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10 J. M. Seigneuri, M. Capponi, Francoise de Bock and Chantal Gross

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SEARCH FOR *RICKETTSIA CONORI* AND *COXIELLA BURNETI* ANTIBODIES IN THE IgM and IgG FRACTIONS OF RABBIT SERA

J. M. Seigneuri, M. Capponi, Francoise de Bock, and Chantal Gross

Ann. Inst. Pasteur. 123:95-108, 1972

Translated by Phebe W. Summers

INTRODUCTION

Serum antibodies produced following bacterial or viral invasion are IgM (19 S) or IgG (7 S). In the majority of cases it is accepted that IgM antibodies are early and detectable in the course of the first weeks after antigenic stimulation. In the second week, they begin to decrease, while IgG become detectable in their turn. IgG persists for a fairly long time in serum.

Following this there appear agglutinating, complement fixing (CF), hemagglutination-inhibiting (HI) antibodies, or others . . . it has been established that the ones which appear rapidly after microbial attach are IgM, the others, found late, belong to the IgG class. Variations among antigens have been noted, and antirickettsial antibodies have not been studied completely in an animal.

In this study, we studied in parallel antibodies elaborated against 2 rickettsiae inoculated into rabbits: the classic one, *R. conori*, and the other classed a little apart, *R. burneti* (*Coxiella burneti*) were studied. On the one hand, we wanted to compare, in whole serum, the evolution of micro-agglutinating (MA), CF, and fluorescent* antibodies; on the other, we

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* The term fluorescent antibody is used here as simplification for the more complete and exact term "antibodies revealed by indirect immuno-fluorescence."

wanted to see to which immunoglobulin classes these different antibodies correspond, if there is evolution in time.

MATERIALS AND METHODS

1. Inoculation of antigens into rabbits and determinations.

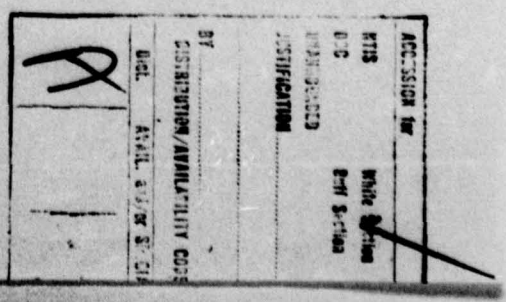
R. conori. After verifying the absence of all serum antibodies in 2 rabbits, they are inoculated by the abdominal dermal (ID) route with an living antigenic preparation obtained by treatment of 3 yolk sacs rich in rickettsiae (Strain Y9) and provided by the Pasteur Institute of Paris. These membranes are homogenized in 18 ml of Bovarnick fluid or SPG, then centrifuged and stored at -20 C. Rabbits are inoculated for the first time with 3 ml of antigenic suspension; this is repeated at 1 and 2 week intervals with 2 ml each time.

R. burneti (Phase II). Two rabbits are inoculated by the ID route with 3.5 ml of antigen inactivated by 80 C heat and provided also by the Institute. The same dose is injected 1 week later. Only 2 injections are carried out, the antigenic properties of R. burneti being better than those of R. conori. (Two rabbits inoculated previously with a living antigenic preparation died on day 20; it was thus necessary to use heated antigen of this strain).

In both cases one draws blood one week after the first inoculation, then weekly for a month, and then monthly for 7-10 months.

2. Fractionation of the Sera.

We separated serum proteins by chromatography on a 3.2-cm column, filled with dextran gel (Sephadex G-200), in a height of about 85 cm.



We used phosphate buffer (pH 7.2) previously filtered and degassed. The delivery in the column is regulated by a pump at about 30 ml/hr. UV absorption of serum fractions are measured by means of a Uvicord II (wave length 280 mμ) connected to a galvanometer registering on a continuous roll of paper (20 mm/hr); one obtains directly the absorption curve of the serum, percentage transmission as a function of dilution.

