Lack of Correlation Between Skin Test and "Tissue" Sensitivities in Guinea Pigs Infected with *Brucella abortus*. (23636)

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The problem of a negative or a depressed skin test for bacterial hypersensitivity of the delayed type in animals known to be infected with the test agent is a very real one. Many reports have dealt with changes in skin sensitivity to tuberculin in humans and other animals infected or presumably infected with *Mycobacterium tuberculosis* during intercurrent infection(1,2), during pregnancy(3,4), during administration of drugs(5), and during changes in age of animals(6) to mention only a few. Data which are derived both from *in vivo* skin tests and *in vitro* "tissue sensitivity tests" are rather scarce(5,7).

During studies on host-parasite relationships using *Brucella abortus* and guinea pig monocytes(8), we found that many guinea pigs which had received large, infecting doses of living *Brucella abortus* did not exhibit positive skin tests after a suitable period of time despite the fact that at autopsy these same animals almost invariably showed splenic and other lesions due to *B. abortus*. We decided to study this phenomenon using *in vitro* technics for determination of "tissue" sensitivity to ascertain if there was a discrepancy between the tuberculin type skin test and *in vitro* "tissue" sensitivity. In recent studies(9), Heilman, Howard and Carpenter reported an *in vitro* system for the study of "tissue" sensitivity in brucella infected animals. Our findings confirm and extend those of these authors.

Materials and methods. Guinea pigs were young adults at the time of infection with *B. abortus*. Strain Lo or strain SA-S‡ was injected subcutaneously in a dose of 0.1 ml of a saline suspension ($BaSO_4$ standard #1) of a 48 hour culture. Animals were killed at stated times and the spleens were removed immediately. Tissue culture methods were those described in a previous paper(10). In some cases pooled, fresh, normal guinea pig serum (30%) and Hanks' balanced salt solution (70%) were used as the medium while in other cases fresh autologous serum and Hanks' comprised the medium. No differences due to this factor were noted. Splenic fragments were imbedded in a chicken plasma clot by using a drop of chick embryo extract as the clotting agent. The proper amount of bacterial extract in 0.5 ml of medium was added as soon as the clot had become firm. Eight tubes, each containing 2 explants, were used for each treatment. The extract of brucella has been described by Pomales-Lebrón (12). For a portion of the experiments an extract of strain Lo was used. An extract of SA-S was used for others. Results were similar with either extract. Saline in place of extract was added to control culture. Cultures were incubated at 37° for 4 days. Skin tests were carried out by intradermal injection of 0.1 ml of a 1:10 dilution of the brucella extract. Certain animals giving negative skin test at this dilution were retested using a higher concentration of extract. After preparation of the explants, some of the spleens were cultured on tryptose agar to determine if viable brucella were present. Crude estimates of the number of recoverable organisms were made (Table I). The statistical significance of differences between extract-containing tissue cultures and saline-containing cultures, based on the migration and degeneration of large wandering cells, was determined according to procedures reviewed by Boyd (11). A p value of 0.05 or less than 0.05 indicates the cultures to which extract had been added showed significantly less migration and

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TABLE I. Results of *In Vitro* "Tissue" Sensitivity Tests and Bacterial Cultures of Normal and Infected Guinea Pigs.

Animal	*B. abortus* strain	Duration infection	Skin test	Tryptose culture of spleen	Probability that control and exp. showed no difference: Control *vs* 1:25 extract	Control *vs* 1:50 extract	Control *vs* 1:75 extract
Normal			Negative	N.D.		p >.05	
"			"	"		p >.05	
Infected	Lo	40 days	Positive	"		p <.01	
"	"	3 mo	"	"		p <.01	
"	"	5 "	Negative*	"		p <.05	
Normal # 33			"	Negative	p >.05		p >.05
Infected 45	SA-S	11 days	"	++++	p >.05†		p >.05
" 30	"	1 mo	"	++	p = .01		p >.05
" 337	"	5 "	"	+	p = .01		p = .01
" 338	"	5 "	Doubtful	Negative	p = .05		p = .05
" 494	"	5 "	Negative	+	p >.05		p >.05

In one instance tissue from a normal noninfected animal was inhibited at 1:75 conc. of extract. No reason can be given for this discrepancy. The high probability (p = .01) left no doubt as to the effect. This had never been observed before during titration of the allergen.

