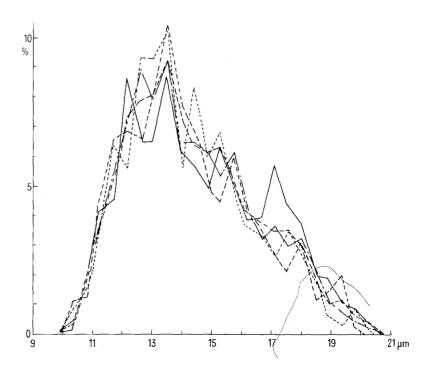
Microspheres (MS) are frequently used for determination of blood flow or circulation characteristics in different organs. Generally radioactive labelled MS are employed. This permits, when the MS are labelled with different nuclides, several measurements under different experimental conditions in the same preparation. Handicaps of this method consist of variations of radioactivity related to random variations of the MS radii<sup>1</sup>, and the fact that only pieces with more than 400 MS give reliable values<sup>2</sup>. In the kidney, size depending redistribution of particulate blood bodies has been additionally suspected<sup>3,4</sup>. For these reasons, the MS method has been seriously criticized and its re-valuation is required, at least when used in the study of the renal circulation<sup>5</sup>. In the present investigation, results are presented which demonstrate that the distribution of MS of 15±5 µm diameter in the kidney is not sizedependent. For this purpose, the size distribution of nonradioactive MS of this diameter is investigated in 4 typical anatomical kidney areas: a) glomeruli of the outer  $\frac{2}{3}$  kidney cortex, b) arteries of this zone, c) glomeruli of the  $\frac{1}{3}$  inner zone, and d) arteries of this area. The postulate is made that a statistical coincidence of the diameter scattering of the administered MS in these zones with that of the injection suspension would exclude a size-dependent distribution of the beads in the observed areas. The investigation is made in vivo and under in vitro conditions as used in hypothermic preservation for transplantation.

Material and methods. In vivo experiments: 1-2·106 MS/kg evenly suspended in 20% glucose solution are given intracardially over a period of 40-60 sec. Thereafter the kidneys are excised, fixed in formalin and embedded in paraffin. In vitro experiments: The kidneys are excised, washed out by gravity with 6% hydroxy ethyl starch (HES) in Ringer or

perfluorotributylamine emulsified in Ringer (Fluosol 43) at 4°C and 73 mm Hg pressure. Thereafter some kidneys are pulsatile perfused during 24 h (80 strokes/min and 60-80 mm Hg pressure) and the rest is stored for the same time at this temperature without perfusion. At the end of the preservation period, all kidneys are again perfused by gravity with 500 ml of the correspondent preservation fluid in which 2 millions MS have been suspended. The kidneys are histologically prepared like those from the in vivo experiments. The experiments are made in mongrel dogs between 17 and 33 kg. Histological preparation and microscopic measurements of the MS: 80-100-µm-thick sections are stained with hematoxilin eosin and mounted for microscopical observation. The cortex of the kidneys in the sections is divided by means of an inkline on the cover glass in 2 zones ( $\frac{2}{3}$  outer cortex and  $\frac{1}{3}$  inner cortex). For the determination of the MS diameter, a TV-camera was installed on a microscope and the MS diameter measured on a TV-monitor by an enlargement of 1660 times. The actual value of the MS size is calculated by comparison

Counted number of MS in the different compartments of the kidneys

Preparation	Kidney area Glomeruli of the outer ½ cortex	Vessels of the outer 2/3 cortex	Glomeruli of the inner	Vessels of the inner
HES	2074	507	549	138
Fluosol 43	353	189	248	116
in vivo	440	92	231	86
N	2867	778	1028	340



Comparison of the diameter scattering von MS from the stock suspension with that in the different kidney areas. — —, stock suspension,—, glomeruli of the outer ½ cortex,—, vessels of the outer ½ cortex; · · · · · · , glomeruli of the inner ½ cortex, - - - - , vessels of the inner ½ cortex.