The quantity of serum introduced in the column is 1 ml, and we recover by use of the collector "Ultro-Rac LKB" 40 fractions of 6 ml each. These fractions are then concentrated 3 times by means of "Centriflo" cones, then mixed 2 by 2 in order to reduce the amount of antigen used for the reactions. We have investigated the antibodies in these fractions, 20 investigations for each serum.

3. Antibody Study.

For each fraction, we studied 3 types of antibodies:

- Agglutinating antibodies, by the microagglutination (MA) method on thin Giroud films with R. conori and R. burneti antigens, furnished by the Pasteur Institute (concentrations 2, 10, 20, 40, 80, 160, etc.).

- CF antibodies, examined by Kolmer technqiue (micro-method) with "Q Fever diagnostic antigen" and "RMSF antigen" supplied by Lederle Laboratories (USA) (concentrations 4, 8, 16, 32, 64, etc.).

- Fluorescent antibodies (FA), studied by indirect immunofluorescence in the Rickettsioses Laboratory of the Pasteur Institute (10, 20, 40, 80, 160, etc.); the antigens, fixed in acetone, are labeled yolk sac rich in

rickettsiae; the fluorescent sera - or conjugates - are rabbit anti-globulin sera from the Pasteur Institute of Garches, diluted 1:100, readings were made with the "Leitz Labor-Lux" microscope (black background, immersion objective 12.3 mm, "BG" filter, and anti-heat filter).

RESULTS

A. ANTI-R. CONORI ANTIBODIES.

1. In whole serum (Fig 1).

Antibody titer is maximal one month after the 1st inoculation (or one week after the 3rd). The titer is almost the same for MA and FA (about 10,000); it is 128 for CF. The decline in titer begins in 2 months and continues to the 3rd month; during the 4th and 5th months, MA and CF antibodies are almost stationary; those of FA rise slightly. This increase is perhaps due to an intercurrent infection. We see this often

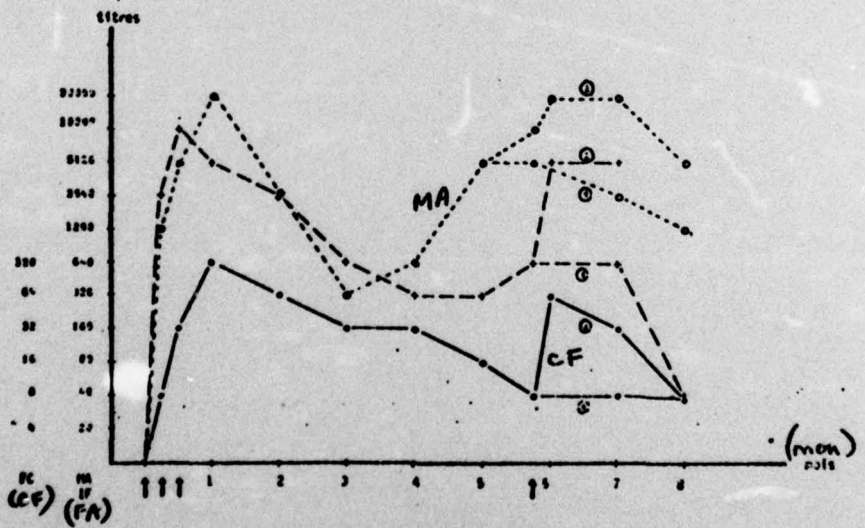


FIG. 1. — R. conori *(sérum total) : évolution des anticorps MA, IF et FC en fonction du temps.*

for MA and FA antibodies in patients ill with nonspecific infections. By 6 months, the MA titer increases also, while CF antibodies continue to decrease slowly. The booster given one rabbit at the end of the 6th month produced in a week a 2nd maximum of production of the 3 antibody types. A slight decrease began about 2 months after this reinoculation. The responses of the 3 types are thus approximately the same.

2. Study of the fractions (Fig 2 and 3).

Fifteen days after the 1st inoculation, or at the time of the 3rd inoculation, MA and FA antibodies are revealed in IgM at the level of the 1st absorption peak.

