ON THE EXISTENCE OF A FACTOR INCREASING TISSUE PERMEABILITY IN ORGANS OTHER THAN TESTICLE

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In an earlier publication a report was made on an infection-enhancing effect obtained with testicle extract. This property is shared by certain other organ extracts but to a far less degree (1). As the enhancing power of testicle extract was later found to be associated with a modification of the skin permeability (2), extracts from various organs have been tested to determine their relative ability to cause this.

The dissociation between *spreading* and *enhancing* action (3) observed with certain tumor extracts, and the necessity of ascertaining where the spreading factor exists in the body, led us to undertake a systematic study of extracts from various tissues by following the spread of India ink particles in the rabbit skin. Such an investigation was also considered as an indispensable preliminary to the study of the physiology of the factor or factors, and of the mechanism of its action.

Method

Adult rabbits of both sexes were used. The extracts were prepared by grinding the freshly removed organs with sand and 1, 1.5, and 2 volumes of Ringer's solution or water. The suspensions were centrifuged and 0.5 cc. of the cloudy supernatant fluid injected intradermally in the flanks of rabbits together with 0.25 cc. of Higgins' India ink, diluted 1:2. A similar control injection of 0.5 cc. of Ringer's solution or water plus 0.25 cc. of the ink was always made in the same animal. The spread of the ink was measured 1 and 24 hours after injection. In some cases precipitation of the tissue extract with 4 volumes of acetone, and re-extraction of the residue with variable amounts of water was resorted to with the hope of purifying and concentrating the factor. The technique, which had been very effective in the case of testicle extract, did not give better results than the use of fresh extract.

EXPERIMENTAL

About 120 tests were carried out, each test involving the extract of an entire organ, or in the case of some extracts, of the pooled organs of 16 different individuals (rats). The average results with testicle extract were included for comparison. The findings are summarized

TABLE I

Occurrence of the Spreading Factor in Various Mammalian Organs

Organ extract tested	No. of tests	Average spreading of 0.5 cc. organ extract plus 0.25 cc. of India ink dilution	Average spreading of 0.5 cc. saline plus 0.25 cc. India ink dilution (control)	Ratio of active spread to spread of control	Positive cases with increased diffusion
		sq. cm.	*sq. cm.*		*per cent*
Rabbit lung	7	27.3	7.2	3.8	100
" spleen	7	18.1	7.2	2.5	100
" liver	7	8.5	7.1	1.2	42
" kidney	10	17.6	7.2	2.4	80
" ovary	2	20.4	4.1	5.0	100
" placenta	2	22.0	9.0	2.4	100
" brain	10	10.4	5.3	1.9	90
" skin	4	10.1	6.5	1.5	100
" blood serum	15	9.8	10.9	0.9	0
" striated muscle	3	3.2	5.4	0.6	0
" testicle	9	55.6	5.8	9.6	100
Rat lung	2	20.3	7.8	2.6	100
" spleen	5	24.2	7.4	3.2	100
" liver	5	26.7	7.4	3.6	80
" kidney	5	18.6	7.4	2.5	100
" brain	4	14.5	6.6	2.2	100
" blood serum	2	7.8	7.8	1.0	0
" testicle	10	40.4	5.8	6.9	100
Guinea pig lung	2	17.0	6.1	2.7	100
" " spleen	2	11.0	5.0	2.2	100
" " liver	2	17.5	5.0	3.5	100
" " kidney	2	9.0	5.1	1.7	100
" " blood serum	1	6.8	6.8	1.0	0
Calf thymus	1	12.0	4.0	3.0	100
Human placenta	1	9.0	4.0	2.2	100
" serum	7	7.4	7.0	1.0	0

in Table I. They clearly demonstrate the existence of spreading factors in all organs studied, although in a proportion far inferior to that existing in testicle, as judged by the India ink spreads. Striated muscle extracts might constitute an exception to this rule. In one investigation they were found inactive.[1] Sera proved inactive in every case.

The quantitative differences in the spreading factor or the extracts studied were further brought out by dilution. Whereas testicle extract still increased the spread of ink when diluted to 1:400 (the highest dilution studied) extracts of other organs were no longer active at 1:10, and only in the case of lung and spleen extracts was there observed some spread at 1:5 dilution.

Another difference between testicle and other organ extracts consisted in the lack of regularity with which the latter yielded a spreading factor, contrasted with the perfect consistency of the former in this respect. This is indicated in the last column of Table I and holds especially for liver. Lung and spleen extracts, although fluctuating in their yields, always exhibited a certain activity in increasing the spread of ink.

