

The Effect of Reticulo-Endothelial Blockade on Dermal Spreading of India Ink

The reticulo-endothelial functions were studied at our laboratory with the aid of radioactive colloids and we worked out a method of testing the colloid absorbing ability of the reticulo-endothelial system (RES) by means of labelled chromium phosphate colloid (GABRIELI¹). Using this method we have studied the dynamics of blockade in the RES and estimated the degree and duration of the blockade induced by different colloidal substances (GABRIELI and HOLMGREN¹).

BOGOMOLETS² proposed the use of trypan blue as an indicator of the functional state of the RES in human beings. In this test the rapidity with which the intracutaneously injected trypan blue travels was followed. Recently ANIGSTEIN and WHITNEY³ pointed out, by means of experimental typhus Rickettsiae infections, the connection between the RES, ground substance and hyaluronidase.

In the experiments reported below, we investigated the effect of blockade of the RES on the spreading of intracutaneously injected India ink.

Methods and Experiments. For our experiments we used fullgrown albino laboratory rats of about 150 g body weight. Hyaluronidase (5 VRU)⁴ dissolved in 0.05 ml physiological saline solution and mixed with the same volume of India ink (the India ink was diluted with an equal volume of water) was injected intradermally on one side of the backs of the rats. The whole

mixture amounted to 0.1 ml. The same mixture, but without hyaluronidase, was injected intracutaneously on the other side. Immediately after injection the size of the ink spots was traced on transparent paper. One hour later, the areas of the two India ink spots were recorded again and compared with the initial values.

In one series (using 33 rats) a blockade was produced by either intravenous or intraperitoneal injections of colloidal iron (50 mg per kg body weight "Ferrivenin", Bengers Ltd., England), while the control animals were given physiological saline solution intravenously or intraperitoneally instead of iron colloid. After 3–4 hours the dermal spreading of India ink was investigated.

In another series we studied the dermal spreading at different times after an intravenous blockade. In this series we used 15 VRU hyaluronidase. We injected colloidal iron (50 mg per kg body weight) intravenously and 3, 4, 7 and 24 hours later the dermal spreading effect of hyaluronidase was investigated, using three animals at a time. Six control rats were injected intravenously with physiological saline solution instead of colloidal iron, and the dermal spreading of India ink was tested 3 and 24 hours later in the same manner as in the blocked animals.

Results. The results after intravenous and intraperitoneal blockade from the first series of experiments are summarized in the table. One hour after intradermal injection of India ink, the area increased much less in the intravenously blocked animals than in the control animals.

The results of the second series proved that the inhibitory effect of the intravenous blockade on the spreading of India ink lasted only a few hours. Three hours after the intravenous blockade the spreading of the India ink had diminished and after four hours it reached a minimum. After five hours we noted an increased spreading of India ink which still remained after 24 hours (see Figure).

Effect of RES blockade upon the dermal spreading of India ink in the presence and absence of hyaluronidase (5 VRU)

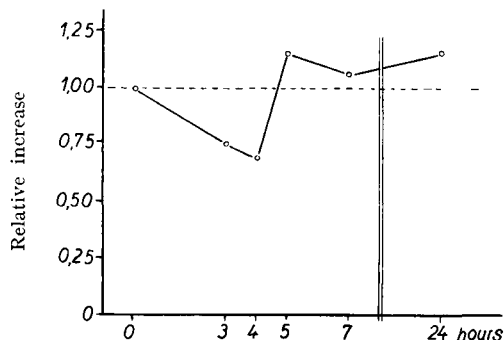
	Area of ink spots in mm ² . Mean (± error of mean)		
	Intravenous blockade (10 cases)	Intraperit. blockade (10 cases)	Controls (13 cases)
<i>With hyaluronidase</i>			
Immediately after injection (a)	116.4	110.4	103.6
1 hour Later (b)	213.4	263.8	317.0
Relative increase* } ($\frac{b}{a}$)	1.88 ± 0.24	2.62 ± 0.33	3.35 ± 0.46
<i>Without hyaluronidase</i>			
Immediately after injection (a)	57.2	59.7	75.2
1 hour Later (b)	92.1	94.9	135.5
Relative increase* } ($\frac{a}{b}$)	1.65 ± 0.23	1.70 ± 0.14	1.99 ± 0.28
<i>Difference in increase</i>			
$d \pm \epsilon_d$	0.23 ± 0.33	0.92 ± 0.36	1.36 ± 0.54
t	0.70	2.56	2.52
P	0.492	0.020	0.012
Significance	No	probable	probable

* Mean of the relative increases of the areas in individual observations
 $d \pm \epsilon_d$ = difference and error of difference
 $t = \frac{d}{\epsilon_d}$
 P = probability that the difference may arise at random (obtained by a t -analysis)

Definition of probability
 $P > 0.05$ no sign
 $0.05 > P > 0.01$ probable sign
 $P > 0.01$ statistical sign

The measure $(\frac{b}{a})$ is approximately normally distributed

If the relative increases in cases with and without hyaluronidase are compared, it is seen that the former values are consistently higher than the latter. If the t -values (third row from the bottom) of the three groups of experiments are pooled, the difference in question becomes statistically significant ($P < 0.001$). The pooling is effected by dividing the sum of the three t -values by $\sqrt{3}$ and using the resultant value as a t -value with infinite d. f.¹.