CF antibodies are found at the beginning and simultaneously in the 2 IgM and IgG peaks and in the region corresponding to the top of these absorption peaks.

Note - It has been shown (J.P. Bringuier, Service of Prof. Sohier) that it is a matter of IgM in the Ouchterlony immunoprecipitation reaction in agar (double diffusion in 2 dimensions); the immune sera used were the following: whole rabbit antiserum, and for mono-specific antisera anti-IgA (α), anti-IgG (γ) and anti-IgM (μ): the antigens were pure IgA, IgG and IgM solutions. The sera were previously concentrated by ultrafiltration.

Diffusion is carried out on the horizontal, at a constant temperature (37 C), in a humid atmosphere, for 48 hr. Reading is made after staining the precipitation arcs with amide black (Fig 7).

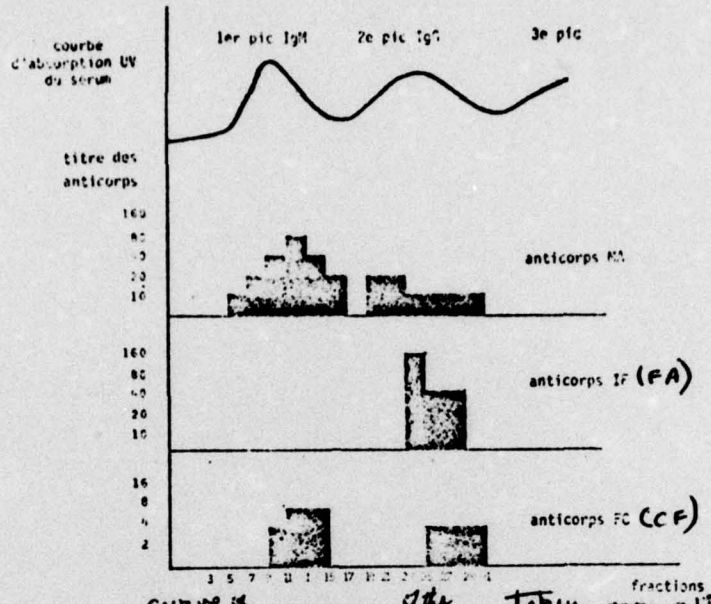


FIG. 2. — R. conori : ~~courbe d'absorption en UV du sérum prélevé 6 mois après la première~~ inoculation, et répartition des anticorps MA, IF et FC dans les IgM et les IgG.

curve of the taken mon after the 1st
distribution of the antibodies in IgM and IgG,

