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Selective Permeability of Skin Capillaries.

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The extensive experimental work on the so-called blood-brain barrier has shown that at least one area of the capillary system, namely, the capillaries of the central nervous system, is endowed with a selective permeability. As has been shown elsewhere,[1] these capillaries are permeable to electropositive and electroneutral substances, impermeable to electronegative ones. These conclusions were based on experiments with toxins, viruses, antibodies, drugs and aniline dyes in rabbits and guinea pigs. The experiments here reported are concerned with the permeability of the cerebral capillaries to aniline dyes in other species and with the permeability of the capillaries of other organs in guinea pigs.

Barbour and Abel[2] reported that after removal of the fore-brain of frogs the rest of the brain was stained by some sulfonated aniline dyes. We, therefore, investigated the permeability of the cerebral capillaries to basic and acid dyes in the intact frog. Frogs weighing approximately 50 g (*Rana pipiens*) were used and the dyes injected into the abdominal lymphsac. The results are recorded in Table I.

It will be seen that the results are different from those obtained in rabbits and guinea pigs. The cerebral capillaries of the frog are permeable to both basic and acid dyes while the cerebral capillaries of rabbits and guinea pigs are selectively permeable to basic dyes.

Vital staining experiments with some acid aniline dyes reported in the literature[1] mostly without references to our problem seem to indicate that the capillaries in some areas of the vascular system are permeable to acid dyes. The number of organs thus far investigated, however, is limited and the distribution of basic dyes has not been studied systematically.

Four ml of 1% solutions of a variety of basic and acid aniline dyes, therefore, were injected intravenously into guinea pigs weighing 300 g. The staining effects were observed macroscopically and are recorded in Table II.

As may be seen from Table II, muscles, epidermis, lungs, liver, kidneys, and adrenals are stained by both basic and acid dyes although not by all of them. The inner surface of the abdominal skin—and the same probably holds true for connective tissue in general—however is selectively stained by acid dyes.

At first sight this result might be explained by a selective affinity of connective tissue for acid aniline dyes. *In vitro* experiments, however, did not support this explanation. In

TABLE I.
Minimal Staining Doses of Basic and Acid Aniline Dyes in mg, in Frogs (*Rana pipiens*) Weighing 50 g.

Basic dyes		Acid Dyes	
Nile Blue	3.0	Eosin	8
Chrysoidine	3.2	Trypan Blue	9
Bismark Brown	6.5	Naphthol Yellow	10
Malachine Green	8.6	Acid Fuchsin	10*
Pyronine	68.0 (faint)	Congo Red	35 (faint)
Neutral Red	180.0 (faint)	Tropaeolin 00	No staining
Methylene Blue	No staining†		

* After acidification with hydrochloric acid.
† Even after acidification and treatment with H_2O_2.

1 Friedemann, U., *Physiol. Rev.*, 1942, **22**, 125.

2 Barbour, H., and Abel, J. J., *J. Pharm. and Exp. Therap.*, 1910, **2**, 163.

TABLE II.
Distribution of Basic and Acid Aniline Dyes in Various Organs.

	Inner surface of abdominal skin	Muscles	Epidermis	Lungs	Liver	Kidneys	Adrenals
			Basic Aniline Dyes.				
Methylene Blue	0	+	+	+	+	+	+
Night Blue	0	—	—	—	—	—	—
Brilliant Green	0	+	+	+	+	+	+
Chrysoidine	0	+	+	+	0 (?)	0	+
Nile Blue	0	+	—	+	—	0	—
			Acid Aniline Dyes.				
Trypan Blue	+	+	+	+	+	+	+
Indigodisulfonate	+	+	+	0	0	0	0
Light Green	+	+	—	0	0	—	+
Eosin	+	+	+	+	—	—	+

0 = No staining. + = Organ stained. — = Not investigated.

these experiments small bits of freshly excised abdominal skin from which the thin muscular layer covering the inner surface was removed were put into varying dilutions of basic and acid dyes in guinea pig serum. The staining effect is recorded in Table III.

TABLE III.
In vitro Experiments. All Dilutions of Dyes Made in Guinea Pig Serum.

