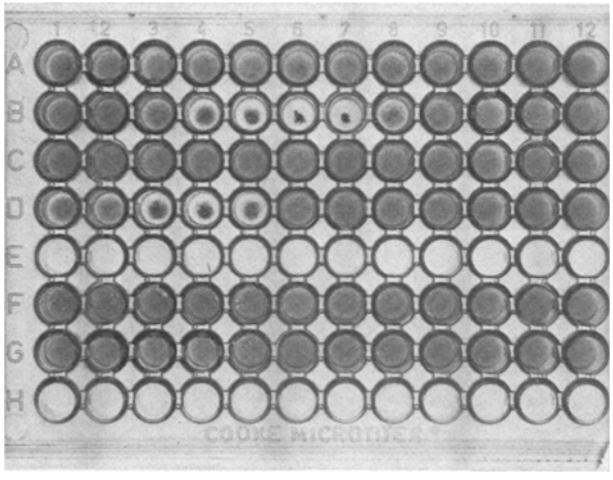

Titration of sera and IgG fractions against concanavalin A.
A) Control serum (anti-Sendai).
B) Anti-Con A serum.
C) Control IgG (anti-Sendai) (1.05 mg/ml in the first well).
D) Anti-Con A IgG (1.25 mg/ml in the first well).
F) and G) same as B) and D) respectively, expect that the erythrocytes were not coated with Con A.
Twofold serial dilutions in PBS from 1 to 11; controls without serum or IgG in 12.

Sensitivity of Ouchterlony diffusion test and 'indirect' haemagglutination assay

	Highest dilutions giving positive results	
	Ouchterlony	Agglutination
Anti-Con A serum	1:40	1:256
Anti-Con A IgG	320 µg/ml	66 µg/ml

Ouchterlony double diffusion tests were run overnight at room temperature. The distance between the wells was 3 mm and each well contained 9 µl of the Con A solution or serum- or IgG dilution, respectively. Immunoprecipitin lines were examined without prior staining of the gels. Agglutination assays were done as described in the text.

The conditions of the incubation (500 µg Con A/ml, 10 min, room temperature) ensure a reproducible preloading of approximately 90% of the available Con A receptor sites[2].

Reaction with antisera. Serial dilutions of antisera in PBS were prepared in microtiter plates (Cooke, Engineering) (25 µl in each well) in advance, and 25 µl of the Con A-coated erythrocytes were added to each well. The plate was shaken for 30 to 60 sec on a Tayo Bussan mixer and read within the next 2 to 3 min.

Results. As seen in the Figure, specific antisera and specific IgG fractions caused massive agglutination (rows B and D, respectively). No agglutination was observed with sera and IgG of unrelated specificity (rows A and C, respectively) or when erythrocytes not coated with Con A were reacted with anti-Con A sera or IgG (rows F and G, respectively).

The anti-Con A serum used here agglutinated coated cells up to a dilution of 1:256 (well B8; 4 assays) and the anti-Con A IgG fraction down to a concentration of 66 µg/ml (well D5). At high concentrations of serum and IgG agglutination was inhibited, suggesting a prozone effect.

As a comparison (Table) we observed immuno-precipitin lines in Ouchterlony plates between Con A (100 µg/ml) and rabbit anti-Con A serum diluted up to 1:40, and rabbit anti-Con A IgG diluted to 320 µg/ml, whereas no reactions were found with the unrelated IgG. A second weak precipitin line found between Con A and undiluted anti-Con A serum was identical with that obtained with undiluted anti-Sendai serum, indicating the presence of serum components different from IgG, but reacting with the lectin. Similarly, in the agglutination assays we observed some slowly developing non-specific agglutination after about 10 min; this, however, could easily be separated from the rapid, specific agglutination shown in the Figure.

Summary. A method for the titration of anti-Con A sera is described. The test is based on the fact that agglutination of Con A-coated human erythrocytes can be prevented by agitation; subsequent addition of Con A-specific antisera and IgG to Con A-coated cells leads to immediate clumping.

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Visualization of Chicken Red Blood Cells in Capillaries by Immunofluorescence

The measurement of regional blood flow distribution using radioactive labelled microspheres has been the object of intensive investigation[1-4]. Difficulties which arise from the rheological properties of these microspheres[2] can be partially avoided by employing radioactive labelled frog red blood cells[5]. Both methods however demand considerable medical preventive precautions and can only be used under closed laboratory conditions. Therefore the development of less difficult methods must be attempted. In the present report, a simple procedure is proposed consisting of labelling red blood cells from chicken by means of a specific antigen-antibody reaction and visualization of the erythrocytes in the capillary vessels.

Materials and methods. a) Preparation of antiserum against chicken red blood cells: Chicken red blood cells are washed with saline solution 6 times by serial centrifu-

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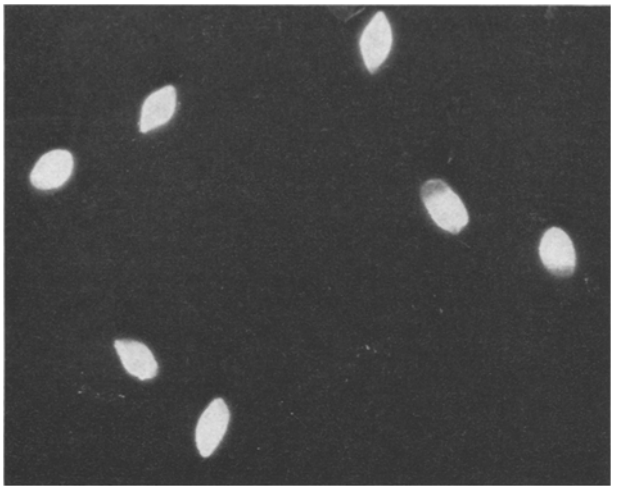

Fig. 1. Fluorescence from chicken red blood cells labelled with anti-red blood cells rabbit serum.

