STUDIES ON INFLAMMATION

II. A Measure of the Permeability of Capillaries in an Inflamed Area

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The accumulation of vital dyes in areas of inflammation has been demonstrated by several investigators. MacCurdy and Evans (1) pointed out that the normal brain and cord always remain free from dye injected intravenously but that areas of damage, such as softening or inflammation, become deeply stained. Bowman, Winternitz, and Evans (2) found that trypan blue injected intravenously stains tubercles in experimental tuberculosis. Subsequently Winternitz and Hirschfelder (3) demonstrated that this dye when injected in experimental lobar pneumonia stains the consolidated area of lung selectively: "The intravenous injection of trypan blue and trypan red gave rise to the usual diffuse staining as described by Bouffard, Goldman, etc., but in addition to this the diseased area of lung showed a much more intense staining than any of the other tissues, while the normal lung tissue was practically normal in color." Lewis (4) found that if the cornea of a rabbit is inoculated with a living culture of the tubercle bacillus, a progressive lesion results characterized by an intense congestion of the conjunctiva. If the animal receives an intravenous injection of trypan red 24 hours or more after such inoculation, the fluid in the anterior chamber of the inoculated eye always becomes colored. Precisely similar results were obtained when abrin was administered in the conjunctiva as an inflammatory irritant. A few years ago McClellan and Goodpasture (5) showed that trypan blue accumulates in lesions of herpetic encephalitis in the rabbit's brain, the injured areas presenting a striking color against the quite unstained healthy brain tissue. Siengalewicz (6) pointed out that general damage to the nervous tissue, such as poisoning with carbon monoxide or with salvarsan, is followed by marked staining of the damaged areas by trypan blue. Ramsdell (7) injected trypan blue into the veins of rabbits and guinea pigs previously treated with foreign serum and found that injection of the same serum into the skin of the ear immediately caused local infiltration of the dye into the adjacent tissue. She regarded the infiltration of the dye as an indicator of edematous changes resulting from toxic injury to the capillary endothelium.

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Okuneff (8) found that a thermal irritant favors the passage of vital stains from the blood stream into the area heated. Kusnetzowsky (9) also observed that the local application to the skin of an irritant such as heat or mustard oil causes an accumulation of trypan blue in the inflamed area when the dye has previously been injected into the blood stream. It has recently been demonstrated by one of us (10) that trypan blue injected into the circulating blood enters the site of inflammation rapidly and is fixed there, so that the tissues are deeply stained. Furthermore trypan blue injected directly into the site of inflammation in the subcutaneous tissue or in the peritoneal cavity is fixed in the inflamed area and fails to reach the regional lymphatic nodes. This work clearly showed that there is not merely a rapid accumulation of the dye from the blood stream into an inflamed area, but also that the dye is held in such an area and is unable to drain away through the regional lymphatics.

The object of the work reported here was an attempt to study quantitatively and directly the rate of change of concentration of trypan blue in the capillaries of an inflamed area. It was thought that such direct studies correlated with the preceding studies on the accumulation of dye from the circulating blood into an inflamed area might give some information concerning the change in capillary permeability with inflammation.

Method

An inflammatory reaction was produced in the peritoneal cavity of frogs (R. catesbiana and R. clamitans) (11) by the injection of about 2 cc. of either 5% aleuronat in Ringer solution or 4% turpentine in olive oil (12). Twelve to twenty-four hours after the injection of the irritant the brain of the frog was pithed, the spinal cord being kept intact in order to preserve a more adequate circulation (13). The frog was then placed on a frog board of the usual type and the abdominal wall was incised from the region of the pubis to the sternum. A loop of intestine was gently drawn out and the mesentery spread over a glass cover on the center of the board, which in turn was placed on the stage of the microscope. 1.5 cc. of aqueous 1% trypan blue was then injected directly into the ventricle. The dye stained the plasma of the mesenteric capillaries almost immediately. The circulation in most cases remained unimpaired. Gradually, over a period of about 10 minutes or less, most of the dye diffused out of the capillaries.

All these observations were duplicated in about the same number of normal animals as controls. Throughout the experiments particular attention was paid to the character of the peritoneal exudate, the appearance of the mesentery and the activity of the capillary circulation. When the circulation is in partial or complete stasis the capillaries may show variable changes in concentration of the dye, and these changes therefore, were studied only in capillaries in which the circula-

tion was active. Although prolonged exposure of the mesentery in a normal frog is sufficient injury to set up an inflammatory reaction, the duration of each experiment was so short that this factor, as judged by the scant migration of leucocytes in our control series, was almost negligible.

