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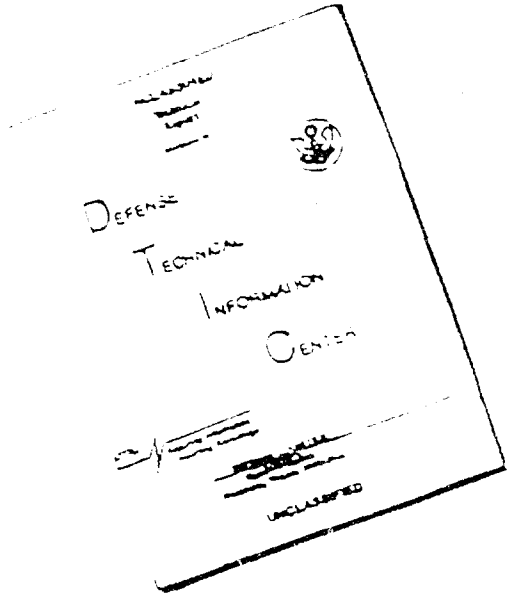

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INTRACUTANEOUS REACTION IN THE DIAGNOSIS OF Q FEVER IN GUINEA PIGS

by

Babudieri, B. & Ravaioli, L.

In the course of research on neutralizing monoserous Rickettsia by means of immune serum, GIROUD discovered in 1939 that immune rabbits given second intracutaneous injections of live Rickettsia, respond with a far more intense local reaction than do virgin control rabbits. (Footnote 1) From this observation, Giroud deduced that there is a dissociation in Rickettsia infection between general and local immunity, and that this is why rabbits who have recovered from such an infection develop cutaneous hypersensitivity. These observations were later confirmed by the findings of Blanc and Noury. (Footnote 2)

Subsequently, Giroud got the idea of applying his findings to the development of a test that would reveal the immune status of an individual. (Footnote 3) For this purpose, he administered an intracutaneous injection to the forearm of the subject under examination of 0.1 cc of a suspension of killed Rickettsia. In each individual given this treatment, a small papula appeared shortly after injection, disappearing within 30 minutes to an hour. Five hours later, there is an infiltration established locally, very minor in the non-immune subjects, never larger in diameter than 4 or 5 millimeters, while in the immune subjects it becomes increasingly evident, reaching a diameter of 40 to 50 millimeters within 28 to 52 hours. This reaction is quite specific, and gives positive results even at two to as much as twenty-five years after the initial infection.

In the study of Q-fever, Mirri recently suggested the use of an intra-palpebral reaction in cattle and sheep. This author reports good results with this procedure, generally agreeing with those obtained via the complement deviation method. (Footnote 4)

Mirri's technique is the following: He uses the Lederle antigen generally used for the complement deviation reaction. It is a heavy suspension of C. Burneti (the Nine Mile strain) prepared from embryo cultures, then concentrated and purified by successive centrifuging and ether extraction. Mirri gives a subcutaneous injection in the lower

eyelid of .2 cc to sheep and 3/8 cc to cattle. The positive reaction is a clearly visible swelling of the eyelid, appearing 3 to 4 days after injection, and persisting for some time.

PATRIGNANI (Footnote 5) in Ancona and LOPES (Footnote 6) at Lipari attempted to use the intracutaneous reaction in diagnosing Q-fever in humans, comparing their results with those obtained from the complement deviation reaction. It does not seem, however, that the results so far obtained are very satisfactory since quite often they got positive intracutaneous reactions from sero-negative subjects.

We determined to inquire more closely into the possibilities of using the intracutaneous reaction, and set ourselves two goals: first, to discover whether or not there is a concordance between this reaction and the complement deviation reaction; and second, to discover how long after infection the reaction becomes positive, and particularly whether or not this positive reaction occurs earlier than a positive complement deviation reaction, and hence whether or not it might be used for early diagnosis.

In our research, we used both the Lederle antigen (Henzerling strain) in a 1:2 dilution, and an antigen prepared by one of us from chick embryo cultures of the Nine Mile strain. In its preparation, we ground the infected vitelline sac, suspended it in fresh physiological solution, then subjected it to repeated centrifuging so as to obtain, insofar as possible, a solution of Rickettsia clear of globules of yolk and other non-specific components. We did not use ether extraction. Our antigen, kept under refrigeration for more than a year, is considerably poorer in Rickettsia than the Lederle preparation, and furthermore contains all the ether-soluble components. We used it undiluted.

We injected it intracutaneously on the shaven abdomen, in doses of 0.1 cc.