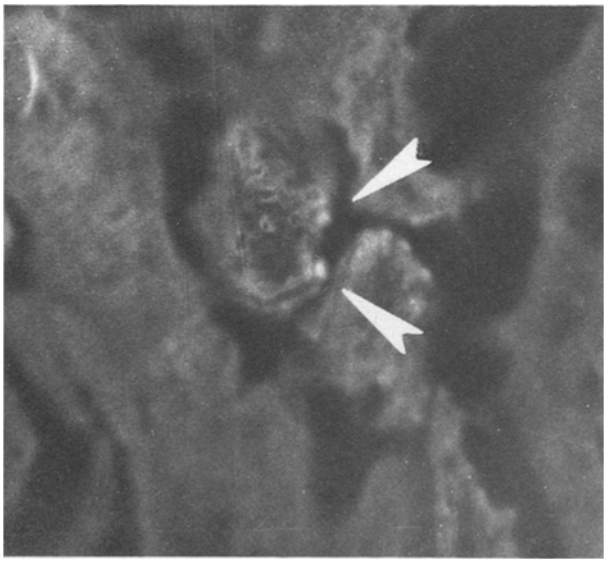

Fig. 2. Histological frozen section of dog kidney demonstrating chicken red blood cells (↑) in a glomerulus capillary.

gation at 3,000 rpm. 0.5 ml packed cell sediment diluted with 0.5 saline is injected i.v. into 4–5 kg rabbits. 5 to 6 times at 1 week intervals, the injection is repeated. 10 days after the last injection, the animals are bled and the blood serum is frozen and conserved at −17 °C.

b) Direct immunofluorescence: The labelling of the antiserum against red blood cells is prepared by conjugating the serum from the rabbits with yellow-green fluorescent FICT[6].

c) Absorption of the antiserum against red blood cells: The labelled antiserum is inactivated for 10 min at 56 °C and then absorbed for 60 min at room temperature with acetone powder made from dog kidney. The mixture is centrifugated at 3,000 rpm. The supernate is used for the incubation of frozen tissue sections.

d) Demonstration of the chicken red blood cells in the capillary vessels (dog kidney): 2 ml washed red blood cells from chicken suspended in saline (10^{11} cells) are given into the aortic arch of a 15 kg bastard dog. After 5 min the left kidney is excized. 2 pieces from the organ are frozen at −20 °C and 4–6 µm sections are prepared. Thereafter the sections are dehydrated for 2 h in 56% ethanol and then incubated for 30 min in the labelled serum at room temperature. After the incubation, the sections are washed 3 times in phosphate-buffered saline (pH 7.2) and then embedded in glycerine phosphate carbonate buffer (pH 9.5)[7]. The preparations are observed in a microscope equipped with UV-illuminator (Ortholux-Fluorescenzmikroskop, Leitz-Wetzlar) and filter system (Fl 40 × 0.65). The photographs were made with the Orthomatcamera (Leitz) and Agfa H P4 Ilford film.

Resultats. Figure 1 show chicken red blood cells labelled with anti-red blood cells rabbit serum. The nucleated chicken red blood cells are elliptical and their bright shape are clearly seen against the dark underground. Flush injection in the aortic arch of 2 ml washed chicken erythrocytes caused no apparent damage in the animals. Pulse rate, blood pressure and respiration were unchanged. Figure 2 shows the histological section of a glomerulus in which 2 red cells are entrapped in a capillary branch. In the kidney medulla and in blood of the renal vein, no chicken blood cells were found.

Discussion. FICT labelled antiserum against chicken red blood cells proved to be specific. A serious limitation of the fluorescent antibody visualization is due to the non-specific fluorescence of the dog kidney tissue. The demonstration of the red blood cells in the capillaries of the organ can, however, be well improved by absorbing the fluorochrome labelled serum with acetone powder from dog kidneys. Theoretical considerations about the possibility and limitations of measuring capillary blood distribution with microspheres or radioactive labelled red blood cells from frog have already been made[2,5] and need no further discussion in this paper.

Washed FICT labelled chicken red blood cells neither agglutinate with dog blood in vitro, nor they seem to aggregate when injected into the arterial circulation of the dog. Because of their size, the red blood cells of chicken are trapped in the capillary vessels and hence can be used to test the blood distribution of organs[2,3]. The intrarenal vascular topography with its portal circulation explains the observation that all the chicken blood red cells are trapped in the glomerulus tuft. Thus, with the use of large size blood red cells, no information on the medullary blood distribution can be obtained. Similarly prepared blood cells of less diameter, however, would pass the capillaries of the glomerulus and distribute homogenously at both cortical and medullar capillary trees.

Summary. Chicken red blood cells labelled with specific rabbit antiserum were used for the demonstration of cortical distribution of blood in the dog kidney by means of direct immunofluorescence. Immunization and labelling techniques are described.

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