A graded series of standards of trypan blue ranging in concentration from 20 mgm. per 100 cc. of water to pure water was made up in glass capillary tubes measuring about 4 to 5 cm. long and about 1 mm. in diameter. These were glued on white cardboard so as to be conveniently handled by the observer. The determinations were made colorimetrically, the attempt being to match the dye in the plasma as seen through the low power objective with the dye in the set of standards. With only a little practice we found this method of estimating the concentration of dye in the plasma to be without difficulty. The observations were usually checked by two people and the mean taken. To avoid preconceptions, one investigator would often make readings while ignorant as to whether the mesentery under observation was inflamed or normal. Readings were made approximately every minute, the last not more than 7 minutes after the first since after that time the dye in the plasma was so dilute as to make accurate determination of the concentration questionable. Although the dye seen through the microscope in the capillaries was matched against standards in glass capillary tubes, nevertheless, since the same technique was used in estimating changes in concentration in both inflamed and normal areas, it is believed that these colorimetric measurements have yielded comparable and valuable data even though we cannot assume that they measure quantitatively the dye within the capillaries.

RESULTS

In Table I there are tabulated the results obtained for changes in concentration of trypan blue in the capillaries of the normal mesentery with disappearance of the dye. The results are expressed in units comparable to milligrams of dye per 100 cc. as determined by the standard scale, and the observations range over a period of seven minutes. The averages of the observed values are represented in Chart I.

If the concentration of dye is denoted by y and the time by x, and if $\log y$ for each concentration is plotted against x, a straight line is obtained. This indicates that the relation between concentration of dye in the capillaries and time follows an exponential curve of the "die-away" type: $y = be^{-ax}$, where b and a are constants, and e is the base of the natural system of logarithms equal to 2.71 . . . By the method of least squares the following equation is obtained:

$$y = 7.63\, e^{-.24x} \qquad (1)$$

TABLE I

Changes in Concentration of Trypan Blue in the Capillaries of the Normal Mesentery

Frog No.	Time in minutes						
	0	1	2	3	4	6	7
	Units comparable to milligrams per 100 cc.						
1	10	4	3	3	2.0	1.6	—
2	10	7	5	3	1.6	1.6	—
3	10	7	7	5	3.0	3.0	2.0
4	8.5	—	4	—	—	—	2.0
5	10	—	—	5	—	1.6	—
6	7	—	—	—	2.0	—	1.3
7	10	—	4	3	—	—	—
8	8.5	—	—	4	—	—	1.06
9	7	5	4	3	3.0	1.5	1.5
Average....	9.00	5.75	4.50	3.71	2.32	1.86	1.57

TABLE II

Changes in Concentration of Trypan Blue in the Capillaries of the Inflamed Mesentery

Irritant	Frog No.	Duration of inflammation	Time in minutes						
			0	1	2	3	5	6	7
		hrs.: mins.	Units comparable to milligrams per 100 cc.						
Aleuronat	10	12 : 30	10	4.0	2.8	1.6	1.3	—	1.2
	11	20 : 30	10	—	—	4.0	1.0	0.0	
	14	15 : 00	10	4.0	1.6	1.06	—	0.73	0.0
	16	18 : 00	10	2.0	—	0.37	—	0.0	
	18	17 : 30	10	—	1.6	0.0			
	19	23 : 00	7	3.0	2.0	1.0	0.7	0.7	0.0
	20	17 : 00	10	5.0	4.0	2.0	1.0	1.0	1.0
Turpentine	12	46	7.8	—	2.8	1.3	0.0		
	13	17 : 30	8.5	3.0	1.06	1.06	—	0.0	
	15	20 : 00	10	7.0	—	4.0	1.3	0.73	0.0
	17	18 : 00	10	—	—	4.0	—	1.3	0.0
Average.....			9.39	4.00	2.27	1.85	0.88	0.56	0.37

From this equation the calculated values of y are obtained:

Time (x) mins.	Concentration of dye (y) Units comparable to milligrams per 100 cc.	
	Observed	Calculated
0	9.00	7.63
1	5.75	6.00
2	4.50	4.72
3	3.71	3.71
4	2.32	2.92
5	—	2.30
6	1.86	1.81
7	1.57	1.42

These values are graphically represented in Chart I. It is seen that there is a fairly close agreement between the calculated and observed figures.