with the division lines of a blood cell counting chamber. The diameter scattering of the MS in the 4 compartments of all kidneys studied and that of a 863 MS sample from the stock suspension are compared with help of the  $\chi^2$  test at a significance level of 5%

Results and discussion: In the table the number of MS in each area of the kidneys studied is shown. The MS from kidneys preserved by perfusion and by storage with HES are given together. The same is made with the Fluosol 43 preserved kidneys. For grafical presentation of the size scattering, the sum of all counted MS in each area is taken as 100 and the number of MS of different size in each zone is expressed in percent of this value. This size distribution in the 4 zones is compared with that of the MS sample from the stock solution. Between the diameter size scattering in the sample and that in the 4 areas there is no significant difference (p < 0.5). The figure 1 shows this distribution. It is asymmetrical with a maximum at 13 µm.

The size distribution in the 4 compartments investigated is identical with the diameter scattering of the injected MS suspension. This finding demonstrates that with the chosen size no preferential redistribution of MS exits that could be attributed to their different diameter.

The method presented permits additionally the study of the MS distribution by direct observation in the trapping vessel. Therefore a greater resolution than that obtained with the radioactive labelled MS method in the observation of the microcirculation can be achieved.

- Acknowledgment. We acknowledge the Fresenius Company, Homburg, Germany, for supply of Plasmasteril (HES) and the Medac Company, Hamburg, Germany, which supplied us with Fluosol 43 and part of the animals used in the experiments. Supported by the Stiftung Volkswagenwerk, grant 111702.
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## Catecholamine levels in newborn human plasma in normal and abnormal conditions and in maternal plasma at delivery

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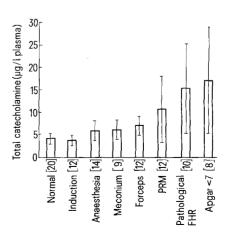
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Summary. High levels of catecholamines have been found in plasma from the umbilical cord of newborn infants, suggesting a release of catecholamine from the fetus during parturition. Plasma catecholamine levels are also elevated in mothers at delivery.

It is well known that the adrenergic nervous system plays a central role in the adaptation mechanisms of the newborn to extrauterine life. The adrenal stores of catecholamines have been found to decline at birth, in both rat and rabbit<sup>2</sup>, while in rabbit the level of plasma norepinephrine increases3. Thus, birth is probably accompanied by an increased release of catecholamines from the newborn animal in these species. It is also known that hypoxia is a powerful stimulus for catecholamine secretion in the fetal sheep<sup>4</sup>. In view of these findings, the purpose of this study was to determine the epinephrine and norepinephrine concentrations in the plasma of the human newborn, in normal and in different abnormal conditions, and also in the plasma of the mother.

Materials and methods. This study was performed on 54 newborn infants and 26 mothers delivered at Maternité Baudelocque, Paris. The infants were divided into 2 groups, according to their clinical conditions, i.e. 'normal' and 'abnormal' infants. The group of 'normal infants' included babies born spontaneously at term, after a normal pregnancy. The group of 'abnormal infants' included seven clinical conditions: induction of labour by oxytocin, anaesthesia of the mother, presence of meconium stained fluid, use of forceps, premature rupture of membranes (PRM), pathological fetal heart rate (FHR) and an Apgar score < 7. All the infants showing one or more of these conditions were included in the 'abnormal' group. Maternal peripheral blood was obtained from healthy pregnant women, during normal spontaneous labour. Blood was collected from the

umbilical cord of infants within 5 min following delivery. Blood samples (about 20 ml) were collected in heparinized tubes placed on ice and containing 10 mg of sodium metabisulfite in order to avoid oxidation of catecholamines<sup>5</sup>. The plasma was separated by centrifugation immediately after collection, and the proteins were removed after addition of 0.2 ml of perchloric acid and a further



Total catecholamines (epinephrine + norepinephrine) in plasma of the human newborn, in different abnormal conditions. Horizontal lines indicate the SEM.