EXPERIMENTAL INVESTIGATION OF THE ABSORPTION OF ERYTHROCYTES FROM THE SUBARACHNOID SPACE

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Erythrocytes appear in the cerebrospinal fluid (CSF) in subarachnoid hemorrhage, complicating trauma or operations and certain diseases of the brain. The outcome of these hemorrhages is largely determined by the degree to which the blood can be removed from the CSF. Treatment of such patients, to be successful, must pay due regard to the removal of blood from the CSF. However, in most clinical investigations of subarachnoid hemorrhage, this problem has been ignored. It is known that blood cells undergo destruction and hemolysis and that the products of this destruction are removed along with the CSF.

Experimental investigations of the absorption of blood or its components (erythrocytes, plasma proteins) have begun to be undertaken only comparatively recently [2, 4-16]. The results of these investigations have been contradictory. Some authors have concluded that blood is easily absorbed from the subarachnoid space. In these circumstances, a large proportion of the erythrocytes are removed from the CSF in an intact form [5, 6, 8, 9, 14, 15]. Others continue to hold the opinion [7, 11, 12, 13, 16] that the blood is retained for a long time in the subarachnoid space, and that the erythrocytes are removed mainly as a result of hemolysis.

The ways in which blood is absorbed from the subarachnoid space are likewise unknown. It has been suggested that the blood is absorbed through the arachnoid villi into the venous sinuses, although there is no direct evidence of this [8, 15]. Another possible route of absorption of blood into the lymphatic stream lies along the perineural spaces of the filaments of the olfactory nerve and the lymphatics of the retro-orbital cellular tissue [8, 9, 14].

In the present investigation the fate of erythrocytes entering the subarachnoid space was examined. The objects of the investigation were as follows: 1) to discover whether erythrocytes are removed from the subarachnoid space only as a result of hemolysis or if they can be removed intact; 2) if the second of these alternatives is correct, to demonstrate by what routes the absorption of the erythrocytes takes place when the pressure in the subarachnoid space is increased.

EXPERIMENTAL

The first part of the investigation was carried out with heterologous erythrocytes. A suspension of the erythrocytes of a cockerel, containing nuclei (labeled cells), made up in physiological saline, was injected into the CSF of animals. The material was injected into the cisterna magna by suboccipital puncture (15 experiments on cats, 3 experiments on dogs), into the cisterna terminalis after laminectomy (10 experiments on cats), and into the lateral ventricles after trephining (10 experiments on cats). The erythrocyte suspension replaced an equal volume of CSF (in the cats, 1 ml into the cisterna magna, 0.5 ml into the lateral ventricle; in the dogs, 4 ml). An increased CFS pressure was created by injecting a larger volume of erythrocyte suspension into the cisterna magna (2 ml) than the volume of CSF withdrawn (1 ml) (5 experiments on cats). The animals were anesthetized with mixtures of different quantities of ether, chloroform, and alcohol. The duration of the experiments ranged from 30 min to 24 h. The animals were killed by electrocution. The CSF was investigated cytologically. The brain, with its meninges, was

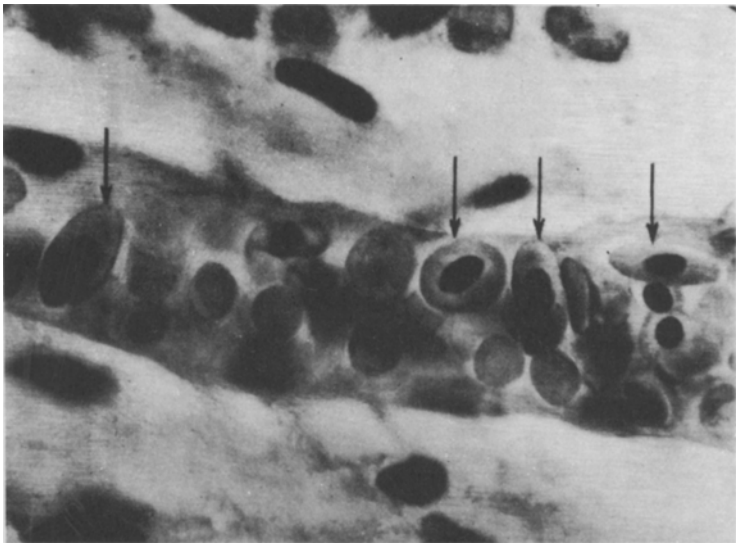

Fig. 1. Nucleated erythrocytes in the internal capillary network of the dura mater of a dog. Injection of a suspension of nucleated erythrocytes into the subarachnoid space. Total preparation of the dura. The arrows point to nucleated erythrocytes among the dog's own blood cells. Photomicrograph. Stained with Ehrlich's hematoxylin and by Lepehne's reaction. Objective 100, ocular 5 (oil immersion).

