necessarily represent the maximal secretory capacity of the stomach.

Summary. Analyses of gastric contents collected during a 3-hour infusion of histamine (maximal stimulation) in unanesthetized, gastric fistula dogs revealed statistically significant negative correlations between $[Na^+]$ and $[H^+]$, and between $[K^+]$ and $[H^+]$. K^+ output, H^+ output, and Cl^- output were positively correlated with output of water during this infusion. $[K^+]$ and volume-rate of secretion were inversely correlated during the first hour of infusion and positively correlated during the second and third hours.

1. Hollander, F., *Fed. Proc.*, 1952, v11, 706.
2. Colcher, H., Hollander, F., *Am. J. Physiol.*, 1959, v197, 1070.
3. Werther, J., Parker, J., Hollander, F., *Gastroenterology*, 1960, v38, 368.
4. Hollander, F., Colcher, H., *Am. J. Physiol.*, 1960, v198, 729.
5. Gray, J., Bucher, G., *ibid.*, 1941, v133, 542.
6. Riddell, M., Strong, J., Cameron, D., *Quart. J. Exp. Physiol.*, 1960, v45, 1.
7. Blair, E., Harper, A., Harris, D., Reed, J., Wilkinson, R., *J. Physiol.*, 1960, v154, 68P.
8. Blair, E., Yassin, A. K., *ibid.*, 1961, v159, 82P.
9. Hanson, M., Grossman, M., Ivy, A., *Am. J. Physiol.*, 1948, v153, 242.
10. Abrams, R., Brooks, F. P., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 278.
11. Hollander, F., *J. Biol. Chem.*, 1931, v91, 481.
12. Cotlove, E., Trantham, H., Bowman, R., *J. Lab. and Clin. Med.*, 1958, v51, 461.
13. Hollander, F., *J. Biol. Chem.*, 1932, v97, 585.
14. ———, *Gastroenterology*, 1961, v40, 477.
15. Marks, I. N., Komarov, S. A., Shay, H., *Am. J. Physiol.*, 1960, v199, 579.

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Failure of Vaccination with Killed Brucellae to Modify Monocyte-Bacterium Interactions.* (27364)

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Despite the claims of a few investigators (1,2), it is generally agreed that exposure of brucella-susceptible hosts to killed brucellae fails to provide any significant and lasting protection against subsequent exposure to live, virulent organisms(3-6). Efficient protection against challenge with virulent organisms apparently results only from the temporary or prolonged establishment of living organisms within the host, and for this reason the use of an attenuated strain of *Brucella abortus* such as strain 19, or of a streptomycin-dependent strain of *Br. melitensis*(7), both of which fail to lead to clinical manifestations, has been advocated. The causes for the failure of organisms, killed in a variety of ways, to elicit efficient protection have remained obscure and difficult to understand, since killed brucellae certainly do lead to production of humoral changes (*i.e.*, formation of agglutinating antibodies) that are usually indicative of an immune state.

Many investigators have stressed the importance of cellular mechanisms in relation to immunity against diseases produced by intracellular parasites such as brucellae, and there have been various studies on interactions *in vitro* between mononuclear phagocytes (monocytes) from different hosts and brucellae of different virulence(8-11). Thus it was shown that monocytes from normal,

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TABLE I. Viable Counts and Intracellular Distribution of *B. abortus* in Cultures Established with Monocytes from Normal, Vaccinated, or Infected Guinea Pigs.

Monocyte donors	Time of examination (hr)	Viable count† per flask ($\times 10^6$)	% monocytes containing 0, 1–10, etc., brucellae*			
			0	1–10	11–20	>20
Normal	2	5.1	10	68	19	3
"	24	15.4	3	27	14	56
"	48	71.0	0	2	4	94
Vaccinated	2	4.1	21	52	20	7
"	24	4.7	6	41	20	33
"	48	16.2	6	20	26	48
Infected	2	3.0	26	68	6	0
"	24‡	3.4	74	24	2	0
"	48‡	1.2	58	24	3	15

* Avg of values obtained on examination of 50 monocytes on each of 2 coverslips.
† Avg of duplicate counts on each of 2 Porter flasks.
‡ No. of monocytes remaining attached to coverslips was less than at 2 hr (7% and 71%, respectively).

susceptible animals (*e.g.*, guinea pigs) allow extensive intracellular multiplication of virulent (S type), but not of avirulent (*e.g.*, R or M type) brucellae(9). In contrast, such intracellular multiplication fails to occur, or is minimal, in animals that are resistant either by virtue of previous infection with virulent brucellae(8) or by virtue of their innate resistance(11); infected guinea pigs provide an example of the former, uninfected rats of the latter.

