THE ACTION OF TESTICLE, KIDNEY, AND SPLEEN EXTRACTS ON THE INFECTIVE POWER OF BACTERIA

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Work reported by Duran-Reynals (1) has shown the existence in certain organs of a substance which we shall call, for purposes of abbreviation, the Reynals factor (2), capable of enhancing to an extraordinary degree vaccinal and staphylococcus infections.

The Reynals factor is not species specific and is present in the testicle, the extracts of which, together with the extracts of epididymis, are by far the most active in this respect of any organ. Extracts of kidney, skin, brain and placenta share the property only to a certain extent. Extracts from retina, muscle and ovary do not modify the infection, whereas those of blood, spleen and bone marrow never enhance and sometimes interfere with, and even suppress the activity of the infectious agent.

As the earlier work on the Reynals factor included observations on only two strains of staphylococci (3), it was thought of interest to try many strains and kinds of bacteria and to test the constancy of the factor and whether its action is widespread.

Methods

For the sake of convenience, the method of preparing testicle extract is described again in this paper. Fresh testicle is removed from an anesthetized or freshly killed animal, stripped of its membranes and the glandular tissue is ground in a sterile mortar with an equal quantity by weight of sterile Ringer's solution. It is then centrifuged, and the supernatant fluid is used for the experiments. To this supernatant fluid is mixed varying amounts of a 24 hour broth culture of active bacteria. The type of broth culture and its pH are, within limits, negligible.

EXPERIMENTAL

The following tables give the results obtained when bacteria are injected simultaneously with testicle extract into the skin of mice and rats. It should be understood that by + and ++ is meant a lesion of about 1 cm. or even less, indurative, without a genuine abscess formation and disappearing in from 2 to 10 days; by the expression +++ is meant a lesion from 2 to 3 cm. in diameter, distinct oedema,

TABLE I

Organism	Number of mice injected		Average resultant lesion		Number of animals dead	
	Testicle extract	Ringer's	Testicle extract	Ringer's	Testicle extract	Ringer's
<i>E. typhi</i>	2	2	++++	++	2	0
<i>B. dysenteriae</i> Shiga.....	3	3	++++	++	1	0
" " Hiss Y.....	3	3	+++	++	0	0
" " Flexner.....	3	3	++	+	0	0
Cholera.....	2	2	+	-	0	0
<i>B. paratyphosus</i> A.....	2	2	++	-	0	0
" " B.....	2	2	+++	+	0	0
Proteus X19.....	2	2	++++	+++	1	0
Proteus X2.....	2	2	+++	+	0	0
Enterococcus.....	2	2	+++	+	0	0
<i>Enteritidis</i>	2	2	++++	+	1	0
Tetragenus.....	2	2	++++	++	2	0
<i>B. prodigiosus</i>	2	2	++++	++	2	0
<i>S. rosea</i>	2	2	+++	++	0	0
<i>Str. erysipelas</i>	3	3	++++	++	1	0
Pneumococci Type III.....	4	4	++++	++	3	0
<i>E. coli</i> , str. 1.....	2	2	+++	+	0	0
<i>E. coli</i> , str. 2.....	2	2	++++	++	1	0
<i>B. violaceus</i>	2	2	++	+	0	0

a central area of suppuration, healing in about 10 to 20 days; and by ++++ is meant a lesion that is acute, with great oedema and with suppuration and ulceration, which may extend the complete length of the abdomen, healing in from 4 to 5 weeks time. These units of degree of infection and the time required for the lesion to heal depend entirely on the virulence of the bacteria used and the dose given.

The effect of the Reynals factor on the lesions produced by many

kinds of bacteria on mice is clearly shown by the intracutaneous injection of 0.15 cc. of an 18 hour broth (pH 7.8) culture of the following organisms together with 0.15 cc. of testicle extract or Ringer's solution (Table I).

TABLE II

	Number of rats injected	Dose staph. culture	Testicle extract	Ringer's solution	Average resultant lesion	Number animals dead
		cc.	cc.	cc.		
Nonhemolytic staph.	2	0.5	0.5	—	++	0
	2	1	1	—	+++	0
	2	2	1	—	+++	0
	2	0.5	—	0.5	±	0
	2	1	—	1	+	0
	2	2	—	1	+	0
Hemolytic staph.	4	2	2	—	++++	2
	2	3	2	—	++++	1
	4	2	—	2	++	0
	2	3	—	2	++	0