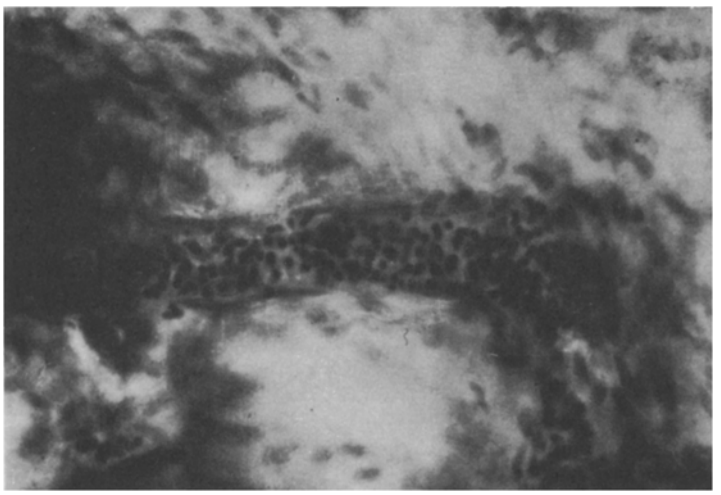

Fig. 2. Nucleated erythrocytes in the lymphatics of the nasal mucosa. Injection of a suspension of nucleated erythrocytes under increased pressure into the subarachnoid space of a cat. A lymphatic filled with nucleated erythrocytes can be seen. Total preparation of the nasal mucosa. Stained with Ehrlich's hematoxylin and Lepehne's reaction. Objective 50, ocular 5.

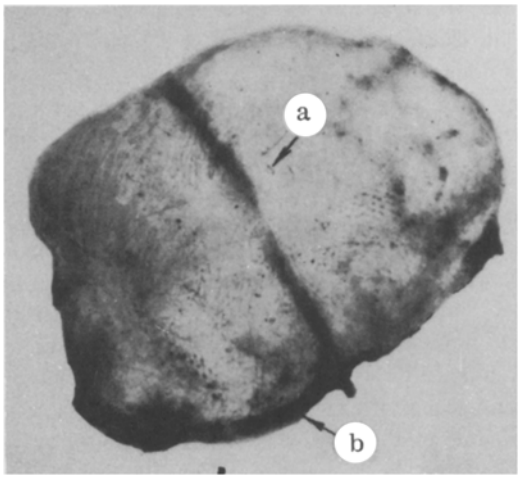

Fig. 3. Macroautoradiograph of the dura of a cat. Injection of P^{32}-labeled erythrocytes into the subarachnoid space. Areas of darkening indicated by arrows correspond to the localization of labeled erythrocytes in the superior longitudinal sinus (a) and the transverse sinus (b).

fixed in 10% formalin for 2-5 days and the dura was isolated. The pia was separated from the brain by fine dissection. The mucous membrane of the nose was removed from the vomer. Film preparations of the meninges and of the nasal mucosa were stained with Ehrlich's hematoxylin or Hansen's iron trioxyhematein. Hemoglobin was detected by Lepehne's benzidene reaction. The films were dehydrated in alcohols of increasing strength, cleared in xylol, and embedded in Canada balsam. The total film preparations were examined under the microscope layer by layer, so that the whole vascular system of the membranes could be studied. Ordinary microtome sections of the brain with its meninges were also prepared.

In the second part of the investigation labeled erythrocytes (auto-and homologous) were used. Cats' erythrocytes were labeled with radioactive phosphorus in vitro, by adding 2-4 μCi of $Na_2HP^{32}O_4$ to 1 ml of defibrinated blood. The cells were exposed at 37°C for 1-2 h. The erythrocytes were repeatedly washed with physiological saline and centrifuged. At the moment of injection the radioactivity of the erythrocyte suspension was 32,000-55,000 pulses/min/ml. Altogether 12 experiments were performed on the cats, including 7 with autologous and 5 with homologous erythrocytes. These were injected by suboccipital puncture, 1 ml of CSF being replaced by 1 ml of the labeled erythrocytes. The radioactivity of the tissue samples (brain, pia and dura, nasal mucosa) and the fluids (CSF, blood) was investigated by means of a type B-2 counting tube. A sample of tissue weighing 50 mg was spread over an area of 2 cm^3. Macroautoradiographs were prepared from the pia and dura, separated into layers and dried on a glass slide, allowing an exposure of 2 weeks with x-ray photographic film.

