Protective Effect of Cattle Sera on White Mice Infected with *Brucella abortus* with the Aid of the Mucin Technic. (17763)

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Dishon and Olitzki(1) produced lethal *Brucella*-infections in white mice with the aid of the mucin technic. The lethal effect of intraabdominal injection of 0.5 cc of a broth culture of the test organism together with 0.5 cc of a 10% mucin suspension was irregular. Since it is difficult to inject more than 1.0 cc of this mixture, the production of regular lethal infections with the aid of concentrated broth cultures has been studied. The experiments showed that injection of 0.5 cc of a 4-fold concentrated 48 hour broth culture together with 0.5 cc of a 10% suspension of mucin regularly produces a lethal infection.

Mucin preparations manufactured by the Armour Laboratories, London, and Winthrop Stearns Inc., New York, were equally effective. To avoid dispersal of the highly infectious material in the laboratory, the *Brucellae* were cultured in centrifuge tubes containing 10 cc of broth. After incubation at 37°C for 48 hours, 3 out of 4 tubes were centrifuged, the supernatant fluids discarded and the sedimented bacteria suspended in the fourth broth culture. The bacterial suspension concentrated in 10 cc of broth contained the growth of 40 cc. After the addition of an equal quantity of the 10% mucin suspension 1.0 cc of the resulting mixture administered intraabdominally produced 100% mortality in white mice.

The protective effect of cattle sera. Sera were kindly provided by Dr. van der Hoeden, Director of the Central State Veterinary Laboratory in Tel Aviv. They were derived from nonimmunized cattle presumed to have had contact with *Brucellae* and from cattle which had been immunized with living "Swiss strain 19". Part 1 of Table I shows the protecting effect of 26 sera. The sera were injected into the dorsal muscles of the mice immediately after the lethal inoculation with the *B. abortus*-mucin suspension. Only sera with an agglutinin titre for *B. abortus* higher than 500 were protective. Moreover, the protective effect was not correlated with the quantity of serum injected. A serum with an agglutinin titre of 1000 administered in quantities of 0.5 and 0.2 cc did not protect, whereas 0.1, 0.05 and 0.02 cc of the same serum protected. In some cases, mice which received 0.5 cc of serum died before the control animals. In general these experiments suggest that the agglutinin titre and the protective effect of cattle sera show no clearcut correlation when the serum is administered after the infection has taken place.

In another series of experiments, cattle sera were injected 24 hours before inoculation with *B. abortus*. A close correlation between agglutinin titre and protective effect was observed. Part 2 of Table I shows that only 10% of the sera with agglutinin titres below 200 gave positive protection tests, while 94% of the sera with agglutinin titres of 200 and more gave positive protection tests.

Correlation between the protective effect and the agglutinin titre. In general sera with agglutinin titres higher than 200 protected the mice when given in quantities of 0.01 cc or more. Table II shows the protective effect of five sera administered in varying quantities 24 hours prior to the inoculation of *B. abortus*. There is no definite correlation between the protecting and agglutinating power of the sera. Three sera with an agglutinin titre of 1000 gave 100% protection at 0.05, 0.05 and 0.01 cc, two sera with an agglutinin titre of 500 gave 100% protection when administered in quantities of 0.02 and 0.05 cc. Thus the ratio between the minimal agglutinating quantity per cc and the minimal 100% protecting quantity varies between 1:10 and 1:50 with an average of 1:30. The doses of serum which give a 50% protection vary between

1. Dishon, T., and Olitzki, L., Proc. Soc. Exp. Biol. and Med., 1949, v71, 698.

TABLE I.
Agglutinin titre and protecting effect of cattle sera injected immediately after or 24 hours prior to the inoculation of *B. abortus*.

Time of admin.	Titre of agglutinins below	No. of sera examined	No. of sera showing protecting effect after the injection of 0.5 cc	0.2 cc
Immediately after the inoculation	200	16	0	0
	200	1	0	0
	500	2	0	0
	1000	3	0	1
	2000	3	1	2
	10000	1	1	1
24 hr. prior to inoculation	200	20	2	2
	200	3	2	2
	500	3	3	3
	1000	1	1	1
	2000	6	6	6
	5000	1	1	1
	10000	3	3	3

TABLE II.
Agglutinin titres and quantitative protection tests carried out with 5 sera.

No. of serum	Titre of agglutinins	No. of deaths after administration of serum (cc) 0.5	0.2	0.1	0.05	0.02	0.01	0.005	0.002	0.001
1	1000	0/2	0/2	0/2	0/4	1/4	3/4	3/4	3/4	4/4
2	1000	0/2	0/2	0/2	0/4	1/4	2/4	3/4	3/4	4/4
3	1000	0/2	0/2	0/2	0/4	0/4	0/4	4/4	4/4	3/4
4	500	0/2	0/2	0/2	0/4	0/4	1/4	1/4	3/4	4/4
5	500	0/2	0/2	0/2	0/4	1/4	1/4	2/4	1/4	4/4

0.012 and 0.004 cc and the ratios between the minimal agglutinating quantity per cc and the 50% protecting doses vary between 1:12 and 1:2 with an average of 1:6. The results show that the agglutinating and protecting powers of cattle sera against *B. abortus* are not closely related. In all instances, however, the serum doses required for 100% or for 50% protection were higher than the minimal agglutinating serum dose.

The bactericidal effect in vivo. Fifty-nine surviving mice were sacrificed on different days between 2 and 4 weeks after the day of infection. None of these animals gave a positive blood culture. Eighteen showed general infections of the abdominal organs, 20 local infections of the spleen and 6 local infections of the liver. It is evident that the administration of serum had not prevented the establishment of the microorganisms in these organs.

Summary. Lethal infections of *B. abortus* may be produced regularly in mice with the aid of the mucin technic by the injection of 0.5 cc of a 4-fold concentrated 48 hours broth culture. Cattle sera with agglutinin titres for *B. abortus* of 200 or higher protect the mice against the infection, when at least 0.05 to 0.02 cc of serum is administered 24 hours prior to infection. The minimal protecting dose and the minimal agglutinating serum quantity per cc are not closely linked. The serum prevents general septicemia and death, but does not prevent the formation of infectious foci in the abdominal organs.

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