A NEW METHOD OF INVESTIGATION OF VASCULAR PERMEABILITY

A. V. Dokukin

From the Laboratory of Pathophysiology and Pharmacology of the Cardiovascular System (Head — Prof. S. V. Andreev) of the Institute of Pharmacology and Chemotherapy (Director — Active Member AMN SSSR V. V. Zakusov) of the AMN SSSR, Moscow

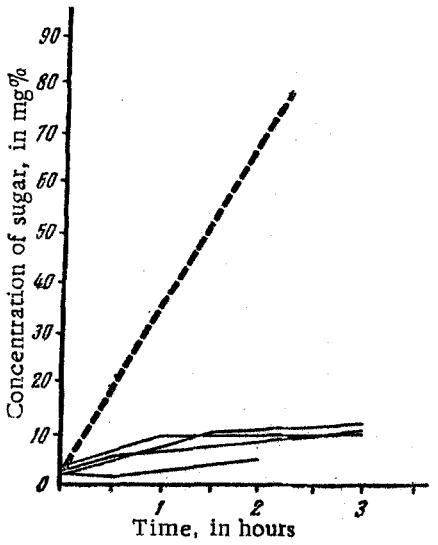
(Received December 19, 1958. Presented by Active Member AMN SSSR V. V. Zakusov)

A widely used method of investigation of the vascular permeability is that of analysis of the contents of the closed natural cavities: the peritoneal cavity [2],the anterior chamber of the eye [3, 4], the cerebral ventricular system [1],and so on. The composition of the fluid which they contain is largely due to the character of filtration through the vascular wall. The degree of permeability of the walls of the blood vessels may be judged from the intensity of penetration into the cavity of substances injected into the blood stream, notably radioactive isotopes. This method has undoubted advantages over other methods of study of the permeability, in which the physiological state of the tissue must knowingly be disturbed (local inflammatory tests, for example). They do, however, have certain defects. The impossibility of estimating the volume of the cavity prevents quantitative interpretation of the results. The great physiological importance of the cavities restricts the use of the experimental procedures which can be used to alter the conditions of filtration. Such procedures include, for example, the creation of an artificial fluid in the cavity of known chemical composition and with given physical constants (osmotic and oncometric, etc.).

Following the suggestion of S. V. Andreev, a suitable artificial cavity for observations was created from an area of the urinary bladder in the rabbit, which was designed for investigation of the permeability of the vessels under chronic experimental conditions.

The operation of isolation of the area of bladder is as follows. On the abdominal wall, immediately above the pubic symphysis, a midline longitudinal incision is made through skin and peritoneum, 2.5-3 cm in length. The urinary bladder is brought out of the peritoneal cavity, the lateral vascular bundles are separated at the junction of the proximal and middle thirds of the bladder (for a distance of 5-7 mm) from its wall, and in the spaces thus formed a ligature is passed in a ring around the bladder. This ligature is then drawn tight, dividing the bladder into two parts. Those vessels to the organ lying above it are left intact. The proximal part of the bladder, in which are situated the orifices of the ureters and urethra, is buried in the peritoneal cavity, and the isolated distal part remains outside it. A suture is placed in the peritoneal incision, constricting the opening to a size which allows free passage of the stretched portion of bladder and the vascular bundles; a continuous skin suture is then inserted.

The operation is not complicated and trauma is slight, and the animals withstand the procedure well. The isolated portion of bladder, from which the urine is first removed and replaced by physiological saline, remains beneath the skin and serves as an artificial cavity. The histological structure of its wall undergoes no essential changes throughout the period of observation (8 months). At any moment the contents of the cavity may be taken for analysis through a special cannula or by puncture of the wall. They form a translucent, slightly opalescent fluid, containing protein (0.05-0.5 parts per 1000), a small number of leucocytes (1-5 per field of vision) and epithelial cells (not in every field). The volume and character of the contents remained unchanged for several months.



Curves of pentration of the blood sugar into a cavity filled with water (continuous lines) and with a 10% solution of ethyl alcohol (broken line).

By injecting various substances into the blood stream (dyes, sugar, radioactive isotopes, etc.) and estimating their concentration in samples taken from the cavity at various times after injection, a curve of penetration of the substance into the cavity from the blood stream is obtained. This curve shows the permeability of the vessels to the substances chosen. In most experiments we did not use foreign substances but estimated the penetration of the blood sugar into the cavity, which was filled with water. It will be seen from the figure that the curve of filtration of sugar is reasonable stable. The injection of substances altering the permeability of the vessels into the cavity has a marked effect on the character of the curve (see Figure).

The volume of the cavity (amounting in various animals to between 5 and 10 ml at atmospheric pressure) is known in each case, and its shape may be regarded as spherical, so that quantitative investigations may be carried out. For example, in assessing the degree of filtration, indices other than the changes in the concentration of the substance injected into the blood stream in unit volume of contents of the cavity may be taken. One such index is the total filtration in a given period of time, equal to Vk, where V is the volume of the cavity and k the final concentration of the substance in its contents.

A still more differentiated index is the filtration per unit area: $Vk/2\sqrt{4.5\ V^2\ \pi}$ in the example shown in the figure, for instance, the total filtration of sugar from the blood into the cavity in 2 hours was 1 mg, and the filtration per unit area 0.045 mg. When the cavity was filled with a 10% solution of ethyl alcohol instead of water, these indices were raised to 6.6 and 0.3 mg respectively.

If various substances are injected into the cavity, the changes in their concentration in successive samples taken from the cavity will give an indication of the filtration of the substances from the cavity into the blood stream.

SUMMARY

The author described a method of forming an isolated cavity from the distal portion of the urinary bladder in rabbits for investigating the vascular permeability in chronic experiments. The urinary bladder is ligated and thus subdivided into two portions. The distal part is led out under the skin. By analyzing the fluid samples obtained from the cavity at different periods after the administration of various substances into the blood or into the cavity, a curve is traced, depicting filtration of the substances from the blood into the cavity and vice versa.

By ascertaining the volume and the area of the cavity, it is possible to conduct quantitative investigations. Building up of artificial contents of a definite chemical composition and with known physical constants in the cavity enables the worker to vary at will the conditions under which the tested substances pass from the blood into the cavity and vice versa, and thereby to study the vascular permeability.