TABLE III

	Rabbit	<i>B. coli</i>	Testicle extract	Ringer's solution	Lesion
		cc.	cc.	cc.	cm.
Experiment 1.....	1	0.1	1	—	2.5 x 8.4
"	"	0.1	—	1	1.8 x 4.7
Experiment 2.....	2	0.5	0.5	—	8.5 x 4.7
"	"	0.5	—	0.5	4.2 x 2.4
Experiment 3.....	3	0.25	0.5	—	8.4 x 5.5
"	"	0.25	—	0.5	1.6 x 1.6
Experiment 4.....	4	0.1	0.5	—	7.7 x 6.1
"	"	0.1	—	0.5	2.9 x 1.7

The effect of the Reynals factor on lesions produced by a 24 hour broth (pH 7.3) culture of active *Staphylococcus py. aureus nonhaemolyticus* and *haemolyticus* injected together with testicle extract in varying doses intracutaneously into rats is shown in Table II.

The results of intracutaneous inoculation of a 24 hour broth (pH 7.3) culture of active *B. coli* together with testicle extract into rabbits are shown in Table III.

Experiments with Kidney Extract.—Extracts of kidney prepared in the same way as those of testicle activate in a lesser degree the staphylococci. So far 24 animals have been inoculated, with 2 strains of staphylococci and kidney extract, including controls. The results from intracutaneous injection into rats of 24 hour broth cultures of

TABLE IV

	Number of rats injected	Dose staph. culture	Kidney extract	Ringer's solution	Lesion	Number animals dead
		cc.	cc.	cc.		
Nonhemolytic	3	0.5	0.5	—	+++	0
	3	1	1	—	+++	0
	3	0.5	—	0.5	+	0
	3	1	—	1	++	0
Hemolytic	3	0.5	0.5	—	+++	0
	3	1	1	—	++++	1
	3	0.5	—	0.5	++	0
	3	1	—	1	+++	0

TABLE V

Resulting lesions from 1 cc. 24 hr. broth culture staphylococci									
Dose spleen extract	Total animals injected	Number of animals with different degree of lesion			Dose Ringer's	Total animals injected	Number of animals with different degree of lesion		
		+	++	+++			+	++	+++
cc.					cc.				
1	5	4	1	0	1	4	1	2	1
1	5	1	4	0	1	4	2	1	1
0.5	7	1	6	0	0.5	5	1	2	2
2	4	3	1	0	2	4	0	2	2

Staphylococcus py. aureus mixed with kidney extract are shown in Table IV.

Experiments with Spleen Extract.—Spleen extract, when added to a suspension of organisms and inoculated intracutaneously into rabbits, has the opposite effect of extracts of testicle and kidney. The lesions are never enhanced, and are in many cases slightly less than those in the controls. The experiments with spleen extract deal only with

two strains of staphylococci. The extract was prepared in the same way and used in the same manner as the two foregoing extracts.

The results of intracutaneous injection of *Staphylococcus py. aureus* (*haemolyticus*) into rabbits simultaneously with spleen extract are shown in Table V.

DISCUSSION

The observations of Duran-Reynals on the action of different organ extracts on vaccine virus and staphylococcus have been extended in the present experiments to 20 different strains of bacteria. Hoffman (4) has shown that testicle extract enhances the infectivity of herpes virus, vesicular stomatitis and Borna disease. It is apparent, then, that we are dealing with a general phenomenon which may well play a role in natural disease.

The nature of the enhancement is not yet clear. Duran-Reynals has shown that vaccine virus injected by any route localizes where testicle extract has been injected intracutaneously, and an extensive lesion results. Other investigators (5, 6, 7, 8) as well as ourselves (9) have proved that extracts of testicle and kidney greatly enhance cell multiplication and growth *in vitro*. On the other hand, the important rôle played by injury and by repair in the onset or localization of infection is well known. It is possible that the Reynals factor from testicle and kidney extracts either acts on the host cells to alter them directly, or else that they are so altered in the processes of injury and repair that they become a more suitable medium for infection. Work is now being carried out on the effects of these extracts on bacteria themselves.

The interfering action of lymphoid tissue on normal and pathological cells may be here recalled (10) in connection with the fact that spleen extract does not enhance the lesions produced by staphylococci, and even in many cases tends to lessen the size of the lesion.

CONCLUSION

The addition of testicle extract to cultures of 20 different bacteria just prior to inoculation enhances their infectious activity to a high degree.

Kidney extracts enhance the infections of staphylococci to a less

degree than testicle extracts, while spleen extracts never give rise to enhancement, and often cause the lesion to be less than would ordinarily be the case.

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