The results obtained for the changes during the first seven minutes in the concentration of trypan blue in the capillaries of the inflamed mesentery are tabulated in Table II. As with the normal values, plotting log y against x points to an exponential type of relationship between the concentration of dye in the plasma and the time (Chart I). The equation obtained by the method of least squares is:

$$y = 6.9\, e^{-.42x} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The calculated and observed values of y, graphically shown in Chart I, are as follows:

Time (x) mins.	Concentration of dye (y) Units comparable to milligrams per 100 cc.	
	Observed	Calculated
0	9.39	6.90
1	4.00	4.53
2	2.27	2.98
3	1.85	1.96
4	—	1.29
5	0.88	0.85
6	0.56	0.56
7	0.37	0.37

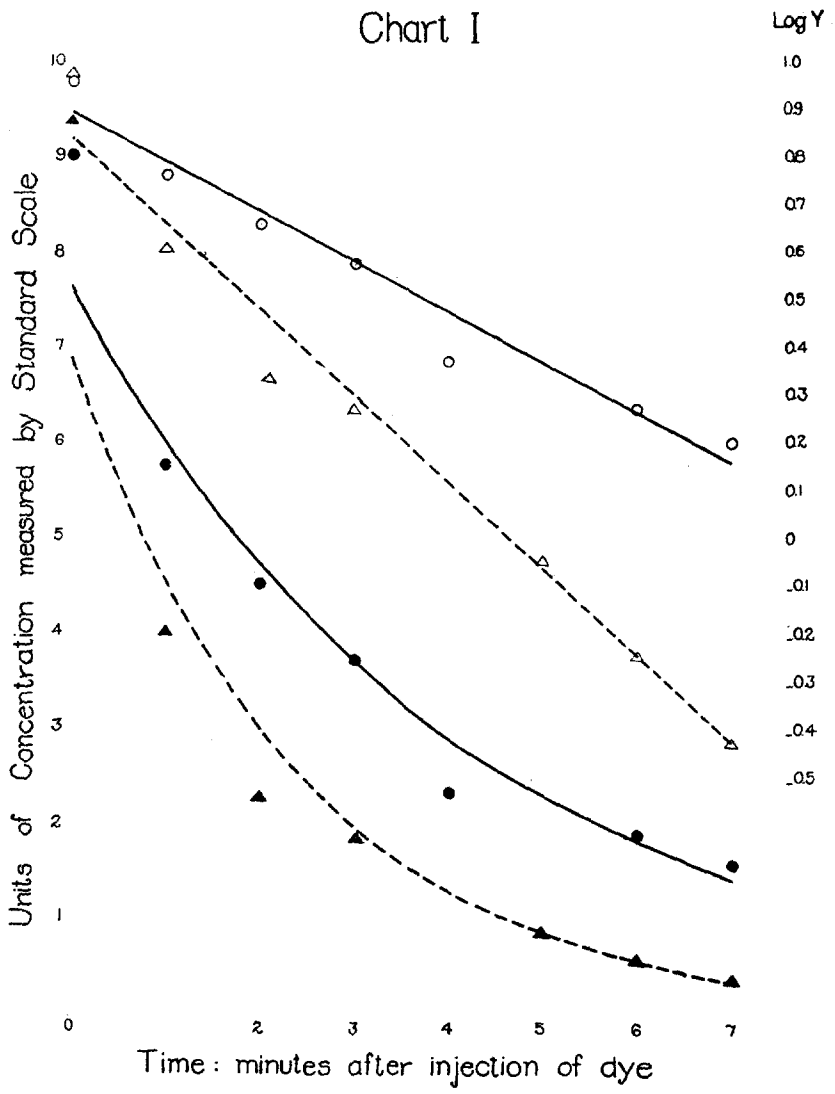

CHART I. The change in concentration of trypan blue in the capillaries of the mesentery. ● observed normal values. ▲ observed values, inflamed mesentery. ——— concentration of dye in capillaries of normal mesentery (calculated). ---------- concentration of dye in capillaries of inflamed mesentery (calculated). ○ logarithm of observed normal values. △ logarithm of observed values, inflamed mesentery.