N.D. = not done. ++++ to + refer to relative amount of growth of *B. abortus* from spleen cultures.

* Tested 2 mo and 4 mo. Negative both times. Cells were, however, capable of suppressing growth of *B. abortus* as would be expected of immune cells (Pomales-Lebrón and Stinebring(8)).

† Probably before allergy had developed.

significantly more degeneration than the controls.

Results. From the data in Table I one may conclude: (a) a definite cytotoxic effect of brucella extract on splenic cells from infected guinea pigs has been observed, (b) a negative skin test is no criterion of "tissue" sensitivity in infected animals, and (c) skin sensitivity and "tissue" sensitivity may not be exhibited even though brucella may be isolated from the host 5 months after infection.

The first conclusion is similar to that of Heilman *et al.*(9) in regard to brucella infections of guinea pigs. The reaction is probably due to the same mechanisms as in the experiments first described by Rich and Lewis(13).

The work of Lurie *et al.*(5) and Heilman and Feldman(7) clearly demonstrates that "tissue" sensitivity and skin sensitivity in the case of experimental tuberculosis are not necessarily correlated. However, the animals studied were treated with hormones or were in the final stages of progressive disease.

Our findings indicate, similarly, that the two tests may be dissociated in experimental brucellosis in untreated animals exhibiting no evidence of intercurrent infections and apparently tolerating the experimental infection well (guinea pigs do not ordinarily succumb to *B. abortus* infection). Therefore, the *in vitro* test is a better indication, in most cases, than is the skin test of sensitivity of the host to a particular allergen. However, it must be noted that in one guinea pig in our series no "tissue" sensitivity could be found and the skin test was negative, but organisms from an infection of 5 months duration could be isolated from the spleen. This requires further confirmation.

Summary. Evidence is presented to show that guinea pigs experimentally infected with *Brucella abortus* and which do not show a positive skin test upon intradermal injection of bacterial extract usually possess a "tissue" sensitivity to this antigen.

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Propagation of Measles Virus in Cultures of Chick Embryo Cells.* (23637)

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Cultivation of measles virus in cultures of chick embryonic tissues and in developing hens' eggs has been reported by a number of workers. Others in similar attempts failed to obtain convincing evidence that the agent could be thus propagated(1,2,3,4,5). These discrepancies depended at least in part upon the fact that in nearly all these studies the only criterion of viral multiplication formerly available in the laboratory consisted in the development of signs of measles in monkeys following inoculation of materials suspected to contain the virus. Since a varying proportion of "normal" monkeys does not develop overt disease even when inoculated with materials known to contain the agent(1,6), it is understandable that the findings of different investigators were not always in agreement.§ In 1954, a more dependable index of viral multiplication was recognized by Enders and Peebles in the characteristic changes which the virus produces in primate epithelial cells (7). Accordingly, a reinvestigation of its capacity to multiply in chick embryonic tissues was undertaken.

At first attempts were made to cultivate in embryonated eggs several strains of measles virus that had been subjected to only a few passages in human kidney cells. No evidence was obtained that any of them multiplied in this host. One of these strains (Edmonston) was added to cultures of chick embryo tissues prepared according to the procedure that Plotz described(8), and with which Rake and his co-workers also reported successful results (3). But again no indication of increase or even persistence of the virus was noted(7). Ultimately adaptation to chick embryos of this strain was attained by Milovanovic, Enders and Mitus(9) after many passages in human renal and amnion cells. In preliminary experiments this chick embryo-adapted virus was found to multiply readily in cultures of chick cells(9), where its behavior will now be described in detail.

Materials and methods. Virus. The Edmonston strain was isolated from the blood of a child in the early acute phase of measles (7). When this investigation was initiated it had been passed serially 24 times in human kidney and 28 times in human amnion cell cultures, and 6 times in embryonated eggs.

Storage of virus. Virus suspensions in the form of infected tissue culture fluids were

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§ At least one of the factors responsible for variation in response of monkeys has lately been defined. Thus Peebles and his co-workers(6) have demonstrated in a high proportion of "normal" rhesus and cynomolgus monkeys maintained under laboratory conditions neutralizing and complement fixing antibodies that react specifically with measles virus. These antibodies presumably arise during the course of unobserved spontaneous infections with this virus or a closely related agent.