RESULTS

I. Experiments with Heterologous Erythrocytes

Examination of the meninges with the naked eye after the various methods of injection revealed that the places where the erythrocytes were concentrated could be distinguished by the reddish appearance of the contents of the subarachnoid space. When the injection was made into the cisterna magna or the cerebral ventricles, within the first hours these red areas were spread throughout the cisternae of the base of the brain, the subarachnoid space of the cerebral hemispheres, and the corresponding space of the cervical portion of the spinal cord. When the injection was made into the cisterna terminalis most of the erythrocytes were found in this cisterna and a smaller number in the subarachnoid space of the thoracic portion of the spinal cord. On microscopic investigation individual nucleated erythrocytes, after various methods of injection, were found not only in clusters, but also throughout the extent of the subarachnoid space. They were found lying freely among the subarachnoid tissue. Their number decreased considerably after 6 h, and at the end of 24 h only solitary erythrocytes were left in the CSF. No nucleated erythrocytes were found in the thin-walled veins of the pia mater. They had not penetrated into the perivascular spaces of the brain. These erythrocytes were found beneath the internal surface of the arachnoid mater and they penetrated into its substance. In some places the penetration of nucleated erythrocytes was observed through the external mantle layer of the arachnoid. When this happened, one pole of the erythrocyte was still inside the membrane while the other projected above the external mantle layer into the subdural space. These appearances demonstrated penetration of erythrocytes through the arachnoid mater into the subdural space along with the outflowing CSF [1,3].*

*The penetration of autologous erythrocytes through the arachnoid into the subdural space was confirmed by direct visual observations in dogs following craniotomy and injection of blood into the CSF. Erythrocytes were revealed on cover slips placed on the outer surface of the arachnoid mater (M. A. Baron and N. A. Maiorova).

Microscopic investigation of the dura showed that individual nucleated erythrocytes lay next to its internal surface throughout its extent. Many of them penetrated beneath the internal mantle layer into the thickness of the dura and some were actually absorbed into its internal capillary network (Fig. 1). Nucleated erythrocytes were found in the large venous collectors of the dura and in its venous sinuses among the nonnucleated erythrocytes of the experimental animal. In blood films taken from the superior longitudinal sinus immediately after sacrifice of the dog, many nucleated erythrocytes could be seen. In blood films from the external jugular vein (this vein collects blood from the brain in dogs and cats) individual nucleated erythrocytes were found.

These observations show that the absorption of erythrocytes from the subarachnoid space takes place along with the absorption of CSF through the whole surface of the arachnoid mater into the subdural space, and from the latter into the blood stream of the dura. The cell count in the CSF, consisting of nucleated erythrocytes, fell rapidly during the first hours, but hemolysis at these times was only very slight. This also points indirectly to the absorption of whole erythrocytes. The rate of absorption, judging by the number of nucleated erythrocytes in the blood stream of the dura, was highest in the first 2 h after injection. In the experiments with elevation of the CSF pressure the same macro- and microscopic findings were obtained as in the preceding groups of experiments. In addition, however, in total preparations of the nasal mucosa, the penetration of nucleated erythrocytes was observed into the perineural spaces around the filaments of the olfactory nerve, and from there into the lymphatics of the nasal mucosa (Fig. 2). This route of absorption must be regarded as supplementary to the main channel, and as being opened when the CSF pressure is raised.

II. Experiments with Labeled Erythrocytes

The highest radioactivity was found in the pia mater (130-200 pulses/min) and the lowest in the dura (30-66 pulses/min). The brain was not radioactive. In the macroautoradiographs of the meninges, the areas of greatest darkening, corresponding to the places of localization of the labeled erythrocytes, were found in the region of the fissures of the subarachnoid space. The region of the superior longitudinal sinus was highly radioactive. It gave a well defined darkening on the macroautoradiograph (Fig. 3). Hemolysis in the CSF was slight. The radioactivity of the supernatant CSF after sedimentation of the erythrocytes by centrifugation was not more than 0.5% of the radioactivity injected. This indicated that hemolysis plays an unimportant role in the removal of erythrocytes. The radioactivity of the blood could be determined if the count was made in a large volume (20 pulses/min/2 ml blood). Hence the results of the radiological investigation also demonstrated that absorption of erythrocytes (auto- and homologous) takes place into the blood stream of the dura.

The results show that whole erythrocytes (nucleated, autologous, and homologous), being cells with no intrinsic motility, are abstracted along the channels of absorption of the CSF. They penetrate through the whole surface of the arachnoid mater from the subarachnoid space into the subdural, and from the latter they are absorbed into the internal capillary network of the dura, returning once again to the blood stream.

This migration of the erythrocytes can take place only because of the high permeability of the arachnoid mater [1, 3, 4], of the internal mantle layer of the dura, and of the endothelium of its internal capillary network. The permeability of the latter is approximately the same as the permeability of the endothelium of the lymphatic capillaries [5]. The internal capillary network of the dura must be regarded as the main channel of absorption of erythrocytes from both the subdural and the subarachnoid spaces.

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