Both equations (1) and (2) appear to be the nearest expressions that describe the observed facts. It is seen that in equation (1) a equals 0.24, whereas in equation (2) it equals 0.42. Since a is an index of the slope and consequently of the rate of change of concentration of the dye it is clear therefore that the rate of fall of concentration of trypan blue in the capillaries of an inflamed area is almost twice as rapid as that found in the capillaries of the normal mesentery.

A question now presents itself. Is the rate of change of concentration of the dye in the capillaries an indication of the rate of passage of the dye outward into the extra-capillary spaces? The dye obviously diffuses outward into the extra-capillary spaces, as can be seen by direct observation, and, of course, by the fact that the dye stains the cells of the extra-capillary spaces. This does not necessarily mean that the fall in concentration of dye within the capillaries is a measure of the amount of dye that passes through their walls, for it is conceivable that there may be other factors that change the concentration of dye within the capillaries in an inflamed area. However, in view of previous studies (10) in which it has been shown that trypan blue injected intravenously rapidly passes into an inflamed area, it is believed that the increased rate of fall of concentration is a measure of increased passage of the dye through the capillary wall.

DISCUSSION

The above observations show that the rate of fall of concentration of trypan blue in the capillaries after intraventricular injection of the dye is greater in the inflamed than in the normal mesentery. Landis (14) showed by micro-injection studies that the rate of passage of dye solution through the normal capillary wall appears to depend upon the level of capillary pressure, not upon capillary diameter. He demonstrated that the rate of filtration through the capillary wall was directly proportional to the difference between the capillary pressure and the osmotic pressure of the plasma colloids. He also emphasized (13) that the vitality of the capillary wall is of the utmost importance, since injury increases its permeability to proteins. At the same time he demonstrated that the increased filtration through the capillary wall with the use of urethane was due not to a stretched endothelium of the dilated capillary, as Krogh originally believed (15), but rather

to a direct injury of the endothelium by urethane accompanied by a high capillary pressure. Furthermore he showed that capillaries injured by alcohol and mercuric chloride appear to be permeable to the plasma colloids and approximately seven times more permeable to fluids than the normal capillary wall. In view of these facts it seems probable that the increased rate of fall in concentration of trypan blue in the capillaries of the inflamed mesentery might also be caused, as with urethane, alcohol, or mercuric chloride, by a direct toxic effect of the inflammatory irritant on the capillary endothelium, which would thus cause an increase of passage of the dye into the extra-capillary spaces. Such direct injury, causing a fall in the osmotic pressure of the plasma colloids resulting from increased capillary permeability, would adequately account for an increased rate of filtration of the dye in an inflamed area and therefore a greater rate of fall in the concentration of dye within the capillaries.

It is true that the factor of increased capillary pressure cannot be completely ruled out. The work of Hirschfelder (16) would however seem to rule out this factor as being of much significance in the increased filtration of dye in inflammation. This investigator showed that when adrenalin was used to reduce the filtration pressure between the capillaries and the extra-capillary spaces, preventing thus the development of edema, still the inflamed area was stained more deeply than the normal area when trypan blue was injected intravenously. For this reason it is believed that the increased rate of fall of concentration of dye in the capillaries of an inflamed area is primarily due to direct injury of the endothelium by the irritant causing rapid filtration of the dye with increased capillary permeability.

The exponential type of equation which seems to describe adequately the rate of change of concentration of trypan blue in the capillaries brings up an interesting point. This type of curve reaches the abscissa line only asymptotically. It would follow that in these experiments the plasma is never completely free of its dye content. This fact is in accord with the work of Grollman (17) who pointed out that a certain amount of such dyes in the circulation is bound by the plasma protein and therefore does not filter out of the capillaries.

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CONCLUSIONS

1. By means of a colorimetric method the concentration of trypan blue in capillaries can be estimated by direct observation and its changes followed as the dye passes out of the circulating blood stream.

2. The change in concentration of trypan blue in the capillaries of both the normal and the inflamed mesentery of frogs can be described by two separate exponential equations of the type: $y = be^{-ax}$.

3. From these equations it is found that the rate of fall of concentration following intraventricular injection of the dye is almost twice as great in the capillaries of the inflamed as in those of the normal mesentery. This difference is a measure of increased permeability with inflammation.

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