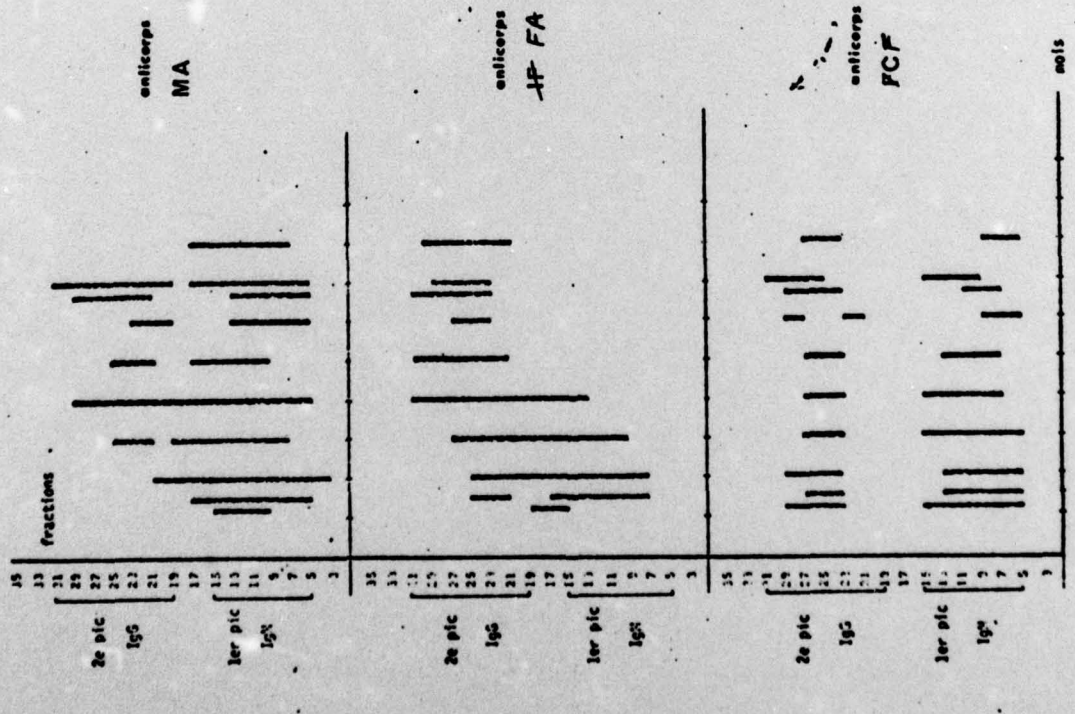


FIG. 3. — R. conori (sérum fractionné) : évolution des anticorps MA, IF et FC en fonction du temps dans les IgM et les IgG.

Evaluation of antibodies is as follows:

a. MA antibodies are found in IgM during the 7 months of the experiment, but at titers less elevated after the 5th month; on the other hand, they are in IgG from the end of the 1st month until the 7th month.

b. FA antibodies are localized as early as the end of the 1st month, entirely in the IgM and IgG; as early as the end of the 3rd month, they are found only in IgG; their amounts are approximately the same as those of MA antibodies (1:160 to 1:320).

c. CF antibodies are separated from the 1st to the 7th month in IgM and IgG, in the portions corresponding to the peak; one finds no antibody in that part of the curve between the 1st and 2nd peaks; their total amount is 1:8, because the fractions are diluted.

Reinoculation of the one rabbit at 6 months did not change the CF antibodies (which remained localized in IgM and IgG), nor the FA antibodies (which remained IgG). They increased the production of MA which was tending to lessen, localizing then mostly in IgM.

B. ANTI-R. BURNETI ANTIBODIES.

1. In whole serum (Fig 4).

Antibody titer is maximal 10 days after the 2nd inoculation (about 20,000, MA: 1280 FA; 32-64 CF). This maximum persists for 3 weeks, then it diminishes for 3 months. At 5 months, the antibodies reach a stable minimal titer (40 MA, 80 FA between 4 and 8 CF). Reinoculation in one rabbit at 6 months induces a week later a 2nd maximum for the 3 antibodies. Almost 20,000 FA, 1280 MA and 16-32 CF. The FA titer drops

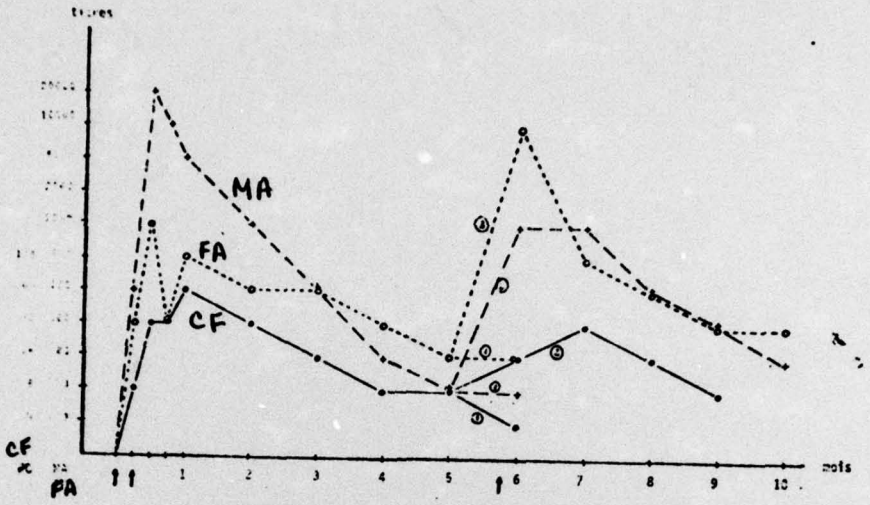


FIG. 4. — R. burneti *(sérum total) : évolution des anticorps MA, IF et FC en fonction du temps.*