In every instance, we compared and checked our results against those of the complement deviation reaction, performed according to the usual technique, and using the Henzerling strain of Lederle antigen.

In our research, it was not our intention to inquire directly into the specificity of the intracutaneous reaction, but we discovered it indirectly because we were trying to check whether its results agreed with those of the complement deviation reaction, leaving aside, at least for the time being, the question of whether or not the latter reactions were always strictly specific, and the allied question as to the meaning to be attributed to positive sero-reactions at a very low level.

In an initial test, we used the two antigens to produce the intracutaneous reaction on 53 healthy seronegative guinea pigs. They were kept under observation for 14 days. During this period, the animals showed no apparent local reaction, except for a few cases where there was a minor area of infiltration which disappeared completely in 3 to 4 days.

Four other guinea pigs, also inoculated with suspected material, produced a complement deviation reaction at a very low level, too low to be accepted as a certain indication that infection had taken place. The results we got from the cutireaction are shown on the following table:

Guinea pigs	Number of days after inoculation	Complement Deviation	Cutireaction with each antigen	
			Lederle	Babudieri
1	37	1 : 4 + 1 : 8 ±	+ +	-
2	37	1 : 8 + 1 : 16 ±	-	-
3	37	1 : 4 ±	+	-
4	21	1 : 4 + 1 : 8 ±	+ +	±

As the table shows, in these cases the Babudieri antigen yielded results that may be considered consistently negative, because the single guinea pig that showed any reaction produced only a very mild one. With the Lederle antigen, however, we got positive reactions in 3 cases, two of them very clear.

In order to evaluate these results, it would be helpful if we knew what significance should be attached to these very slightly positive sero-reactions. This is not a very simple matter. We would point out, however, that two of the first three guinea pigs were given a few drops of a weak suspension of weakened Rickettsia in the conjunctival sac, and the third the same amount orally. Infection probably did not occur, but there is always a possibility that the small quantity of antigen inserted into the system was enough to produce a small number of antibodies, and that therefore the sero-reaction, weak though it was, might actually have a specific significance of theoretical, if not practical, interest. The 4th guinea pig was inoculated subcutaneously with material that was almost certainly sterile. Its mate, inoculated at the same time with the same material, gave a completely negative

sero-reaction. It is not possible, either, that the mildness of the sero-reaction was due to too short an interval between inoculation and blood-sampling, because a second complement deviation test made 20 days later gave us precisely the same results as the first.

Lastly, we performed the intracutaneous reaction test on a group of 13 guinea pigs that had been infected 26 to 173 days earlier, all of which showed a markedly positive sero-reaction (> 1:16). Our results are shown on the following table:

Guniea Pigs	Days since infection	Strain of C. Burneti used	Cutireaction with each antigen:	
			Lederle	Babudieri
1	173	G. A.	±	+ +
2	120	"	+ + +	+ +
3	120	"	+ + +	+ +
4	45	Cap.	+	±
5	45	"	+	+
6	37	N. M.	±	+ +
7	37	"	+ +	+
8	30	G. A.	+	+ +
9	30	"	±	+ +
10	30	"	-	-
11	26	"	+ +	+ +
12	26	"	+ +	+ +
13	26	"	+ +	+ +

G. A. - Grottazzolina. - Cap. - Capodistria. - N. M. - Nine Mile

As the table shows, there is generally a pretty fair correspondence between the results of the sero-reaction and those of the cutireaction. In one single case, the latter produced totally negative results with both antigens. In the others, the Lederle antigen gave us nine strongly

positive and three mildly positive reactions, while the Babudieri antigen gave eleven strong and one mild. It should be noted that guniea pig number 10, which produced negative reactions with both antigens, was, like number 9, which also reacted mildly to the Lederle antigen, the only one infected in the conjunctival sac; all the other animals were infected either orally or intracutaneously. It is possible that the mode of introduction of the pathogenic germs exerts some influence on the results of the cutireaction.

If you compare results obtained with the two antigens, you see that although in many cases the reactions were practically the same, there were others in which there was no such correspondence, with the Lederle antigen giving the strongest reaction in some cases, and the Babudieri in others. If we compare these non-corresponding cases with the sero-reaction results, including the mild reactions of the latter type with the rest, we note that the Lederle antigen usually, though not always, seems to possess greater sensitivity, while the Babudieri antigen is considerably more specific.

If we consider the number of days after inoculation in which the cutireaction reaches its greatest intensity, we note that the Lederle antigen produces the strongest reaction between the 6th and 10th days, while the Babudieri reaction is strongest between the 5th and the 15th. The same holds true of the onset of the reaction, which is usually five days after administration of the Lederle antigen, and seven days after administration of the Babudieri.

