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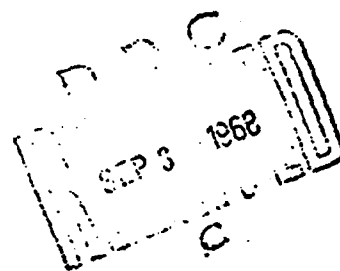
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THE FLUORESCENT MICROSCOPIC DESCRIPTION OF RICKETTSIA BURNETI
AND THEIR PHOTOGRAPHIC REPRODUCTION

H. Urbach
M. Sprossig

16 April 1965 .

THE FLUORESCENT MICROSCOPIC DESCRIPTION OF *RICKETTSIA BURNETI*
AND THEIR P. O. ORIGINALS REPRODUCTION

Following is the translation of an article by R. Urbach
and H. Grosser, published in Zent. Blatt. I. Orig. 161:
39-44, 1954. Translated by B. MacDonald.

For the first time in German literature R. Urbach (1)
in 1946 and 1947 reported on the microscopic description of
Rickettsia burneti (R. burneti) and by this opened an equi-
lateral sphere of action, that by the study of these organisms
and the diagnostic of Q-fever is characterized with microscopic
methods.

Since this time a series of works have appeared, bear in
mind the morphological view point in the illustration of *R. burneti*
with the light and electron microscope (2). As yet the
process most frequently used of microscopic visualizations with
the help of the light microscope avails itself of the color
technique according to Giemsa or with Victoria Blue according
to Herzberg. In order to preserve easily visible pictures, it
is necessary to administer to the Giemsa dye a 1-3 daily
preservation of freshly made preparation, in the Victoria blue
dye a once daily preparation is enough.

It is nevertheless often necessary on a technical working
basis, as soon as possible, after production of a preparation to
make a decision about it, if the raw material used is an agita-
tor producer or further treatments are necessary and so on.
Especially in the production of antigen from vitelline sac cul-
tures from *R. burneti* the question of exciters to the complement
fixation reaction is very significant, since on their reaction
depends further treatment and processing of vitelline sac ma-
terial to the antigen. The work undergoes an objectionable in-
terruption, when first a day must be waited for in order to
provide a basis to decide on the dye-technical production of
Rickettsia. For us the necessity appears from what has been
said, after seeking a process of preparation, that immediately
after production of the preparation demands a short lasting treat-
ment of the same and yet guarantees a good general view in the
microscope investigation. The advised requisition under consider-
ation appears appropriate to us, for the valuation the fluorescent
microscopy is referred to.

The foundation for the development of the fluorescent mi-
croscope took place in 1904 by A. Kohler, Zeiss-Jena, by the
knowledge that biological objects fluoresce with exposure to
monochromatic Ultraviolet (UV) rays. A. Kohler and H. Siebert
demonstrated in 1908 in Vienna the method of dark field illumi-
nation with UV, this simultaneously was the first knowledge pro-
duced about fluorescent microscopes. H. Lehmann, Zeiss-Jena,
improved the filter, so that only UV-rays between 280 nm and
400 nm penetrate the object. A carbon arc lamp serves as a
UV source. The firm C. Reichert developed a similar design in
1911, however they found iron carbon arcs applicable to UV pro-

Question. S.V. Frowd made in 1914 the first fluorescent coloring and S. Jenner and in 1925 colored animal material with fluorescent substrates (fluorochromes). It is the especial merit of Hattingers, later H. Hattingers (fluorochromes) have been reported on their usefulness in fixed cuts and new color effects, the so called secondary fluorescence. In 1937 H. Hattingers had then referred to the fluorochromes in the production of protozoa bacteria and different virus breeds.

The principle of fluorescent microscopic detection is known afterward that the fluorochrome impregnated objects to a visible and invisible UV rays change to visible light, through which the fluorochromed substrates are observed to be self lighting bodies and with the usual optic of a microscope can be perceived. The primary or self fluorescence is less significant for medical microbiology. On the contrary fluorochromes which absorb selectively as dye material on special elements must be used. These phenomena are reduced to an electrochromophore or electrostatic process. In order to utilize the fluorochromes with objects described, dehydrated smear preparations with fluorochromes can be prepared. Furthermore, it is also possible to handle the culture media with such fluorochromes, which were taken out from the developing micro-organisms in the nutrient medium. Finally the possibility still exists, to administer to host organisms fluorescing chemotherapeutics and to diagnose the accumulated fluorescent flashing substrates in parasites.