The relative increase in rats of the spots of India ink (0.1 ml containing 15 VRU hyaluronidase) given intradermally at different times after i. v. administration of 50 mg per kg. body weight of colloidal iron. The relative increase was calculated by comparison of the areas, immediately and 60 minutes after the injection of India ink, and by division of this value with the mean value from 6 control animals, which were injected with physiological saline solution instead of colloidal iron. Each circle represents 3 experiments.

Discussion. The RES may be said to consist of the lymphatically and the haemovascularly located phagocytic elements. If a colloid is injected intravenously, it is taken up by the haemovascularly located reticulo-endothelial cells. One cannot find iron colloids in the lymphatic macrophages after intravenous iron injection (ANDERSSON and REXED²). In the above mentioned experiments the hyaluronidase effect was tested intradermally. It is of interest to notice that after intravenous blockade we obtained a marked inhibition of the spreading of India ink.

Using Ferrivenin the inhibiting effect in the blocked animals lasted a relatively short time. After five hours we could no longer show any inhibition of the spreading of India ink. This result was similar to that which we obtained when we investigated the blocking effect of Ferrivenin with the aid of radioactive colloids. The phagocytic ability of the RES was resumed in a few hours.

We intend to make a further study of the RES and the spreading effect. E. R. GABRIELI³ and M. CAMPANI

Department of Experimental Histology, Karolinska Institutet, Stockholm, April 2, 1951.

Zusammenfassung

Die intradermale Ausbreitung von chinesischer Tusche in Gegenwart von Hyaluronidase wird vermindert, wenn das retikuloendotheliale System mit kolloidalem Eisen (Ferrivenin) blockiert wird. Dieser Effekt ist größer, wenn Ferrivenin als blockierendes Agens intravenös anstatt intraperitoneal angewendet wird. Die Wirkung hält nur wenige Stunden an. Es wird auf die Ähnlichkeit verwiesen, die zwischen der Ausbildung der Blockade und der verminderten Ausbreitung der Tusche besteht.

¹ G. GOLDBERG *et al.*, Acta physiol. Scand. 22, suppl. 77 (1950).

² N. ANDERSSON and B. REXED, Nordisk. Med. 42, 1704 (1949).

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Autoradiographic Analysis of the Distribution of Labelled Ca and P in Bones

A microphotographic-photometric study of the X-ray absorption of bone ground substance revealed that minerals are unevenly distributed in the latter¹. Resorption and new formation of bone tissue determine changes in the distribution of minerals in compact and spongy bone. The Ca content in recently formed pieces of secondary bone (e.g. Haversian systems, *osteons*) is in general from 10 to 12% lower than the Ca content in the fragments of the older periosteal bone or in the interstitial remnants of previously built osteons; the mineralization of the newformed osteons undergoes a slow and progressive increase.

Autoradiographs made from ground sections of bones compacta of young dogs a few hours after administration of labelled P, showed a greater activity of the matrix in recently laid down osteons than in the older primary bone tissue; viz., a larger number of labelled atoms is incorporate in the ground substance of the relatively less calcified pieces of secondary bone.

However, it remained open to question whether the different deposition of labelled atoms in the various structures of bone depends on the different situation of the latter in regard to the blood vessels, or on different physico-chemical properties of the ground substance itself. It is obvious that mineral atoms of the blood can be adsorbed more easily in the ground substance which neighbors the vascular channels than in the matrix which lies at a greater distance from blood vessels, namely in the interstitial systems.

A different technique was applied in this research to obtain further information on the alternative mentioned.

Ground sections of fresh bone compacta from 25 to 45 micra in thickness were kept from a few hours to a few days in solutions of radioactive Calcium chloride². The sections, thoroughly washed in distilled water and dried at room temperature, were pressed against the emulsion side of photographic plates or films (C2 Ilford Nuclear Research plates, Ferrania X-ray films)³. This procedure has the advantage that the radioactive Ca comes in ready and almost uniform contact with each single particle of the bone section; thus the possibility that a different deposition of labelled atoms in the various structures of bone might depend on the topography of the latter in regard to blood vessels was ruled out.

Figures 1 and 2 illustrate the Roentgenmicrophoto (negative) of a cross section of the diaphysis of the femur (man 47 years old) with its corresponding autoradiograph after treatment with labelled Ca. It is apparent (fig. 2) that the radioactivity is not uniform throughout compacta. The comparison in the same section of the degree of radioactivity (fig. 2) with the degree of calcification of the various structures (fig. 1), demonstrates that a greater amount of radiocalcium is deposited in the ground substance of recently formed osteons; the Ca content of the latter is from 6 to 16% lower than that of the neighboring older bone tissue.

The distribution of Ca⁴⁵ in the ground substance appears to be exactly the same as described above when

¹ R. AMPRINO and A. ENGSTRÖM, Boll. Soc. ital. Biol. sper. 26, 148 (1950); Acta anat. (in press).

² The author wishes to express his thankfulness to Prof. P. LA-CROIX, Louvain, who kindly supplied the Calcium chloride for these experiments.

³ Technical details and a complete description of results will be published elsewhere.