The spread induced by the various organ extracts was similar to that obtained with testicle extract, but took place much more slowly. Nevertheless, lung and spleen extracts caused in certain cases considerable spreading in the hour following injection. In general though, the amount of the ink distributed through the area in which spread occurred was much less marked than in the case of testicle extract. The areas of spread when testicle extract was used looked always blackened by the ink, while the skin modified by the extracts from other organs, as shown by the spread of ink particles through it, was regularly lighter. Another detail to be mentioned is that the skin thickening (probably due to edema) was greater in the case of the latter. Skin treated with testicle extract was not swollen. Some of these points are illustrated in Table II, in which the areas and the density of the spreads produced by extracts prepared with the organs of a single rabbit were recorded 1 and 24 hours after injection.

[1] Spinelli (6) has also found a spreading power in thyroid extracts. Muscle extracts, taken as control, were inert in 8 of his 15 trials. Testicle extracts were always active.

Summarizing we can state that practically every one of the 12 organs studied contains in widely varying amounts factors increasing tissue permeability, as shown by the spread through it of India ink, and that testicle and epididymis, lung and spleen seem to be the most active.

The possible rôle played by the testicle in bringing about the presence of the spreading factor in the other organs has been investigated. Their property of increasing tissue permeability was found to be retained after castration. The results obtained with rats' organs

TABLE II

Progression of the Spread with Various Organ Extracts

	Readings after 1 hr.		Readings after 24 hrs.	
	Area of spread	Density of spread	Area of spread	Density of spread
	sq. cm.		*sq. cm.*	
Ringer's solution plus India ink (control)	4.1	±	4.2	±
Spleen extract	14.6	+++	29.0	+++
Lung "	9.0	+	36.9	+++
Kidney "	8.1	+++	17.6	+++
Brain "	6.2	++	10.5	++
Liver "	8.4	++	8.0	++
Muscle "	4.4	+	4.9	+
Testicle " (average of 9 tests)	19.4	++++	55.6	+++++

tested 5 weeks after castration have been included in the table. This observation indicates that the spreading factor is a usual constituent of these organs.

COMMENT

The results of the present investigation clearly demonstrate the existence in nearly every organ tested of a factor active in increasing tissue permeability. It was shown in previous work that the same organ extracts possess the power of enhancing bacterial and virus lesions (1). In the case of extracts from testicle (2) and from invasive bacteria (3) a direct correlation was found to exist between the degree of spreading and the intensity of enhancing power. These observations make it probable that the same correlation may exist in

most of the organ extracts studied. Further experiments are necessary to test this assumption. It is sustained by the fact that certain azo compounds which are active spreading agents also enhance and increase the size of infectious lesions (4). The fact deserves mention, however, that spleen extracts not only failed to enhance vaccinal or staphylococcus lesions, but sometimes exhibited a definite inhibiting action. An analogous dissociation of the properties of organ extracts regarding the power to increase tissue permeability or to enhance infections has been met with in the case of certain melanomas (5). Likewise, extracts of some transplantable sarcomatous growths failed to enhance infectious lesions, although rich in spreading factor. In such cases there was an indication that an anti-infectious agent was present in the tissue extracts, in addition to the spreading factor.[2] For this reason we consider the permeability test more reliable for the detection of the factor in organ extracts than the original method of the production of lesions. Blood serum is completely devoid of both enhancing and spreading power.

Work is under way on the chemical relationship between the testicle factor and the factor obtained from other organs. The possibility of quantitative or qualitative variations at different periods in the life cycle is also being considered.

SUMMARY

Many of the organs from animals of both sexes, including the ovary, have been found to contain in various proportion a factor or factors increasing tissue permeability. The potency exhibited by such active extracts was always less than that of extracts from testicle. Blood serum was found to be devoid of any spreading property.

[2] In support of this view we can adduce the fact that, while extracts prepared from testicle immune to vaccine virus still retain to the same extent as normal testicle the power of increasing dermal permeability, they fail to enhance the virus lesions and indeed actually suppress them. The immune testicle extract may have spread the vaccine suspension over a large area, but it has spread an inactivated material. It is worth noticing in this connection that Felton (7) has increased the virulence of pneumococcus by repeated automatic transfers in lung medium and decreased it by similar transfers in spleen medium.

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