We can say, in conclusion, that the cutireaction agrees very well with the sero-reaction when the latter is negative. When the sero-reaction is positive, however, there is frequently a discrepancy; in such cases, therefore, it can be used as a sound standard for orientation, but cannot be taken as an absolute test. It has importance only if it is very strongly positive. Further research will be required to determine which of the two reactions is more specific in cases where they disagree. Among the two antigens we used, we preferred the Babudieri antigen, even though it is slower-working.

The second aim of our study was to see whether the intracutaneous reaction had the qualities that would make it suitable for use in early diagnosis of infection.

For this purpose, we infected a group of guinea pigs subcutaneously with the Grottazzolina strain. At measured intervals, these animals were subjected to both the complement deviation test and the intracutaneous reaction test with both antigens. The following table shows the results obtained.

Guniea pigs	Number of days since infection	Complement Deviation	Cutireaction with each antigen	
			Lederle	Babudieri
1	3	1 : 4 -	-	-
2	3	1 : 4 -	-	-
3	3	1 : 4 ± 1 : 8 -	-	-
4	5	1 : 4 -	-	-
5	5	1 : 4 ± 1 : 8 -	-	-
6	7	1 : 64 + 1 : 128 -	?	? (+)
7	7	1 : 4 -	-	-
8	7	1 : 256 + 1 : 512 -	?	? (+)
9	7	1 : 512 + 1 : 1024 -	±	-
10	9	1 : 32 + 1 : 64 ±	?	? (+)
11	9	1 : 32 + 1 : 64 ±	-	±
12	10	1 : 128 + 1 : 256 -	-	-
13	10	1 : 64 + 1 : 128 -	-	-
14	11	1 : 256 + 1 : 512 -	±	-
15	11	1 : 2048 + 1 : 4096 ±	?	? (+)
16	16	1 : 1024 + 1 : 2048 ±	-	-
17	16	1 : 512 + 1 : 1024 ±	±	-
18	26	1 : 512 + 1 : 1024 ±	+ + +	+ +
19	26	1 : 512 + 1 : 1024 ±	+ + +	+ +
20	26	1 : 512 + 1 : 1024 ±	+ + +	+ +
21	30	> 1 : 16	+ +	+

(+) The guinea pig died within 5 days after the cutireaction.

The table shows clearly that the intracutaneous reaction becomes positive later than does the sero-reaction. If, as in our case, the infection dose is fairly high, the complement deviation tests in most cases give reliable results as early as the 7th day, whereas the intracutaneous reaction test does not reveal a positive reaction, if any, until the 16th to the 26th day. Consequently, we feel that, quite apart from its degree of concordance with the complement deviation reaction, the intracutaneous reaction is not suitable for early diagnosis.

To sum up, our research shows that, at least in guinea pigs, the intracutaneous reaction tests is worth using in diagnosis of infection with C. Burneti, so long as no absolute value is attributed to the findings, particularly if they are negative. It is of no use, however, in early diagnosis. Our conclusions, of course, refer only to guinea pigs. It is common knowledge that the sensitivity of the various animal species to intracutaneous introduction of foreign substances can vary widely, and it is highly probable that the human species may show frequent and marked receptivity to the non-specific factors undoubtedly present in the antigens we used. This would explain the dubious results of research of this type on human subjects. For similar reasons, our research gives us no grounds for an opinion, either positive or negative, as to the reaction suggested and tested by Mirri in cattle and sheep.

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ABSTRACT

The authors, B. Babudieri and L. Ravaioli, engaged in the research project covered in this paper with the aim of discovering whether or not there is a direct relationship and concordance between the results produced by the complement deviation reaction and the intracutaneous reaction in diagnosis of Q-fever in guinea pigs. They were concerned with the length of time after infection at which the positive reaction appears, in order to determine whether or not it might provide a tool for early diagnosis of Q-fever.

Throughout their experiments, the authors used both the Lederle antigen (Henzerling strain) and one of their own, prepared from chick embryo cultures of the 9-mile strain. In every case the authors compared and checked their results against those of the complement deviation reaction.

They noted that the Lederle antigen usually, though not always, possesses greater sensitivity, while the Babudieri antigen (of their own preparation) is considerably more specific.