rapidly during the following months, while MA and CF remain stationary. It should be noted that CF peak antibody, at the time of this reinoculation is conspicuously low. Finally, the titer of all these antibodies diminishes evenly from the 8th month. The evolution of these 3 types of antibody is, therefore, parallel. FA antibodies are perhaps a little less stable than MA, but FA and MA are equally sensitive; the CF reactions a little less sensitive, but more stable.

2. Study of the fraction (Fig 5 & 6).

a. MA antibody. For the first 5 months, one finds them almost exclusively in the IgM absorption peak. It is only after reinoculation at 6 months that the antibodies change slightly into the IgG and are found only with difficulty in IgM.

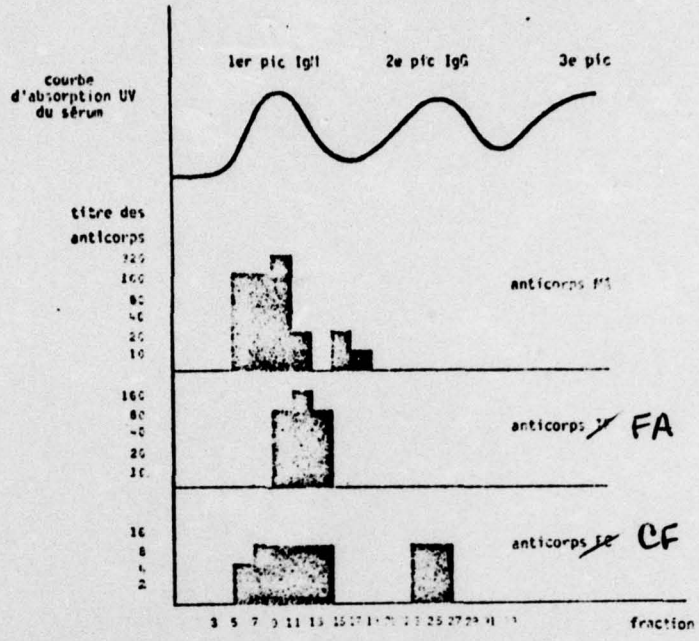


FIG. 5. — R. burneti : *courbe d'absorption en UV du sérum prélevé 3 semain la première inoculation, et répartition des anticorps MA, IF et FC dans les IgM et*

