If a mild phase-2-like reaction has occurred, it would have been obscured by the non-specific reaction.

Specifically, the results supported the thesis that the different phases of an active immediate local hypersensitive reaction are the expression of differently reacting specific antibody molecules. More generally, attention is directed to the need for a more comprehensive criterion of the combination of antigen and antibody *in vivo* than the standard at present used, the passive cutaneous anaphylactic reaction. The diffusion of colloids from blood vessels in an area of inflammation may actually be inhibited if œdema formation is active and rapid[6]. Since phase 1 reactions are characterized by rapid œdema formation, failure to detect the responsible antibodies by the passive cutaneous anaphylactic reaction may be due to such an inhibition. Of course, it may, instead, be due to a lack of 'fixation' to the tissues of the responsible antibodies. The inhibition theory is presented as a possible alternative explanation which requires substantiation.

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Effect of Exposure of Very Young Calves to Virulent *Brucella abortus* on their Serological Response to Re-infection by the same Organism at 6 Months of Age

In the course of a *Brucella* eradication programme in an adult vaccinated herd certain anomalies were noted in the immune responses of 6-months-old calves to *Brucella abortus* strain 19 vaccination. Calves were routinely tested for *Brucella* sero-agglutinins immediately prior to vaccination. A proportion of these calves had positive titres at this stage, and after vaccination a few of them showed no, or very little, rise in agglutinin titre. On checking the clinical status of the dams of such calves it was found that they were *Brucella*-infected animals. It seemed likely that these calves had been exposed to *Brucella abortus* at a very early age and often for a prolonged period. Therefore it was decided to set up a preliminary experiment to investigate the immune response of calves which had been experimentally exposed to virulent *Brucella abortus* during the early days of life.

Three 1-day-old heifer calves were exposed by the oral route to virulent *Brucella abortus* 544 (3-days-old culture grown on serum-dextrose agar).

The first calf (*IT*/3) received 1×10^8, the second (*IT*/1) 2×10^8 and the third (*IT*/4) 1×10^{11} organisms (total count) daily for the first 15 days of life. At six months of age they were injected, together with 3 control calves (3*C*) of the same age and sex but no previous *Brucella* experience, with 6×10^{10} *Brucella abortus* 544 (total count). They were bled at regular intervals after challenge and the sera tested within two days for *Brucella* sero-agglutinins and complement fixing antibodies. The agglutination test was carried out in the conventional manner for the serological diagnosis of *Brucella*[1,2]. Serum dilutions started at 1:2, 1:5, 1:10, etc., and the end titre was taken as the highest dilution at which there was 50 per cent agglutination. The complement fixation test was performed as described previously[3] starting at 1:5 dilution of the serum.

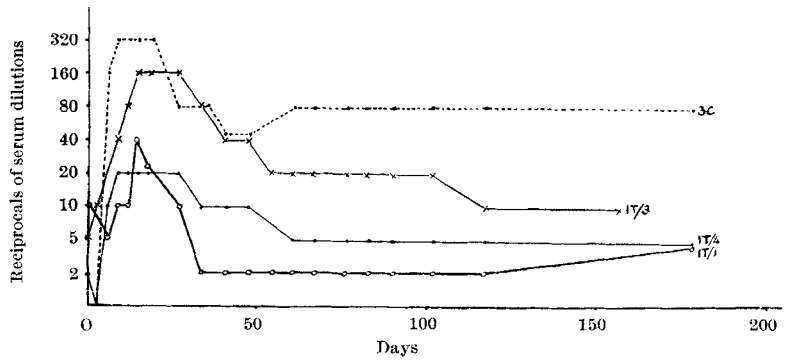

Fig. 1. Results of agglutination tests for *Brucella* sero-agglutinins. Calf *IT*/3 received 1×10^8; *IT*/1, 2×10^8; and *IT*/4, 1×10^{11} *Brucella abortus* 544 daily by the oral route for the first 15 days of life. 3*C* represents 3 control calves which had no *Brucella* experience until 180 days old. All six calves were challenged with 6×10^{10} *B. abortus* 544 at 6 months of age marked O on the graph

The results of the agglutination tests are presented in Fig. 1.

Fig. 1 shows that the 3 control calves (3*C*) gave a vigorous immunological response following injection by *Brucella* and were still showing elevated agglutinin titres 6 months after exposure. The 3 calves which had previous experience of *Brucella* infection showed a similar but less-pronounced serological reaction to exposure to *Brucella*. The rate of decline of agglutinin titres was much greater in the cases of neonatally infected animals than in the controls.

The complement fixing titre of the 3 control calves became positive at 1:5 serum dilution 5 days after infection, reached its peak titre of 1:40 by the end of the third week and is still 1:10 six months after infection.

The complement fixing titre of calf *IT*/3 is comparable with that of the control calves, but the titre of the other two (*IT*/1 and *IT*/4) never exceeded 1:10 and remained intermittently positive at 1:5 serum dilution for over a period of 6 months even when agglutinin titres were $< 1:10$.

Diminished or changed immunological responsiveness to *Brucella* after either chronic infection or heavy experimental exposure to these organisms has been reported during the past years[4,7]. The present and previous findings[3] support these observations. It is suggested that heavy exposure of the neonata to *Brucella* may alter their ability to give normal serological response on re-exposure to these bacteria. The possibility of such calves remaining infected until they are adults should be re-examined and the effect of re-infection in later life, both on immune response and in provoking abortion, should be studied.

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