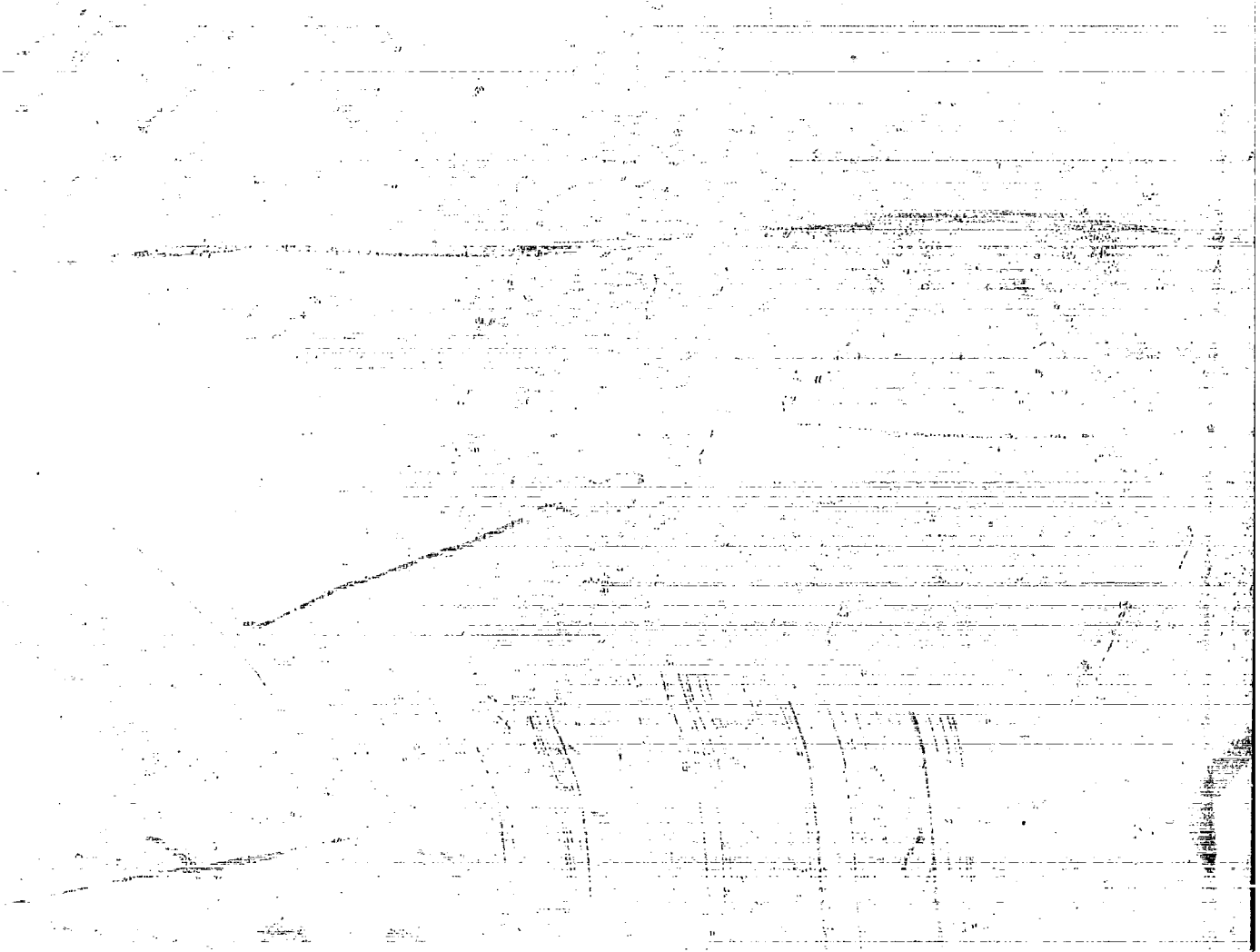
However, the Lederle antigen reaches its peak of reaction strength from 6 to 10 days after inoculation, while the Babudieri reaction is strongest from 5 to 15 days after inoculation. The onset of reaction, like its peak, is earlier with the Lederle antigen than with the Babudieri (5 days as against 7).

In conclusion, the authors noted that the intracutaneous reaction coincides quite neatly with the serum reaction when the latter is negative. Deviation is quite common, however, when the serum reaction is positive. In such cases the cutireaction is of value as an aid in orientation, but cannot be taken as an absolute test. Further research alone will show which of the two reactions is more specific in cases where they conflict.

The authors' data shows that, aside from its degree of concordance with the complement deviation reaction, the intracutaneous reaction is not a suitable tool for early diagnosis.