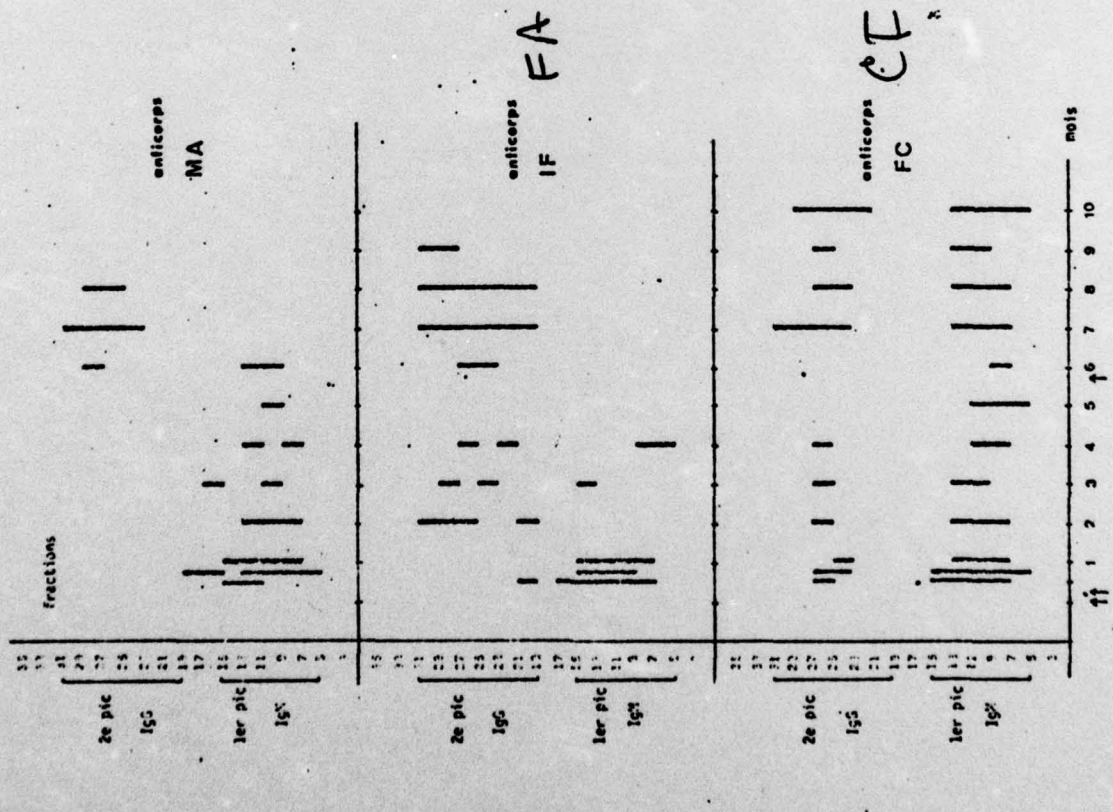


FIG. 6. — R. burneti (sérum fractionné) : évolution des anticorps MA, IF et FC en fonction du temps dans les IgM et les IgG.

b. FA antibody. During the first month, they are found uniquely in IgM. At the end of 2 months, they are all in IgG. One month after reinoculation they are only in IgG: they are found in all fractions of this peak, and the titers are elevated (320 vs 20 one month earlier).

c. CF antibody. They appear at first in the 2 peaks and persist there in a similar way after reinoculation. Titers do not vary much.

J. M. SEIGNEURIN ET COLL.

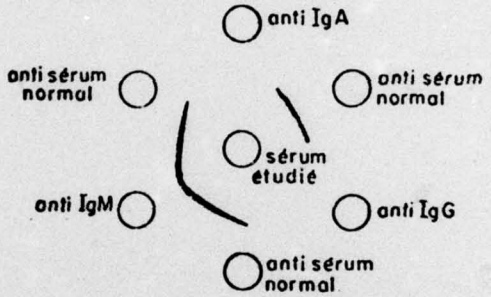


FIG. 7. — *Immunodiffusion sur gel. Vérification de la fraction IgM.*

N. B. — *Légende commune aux figures 1 et 4 :*

anticorps MA ——+——+—

FA anticorps IF ----o-----o--

CF anticorps FC ——•———•—

↑ inoculation ou réinoculation

① lapin non réinoculé

② lapin réinoculé

DISCUSSION

A. WHOLE SERUM ANTIBODIES.

The anti-R. conori and anti-R. burneti antibodies evolve in similar manner as a function of time. The maximum titers are reached 15 days after primary inoculation in MA for both, and in FA for only the latter; one month later, in FA for R. conori and CF for both.

Maximum titers obtained for CF are much less elevated than those for MA and FA (titers: 320-640 vs. 10,000-20,000). The microagglutination and immunofluorescence tests are slightly more sensitive. This is in agreement with studies of one of us (6) which found that at the time of acute illness, the antibody responses of MA, FA and CF were comparable, if not always equal, as opposed to chronic rickettsial cases in which MA is often the only one to have a positive response. On the other hand, it should be noted that after reinoculation it is the FA reaction which has the greatest sensitivity.