We now have examined the fate of intracellular brucellae within monocytes from guinea pigs vaccinated with formalin-killed *Br. abortus*. It is the purpose of this communication to describe how the failure of such vaccination to protect against subsequent challenge is correlated with a failure to cause the type of modification of host cell-bacterium interactions that is typical of phagocytes from resistant hosts.

Materials and methods. Male guinea pigs of the Hartley strain, weighing initially about 350 g were used. Killed cells for vaccination were prepared from virulent *Br. abortus*, strain SA-S(9), by exposure to 0.04% formalin, appropriate tests for the absence of living organisms being made prior to use. Vaccination was performed either by a single intramuscular injection of approximately 1×10^9 killed cells into the thigh 2 or 4 weeks prior to monocyte harvest, or by 2 such injections, spaced 3 months apart, 4 and one month prior to monocyte harvest. The data were essentially identical in all instances, and this report will be restricted to a compilation of results obtained with a single vaccination. All guinea pigs were bled prior to vaccination, and again at time of sacrifice. Brucella agglutinins were titrated by tube agglutination.

Technics employed in the monocyte maintenance cultures have been described(8,9,11). Briefly, monocytes were harvested 48 hours after an intraperitoneal injection of 20 ml of 0.85% saline, and suspended, at a concentration of approximately 2×10^6 per ml, in a medium consisting of 30% autologous serum in Hanks' BSS. *Br. abortus*, SA-S, was added to a final concentration of about 5×10^7 per ml, and aliquots of the mixture introduced into Porter flasks containing flying coverslips. After 2 hours' incubation at 37°C, when the monocytes had ingested brucellae and had become attached to the floor of the flask, the supernatant medium was replaced by 30% serum-Hanks containing streptomycin at a final concentration of 10 μg per ml. This concentration of streptomycin is capable of killing all extracellular brucellae without killing any of the intracellular organisms(8). Cultures were examined periodically, usually 2, 24 and 48 hours after initiation, by removing flying coverslips for direct observation, as well as by performing viable counts on the bacterial contents of whole cultures.

Results and discussion. Table I, compiled from typical examples collected in studies with monocytes from 12 animals, shows a

comparison of events occurring in monocytes harvested from a) normal, unvaccinated animals, b) animals vaccinated with killed brucellae, and c) animals that had been infected with live, virulent brucellae. The extent of intracellular multiplication of smooth, virulent brucellae in these cells is reflected both by direct counts of the number of bacteria per individual stained monocyte and by assays on the number of viable cells recoverable from the same monocyte-brucellae cultures 2, 24 or 48 hours after initiation of the cultures. On the basis of these criteria, the typical multiplication of brucellae took place within monocytes from normal, susceptible animals and very little multiplication occurred within monocytes obtained from infected, immune animals (infected 6 months prior to monocyte harvest). The events within monocytes from animals exposed to killed brucellae resembled those occurring within cells from normal, susceptible hosts rather than those typical for infected, immune animals, *i.e.*, the brucellae multiplied intracellularly, but the extent of such multiplication was somewhat less than in "normal" monocytes. These trends have been confirmed by observations with additional animals.

The vaccinated animal chosen as an example for Table I showed a serum agglutinin titre of 1:1280; other animals which yielded essentially identical results showed agglutinin titres ranging from 1:640 to 1:2560. (The infected animal of Table I showed a titre of 1:576.) It was, therefore, considered possible that the lesser extent of intracellular multiplication observed in cultures containing cells and serum from vaccinated donors, compared to normal donors, may have resulted from agglutination of the bacteria prior to ingestion. To test this, one aliquot of monocytes from the vaccinated guinea pigs was maintained (for the 48-hour experimental period) in the presence of autologous serum that had been drawn prior to vaccination and stored at −20°C, while a second aliquot was maintained for the same period in fresh, *i.e.*, antibody-containing, serum. As a control stored (frozen) and fresh autologous sera also were used with monocytes from normal animals. Under these various conditions no significant differences in numbers of brucellae ingested by monocytes from normal or vaccinated donors could be observed, regardless of whether stored (frozen) or fresh sera were used. Also, the extent of intracellular multiplication was independent of the antibody titre of the extracellular medium, monocytes from vaccinated donors again supporting intracellular multiplication of brucellae. As in the prior tests, the extent of growth within cells from vaccinated guinea pigs, however, remained less than that in cells from normal animals both in autologous pre- and post-vaccination sera.