In guinea pigs, the only species covered in these experiments, the intracutaneous reaction test is helpful in diagnosis of C. Burneti infection, but not reliable as a proof of non-infection. It is of no help whatever in early diagnosis. However, since sensitivity to intracutaneous introduction of foreign substances varies widely from one animal species to another, it is quite likely that humans may show a

high incidence of receptivity to the non-specific factors present in the antigens used. This would explain the dubious results hitherto obtained in research of this kind of human subjects. The authors have not sufficient information to form any opinion as to the reaction suggested and tested by Mirri in cattle and sheep.



SUMMARY

The authors compare, in guinea pigs, the results of diagnosis based upon complement-deviation and cutireaction, obtained by means of (ether-extracted) Lederle antigen and (not ether extracted) Babudieri antigen.

The experiments showed, that the cutireaction does not yield any local reaction, or only a very slight one, in healthy animals.

In clearly seropositive animals, the reaction appears mostly, although not constantly, positive. In weakly seropositive animals the cutireaction is sometimes positive, sometimes negative.

While the seroreaction generally turns positive already after 7 days, the cutireaction takes 16 days after infection to show positive results; it is therefore not suited for early diagnosis.

As to the two antigens, the Lederle-one seems to be often, but not always more sensitive, while the Babudieri-one appears more specific.

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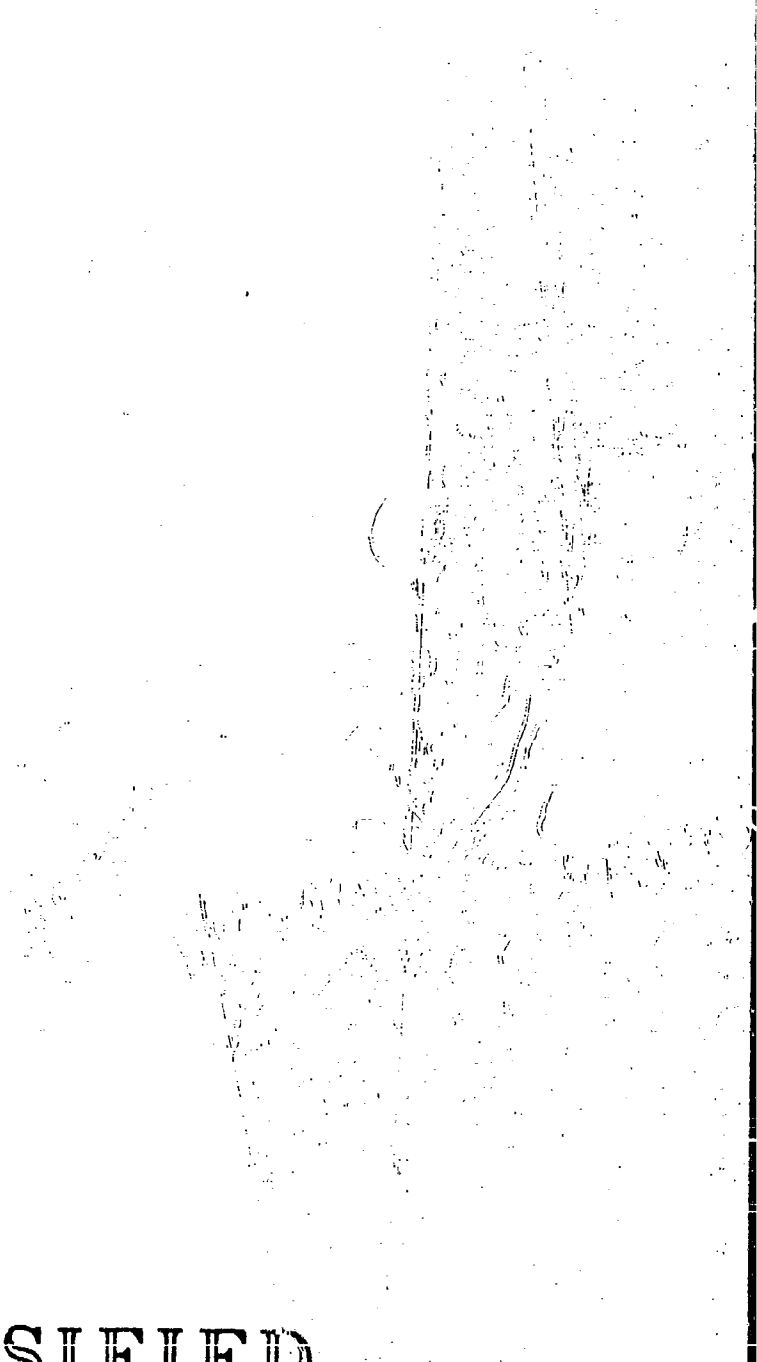

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