B. DISTRIBUTION OF THE ANTIBODIES IN IgM AND IgG CLASSES.

It is a general rule for viruses or even bacteria that the IgM antibodies are always synthesized at an early phase of the immune reaction and that, somewhat later, IgG appears to occur at the same time as the decline in IgM. A later stimulation induces only IgG antibodies. There is also some production of IgM, but of weak intensity and short duration. Occasionally, production of IgM is only a transient episode with the appearance of the other immunoglobulins, occasionally they continue to by synthesized.

Recalling results obtained for virus, Bellanti et al (2), studying adenovirus neutralizing antibodies in patients, found a prolonged IgM response at the same time as there was increased production of IgG antibodies (50-60 days and even 90 days after the start of infection).

Brown, Baublin and O'Leary (5) studied by the FA method for a period of 3 months, IgM, IgG and IgA produced during mumps; in whole serum and in

IgG, the titers are elevated from the onset and remain unchanged for 3 months; IgM antibodies are rarely seen after 50 days. Finally, Leonard, Schmidt and Lennette (11) found during varicella (primary infection) IgM and IgG antibodies, and only IgG during herpes zoster (secondary infection).

For bacteria, the studies conducted in human and animal brucellosis (1) showed that IgM and IgG antibodies appeared simultaneously, the first diminishing soon, the second persisting in chronic cases. In man, it is thought that IgM constitutes the principal agglutinating antibodies, whereas IgG fixes complement. One cannot make this distinction in bovines.

In the case of Mycoplasma pneumoniae, Bringuier, Barbe, Bosshard and Sohier (4) most often found in man IgM antibodies by the CF test until the 15th day. IgG antibodies are produced generally after day 15; Fernald (cited by these authors) found IgM antibodies 6 months after onset of illness; at 12 months, the titer is very weak and only IgG persists.

Rickettsial studies have furnished the following:

In the typhus group IgM antibodies are associated with the primary response, IgG appear later (3,6 bis). For R. burneti, agglutinating and CF antibodies are IgG (12). As a function of phases I and II, some more complete studies (8,10) showed that CF antibodies appear rapidly after inoculation: 7-30 days for Phase II, after 30-40 days for Phase I.

Our study showed that, very quickly after primary inoculation CF antibodies are localized at the end of the first week in IgM and in IgG for R. conori, and at the end of the second week for R. burneti. They persist in these 2 immunoglobulin classes during the entire time of the experiment: 7 months for R. conori, 10 months for R. burneti. IgM fractions of late samples were verified by MA. On the other hand, MA

and FA antibodies seem to follow a more classic evolution; FA antibodies localize even with the IgM for the 1st month, move progressively to the IgG, where they localize solely to the end of the 5th month. The curve of evolution of MA antibodies is less clear, in regard to R. conori: these antibodies are often revealed in the fractions intermediate between the 2 absorption peaks; after the 5th month, one again finds, frequently, IgM antibodies.

Reinoculation, determined from the beginning of the experiments to the end of 6 months (a point where MA and FA antibodies are somewhat localized at the level of IgG), does not induce a marked difference in the separation of IgM or IgG antibodies between rabbits reinoculated and those not. Significant differences exist only in the antibody titers. One sees the same CF titers in IgM and IgG, FA titers, generally more elevated in IgG, and MA often higher in IgM, after the first inoculation. It should be noted that the level of FA, which is usually more elevated than the others, is sometimes lower. This is often seen in human rickettsioses, and is perhaps due to the antigens used or to the difficulty of showing FA in high dilutions of serum. It is necessary therefore to use more specific fluorescent anti-γ, anti-μ, anti-α sera. This has been done in other studies and we ourselves propose to use these sera in our next study.

It is clear that, in experimental rickettsioses of the rabbit, diagnosis of recent infection by investigation of IgM and IgG antibodies, relies more on FA, than on CF. This supposes always that one finds IgM antibodies during 4-5 months after onset of infection.

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