Thus, while the intracellular multiplication of *Br. abortus*, SA-S, within the monocytes of vaccinated guinea pigs was found to be less than in monocytes from normal, susceptible (unvaccinated) animals, it nevertheless did not approach the reduction of intracellular multiplication typical for immune, infected guinea pigs. It is, therefore, tempting to speculate that the generally encountered failure of killed brucellae to protect against natural brucellosis, or against experimental brucellosis that mimics natural infection, may be associated with a failure of killed organisms to modify cellular factors of the host. In a typical susceptible host, protection against challenge with virulent brucellae may require a modification of both humoral and cellular factors, and it would appear that while live virulent organisms are capable of producing such changes, killed organisms, although producing alterations in serum factors, are insufficient for producing the characteristic cellular alterations. (Similar conclusions were reached recently for *Salmonella enteritidis* in studies by a group of Japanese investigators (12) which came to our attention after the conclusion of our tests.) Some of the reported success in protecting animals against experimental brucellosis with the aid of killed vaccines or bacterial extracts(1) may be attributable to the use of relatively resistant hosts, such as mice. It has been shown previously(11) that in such hosts extensive intracellular multiplication of brucellae in monocytes does not occur, and it may be argued that this may reduce the need for si-

multaneous alteration of humoral and cellular factors. Alteration of humoral factors, achievable by killed cells or their components, may suffice to curb the relatively atypical experimental infections produced in such hosts. The situation appears to be more complex in a typical susceptible host like the guinea pig, at least as far as any lasting and significant protection is concerned. Jones *et al.*(13) did observe that a killed S or R adjuvant vaccine can produce slight protection in guinea pigs but the resulting increase in ID_{50} was minor compared with that obtained after use of live vaccines. It will now be of interest to determine whether in typical susceptible hosts, like the guinea pig, full protection can be achieved with the aid of killed brucellae when the animal is simultaneously exposed to other agents, *e.g.*, meprobamate, which recently have been shown capable of modifying cellular responses (14). Under these conditions the drug may lead to the required alteration of cellular properties while the killed brucellae modify humoral factors.

Summary. *In vitro* studies with guinea pig monocytes showed that vaccination of the donor with formalin-killed *Brucella abortus*, failed to result in development of certain cellular properties that are typical for phagocytes obtained from donors that are immune by virtue of innate resistance or prior infection. That is, while brucellae fail to undergo intracellular multiplication in monocytes from immune donors, they do multiply in monocytes from donors treated with killed vaccine, even though the extent of such multiplication was found to be less than in monocytes from normal, susceptible donors. An attempt has been made to relate this finding to the problem of effective immunization against a pathogen like brucella, which presumably requires an effective alteration of both humoral and cellular factors.

1. Live, I., Sperling, F. G., Stubbs, E. L., *J. Infect. Dis.*, 1945, v77, 16.

2. Elberg, S., Herzberg, M., Schneider, P., Silverman, S. J., Meyer, K. F., *J. Immunol.*, 1951, v67, 1.

3. Bang, B., *J. Comp. Path. Therap.*, 1906, v19, 191.

4. Gwatkin, R., *J. Infect. Dis.*, 1931, v48, 331.

5. Traum, J., in Brucellosis, A Symposium. . . ., A.A.A.S., Washington, D. C., 1950, 225.

6. Spink, W. W., *The Nature of Brucellosis*, 1956, Univ. of Minnesota Press, Minneapolis, p268.

7. Herzberg, M., Elberg, S. S., *J. Bacteriol.*, 1955, v69, 432.

8. Pomales-Lebrón, A., Stinebring, W. R., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 78.

9. Braun, W., Pomales-Lebrón, A., Stinebring, W. R., *ibid.*, 1958, v97, 393.

10. Holland, J. J., Pickett, M. J., *J. Exp. Med.*, 1958, v108, 343.

11. Stinebring, W. R., Kessel, R. W. I., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 412.

12. Mitsuhashi, S., Sato, I., Tanaka, T., *J. Bacteriol.*, 1961, v81, 863.

13. Jones, L. M., Thomson, P. D., Alton, G. G., *J. Comp. Path.*, 1958, v68, 416.

14. Kessel, R. W. I., Boughton, J., Braun, W., *Science*, 1961, v134, 1882.

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Effect of Nitrofurazone on Release and Activity of Endogenous Serum Pyrogen.* (27365)

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It is generally thought that bacterial endotoxins produce fever by injury to leukocytes with release of a pyrogen which acts upon the hypothalamic thermo-regulatory centers resulting in an elevation in temperature. This fever-producing material which has been termed *endogenous serum pyrogen* also has been found in animals made febrile with influenza viruses and in the fevers associated with experimental streptococcal, pneumo-

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