Dilution of dye	Eosin	Methylene Blue	Trypan Blue	Night Blue
1:500	—	—	+	—
1:1,000	+	+	±	+
1:2,000	+	+	0	+
1:4,000	+	+	0	+
1:8,000	±	+	0	±
1:16,000	0	±	—	0
1:32,000	0	0	—	0
1:64,000	0	0	—	0
Cv	1:8,000	1:16,000	1:1,000	1:8,000

As may be seen from Table III basic dyes stain the skin even in higher dilutions than do acid dyes. The objection may be raised that the staining properties of the abdominal skin may undergo a rapid change after removal from the animal body. Transplantation experiments with skin, however, show that excised skin retains its viability during the time consumed in our *in vitro* experiments. Also, respiration of excised tissues remains intact for many hours. There is no reason, therefore, for supposing that the staining properties of the abdominal skin *in vitro* are essentially different from those in the intact animal. Nevertheless, this objection was met by the following experiment: In the anesthetized living guinea pig a flap of abdominal skin was formed by severing the skin in the middle line and along Pouparts ligament. The thin muscle layer on the peritoneal surface of the skin was removed with a forceps and drops of various dilutions of methylene blue and eosin in guinea pig serum placed on the inner surface of the abdominal skin at some distance from the cuts. Both dyes stained in a dilution of 1:10,000.

The *in vitro* experiments do not necessarily express any affinity of the dyes for connective tissue. Primarily probably both dyes penetrate the skin by way of diffusion. There is no reason why they should not do so from the vascular system provided the capillaries of the skin were perfectly permeable to both basic and acid aniline dyes. On this assumption the minimal staining concentration in the blood plasma (Cb) should be identical with the one observed *in vitro* (Cv). It will be shown presently that Cb can be calculated by dividing the intravenous minimal staining amounts of the individual dyes through the plasma volume (1/25 of the body weight). As a matter of fact, according to Wittgenstein and Krebs[3] basic aniline dyes leave the vascular system much more rapidly than do acid dyes. In previous investigations on the permeability of the cerebral capillaries,[4,1] however, it was shown that within the limits of experimental error Cb and Cv were identical for basic dyes. This result shows that the staining effect is determined by the initial

[3] Wittgenstein, A., and Krebs, H. A., *Pflügers Arch.*, 1926, **212**, 268, 282.

[4] Friedemann, U., *J. Immunol.*, 1937, **32**, 97.

TABLE IV.

In vivo experiments. All dyes injected intravenously in 1% solution. Animal killed 3 minutes after injection, by ether anesthesia.

	Wt of guinea pig in g	Dye in mg	Concentration in blood plasma	Result	Cb
I. Eosin	340	11	1:1,200	+	1:2,400
	460	7.6	1:2,400	±	
	300	2.5	1:4,800	—	
II. Trypan Blue	425	28	1:600	+	1:600
	375	12.5	1:1,200	—	
III. Methylene Blue	325	44	1:300	—	>1:300
	300	20	1:600	—	
IV. Night Blue	225	30	1:300	—	>1:300
	265	18	1:600	—	

Plasma volume is calculated by dividing the body weight of the experimental animal by 25.
+ = Strongly colored. ± = Slightly colored. — = Uncolored.

TABLE V.
Cb, Cv, and Cb/Cv.

	Cb	Cv	Cb/Cv
Eosin	1:2,400	1:8,000	3.3
Trypan Blue	1:600	1:1,000	1.7
Methylene Blue	>1:300	1:16,000	>53
Night Blue	>1:300	1:8,000	>27

concentration of the dye in the blood plasma. Cb, therefore, was calculated for eosin, trypan blue, methylene blue and night blue and the results are recorded in Table IV.

The Cb, Cv, and Cb/Cv values for the 4 dyes are recorded in Table V.

As may be seen from Table V the Cb and Cv values for acid dyes are not identical but very close to each other. On the contrary, the Cb/Cv values for basic dyes are far above 1. These experiments show that the capillaries of the skin are permeable to acid dyes, impermeable to basic dyes.

It may be emphasized that the colloidal or non-colloidal character of the dyes is apparently of no significance. For there are colloidal and non-colloidal dyes in both groups. The permeability of the cerebral capillaries was likewise found to be independent of the degree of dispersion of the dyes. The decisive factor is apparently the electrical charge. We thus reach the conclusion that both the cerebral capillaries and those of the skin (and probably of the connective tissue in general) are endowed with a selective permeability to polar substances. But one is the reverse of the other. The theoretical aspects of these findings will be dealt with in a separate paper.