

11842 P

Variation in Immune Response to *Brucella abortus* Depending on Route of Administration.

PAUL O. HAGEMAN AND J. A. DOUBLY. (Introduced by David P. Barr.)

From the Department of Medicine, Washington University School of Medicine, St. Louis.

A perplexing diagnostic problem is presented by the combination of vague complaints, low titer *Brucella* agglutination, and positive Brucellergen skin test. Whether such findings indicate past or present infection or whether they lack significance is a fundamental problem.

It was thought that the ingestion of milk containing *Brucella* organisms killed by pasteurization might be responsible for this confusion by stimulating the formation of agglutinins. This hypothesis seemed especially plausible, since heat-killed *B. typhosus* given orally and intravenously yields similar immunological responses, and particularly because of the similarity in the accepted pathogenicities of typhoid and undulant fevers. The investigation of the immune reactions to *Brucella abortus* following injections by these routes was undertaken.

Technic. A stock culture of *Brucella abortus* (National Institute of Health strain No. 456) was grown in 1% dextrose beef infusion broth aerobically at 37°C for 48 hours. The flasks were then heated in a waterbath for 30 minutes at 60°C. Samples of the heated culture showed no growth after 4 days' incubation at 37°C.

Part A. Three groups of 4 rabbits each were used:

Group I received the heat-killed organisms suspended in milk by stomach tube. After centrifuging the heated culture for 20 minutes at 3,000 r.p.m., discarding the supernatant fluid, the organisms were resuspended in the same volume of fresh sweet pasteurized whole milk.

Group II received the heated broth culture intravenously.

Group III served as controls, received no injections, were kept on a regular diet and bled at the same intervals as Groups I and II.

During the first week 0.5 cc of culture or its milk suspension equivalent was given for 3 successive days, in the second week 1 cc was given on 3 successive days, and so on, 1.5 cc in the third week, and 2 cc in the fourth week. Blood was drawn for agglutination tests with the homologous strain before the first dose of organisms was

TABLE I.
Agglutination Titers.

Rabbit Group	Days						
	1	Injections begun	7	14	21	31	
Part A	I	0*	oral	0	0	0	0
	II	0	intravenous	1:1040†	1:5120	1:5120	1:4480
	III	0	—	0	0	0	0
Part B	I	0	oral‡	0§	0	0	0
	II	0	intravenous	1:640	1:1280	1:1280	1:1280

*0 = No agglutination in dilution of 1:20.

†Figures quoted are averages for the group.

‡Oral dosage of organisms in Part B is 20 × that in Part A.

§One rabbit in this group died of a milk pneumonia because the injection was given intrabronchially. Titer of 1:20 was present just before death.

given, and at weekly intervals thereafter, and again 10 days after the final dose had been given.

Part B. A second batch of heat-killed broth culture of *Brucella* was prepared as described above. Two groups of 4 rabbits each were used, one receiving by stomach tube the same volumes of a milk suspension of the heat-killed *Brucella* culture concentrated 20 times (*i.e.*, 0.5 cc milk contained the organisms from 10 cc of the original culture), and the other receiving the heated culture intravenously in the same doses employed in Part A. Bleedings were made at the same intervals as in Part A.

Results. See Table I.

Part A. Rabbits receiving heat-killed *Brucella* organisms by vein developed agglutinins for that organism, while the same number of organisms given orally failed to stimulate the formation of agglutinin response.

Part B. Increasing by 20 times the number of organisms given orally failed to stimulate formation of agglutinins.

Discussion. Data presented here clearly indicate that, under the conditions of this experiment, heat-killed *Brucella* organisms introduced into the intestinal tracts of rabbits do not lead to the formation of agglutinins. The antigen is either not absorbed or is altered sufficiently by digestive processes so that it loses its antigenicity. In view of these results, ingestion of heat-killed *Brucella* organisms would not seem important as a cause for low agglutination titers for this organism. Likewise attempts to immunize against this infection by oral administration of non-viable *Brucella* organisms would seem futile.

Summary. Rabbits develop agglutinins for *Brucella abortus* following intravenous injections but fail to do so after sizeable doses are introduced into the stomach.