For our purpose only the fluorochroming of smear preparations is applicable. Some fluorochromes can cause a polychromatic flash. Their application always results in strong dilutions such as 1:55 to 1:1000000 and for short times of a few seconds or a minute. In the case of objects which could not be viewed in their behavior processes fluorochromes, it is recommended then in the event of great dilution in order to overlook the duration of color time. By this means overcoloring can be avoided. The fluorochrome solutions were used for a preparation only once. Therefore the use of dye bath is not recommended, the use can be checked in slide series. The stability of the preparation should be guaranteed for 1 year. Invert glass the reduction of the light intensity of the fluorochrome itself manifested itself with repeated and longer lasting exposure of the preparation to the UV ray path with the fluorescent microscope.

1. Fluorescentmicroscopic Research

In our fluorescent microscopic research we make use of large luminescence equipment with the carbon arc lamp of the Zeiss Jena firm. The curved light is steratized directly by a cooling bulb with a 4% solution of copper sulfate, whereby the light wave red portions are disconnected, in order to pass a series of different thicker line and wave lengths (UV 0.15 mm; 0.2 mm; 0.3 mm; 0.4 mm; 0.5 mm; 0.6 mm; 0.7 mm; 0.8 mm; 0.9 mm; 1.0 mm; 1.1 mm; 1.2 mm; 1.3 mm; 1.4 mm; 1.5 mm; 1.6 mm; 1.7 mm; 1.8 mm; 1.9 mm; 2.0 mm; 2.1 mm; 2.2 mm; 2.3 mm; 2.4 mm; 2.5 mm; 2.6 mm; 2.7 mm; 2.8 mm; 2.9 mm; 3.0 mm; 3.1 mm; 3.2 mm; 3.3 mm; 3.4 mm; 3.5 mm; 3.6 mm; 3.7 mm; 3.8 mm; 3.9 mm; 4.0 mm; 4.1 mm; 4.2 mm; 4.3 mm; 4.4 mm; 4.5 mm; 4.6 mm; 4.7 mm; 4.8 mm; 4.9 mm; 5.0 mm; 5.1 mm; 5.2 mm; 5.3 mm; 5.4 mm; 5.5 mm; 5.6 mm; 5.7 mm; 5.8 mm; 5.9 mm; 6.0 mm; 6.1 mm; 6.2 mm; 6.3 mm; 6.4 mm; 6.5 mm; 6.6 mm; 6.7 mm; 6.8 mm; 6.9 mm; 7.0 mm; 7.1 mm; 7.2 mm; 7.3 mm; 7.4 mm; 7.5 mm; 7.6 mm; 7.7 mm; 7.8 mm; 7.9 mm; 8.0 mm; 8.1 mm; 8.2 mm; 8.3 mm; 8.4 mm; 8.5 mm; 8.6 mm; 8.7 mm; 8.8 mm; 8.9 mm; 9.0 mm; 9.1 mm; 9.2 mm; 9.3 mm; 9.4 mm; 9.5 mm; 9.6 mm; 9.7 mm; 9.8 mm; 9.9 mm; 10.0 mm; 10.1 mm; 10.2 mm; 10.3 mm; 10.4 mm; 10.5 mm; 10.6 mm; 10.7 mm; 10.8 mm; 10.9 mm; 11.0 mm; 11.1 mm; 11.2 mm; 11.3 mm; 11.4 mm; 11.5 mm; 11.6 mm; 11.7 mm; 11.8 mm; 11.9 mm; 12.0 mm; 12.1 mm; 12.2 mm; 12.3 mm; 12.4 mm; 12.5 mm; 12.6 mm; 12.7 mm; 12.8 mm; 12.9 mm; 13.0 mm; 13.1 mm; 13.2 mm; 13.3 mm; 13.4 mm; 13.5 mm; 13.6 mm; 13.7 mm; 13.8 mm; 13.9 mm; 14.0 mm; 14.1 mm; 14.2 mm; 14.3 mm; 14.4 mm; 14.5 mm; 14.6 mm; 14.7 mm; 14.8 mm; 14.9 mm; 15.0 mm; 15.1 mm; 15.2 mm; 15.3 mm; 15.4 mm; 15.5 mm; 15.6 mm; 15.7 mm; 15.8 mm; 15.9 mm; 16.0 mm; 16.1 mm; 16.2 mm; 16.3 mm; 16.4 mm; 16.5 mm; 16.6 mm; 16.7 mm; 16.8 mm; 16.9 mm; 17.0 mm; 17.1 mm; 17.2 mm; 17.3 mm; 17.4 mm; 17.5 mm; 17.6 mm; 17.7 mm; 17.8 mm; 17.9 mm; 18.0 mm; 18.1 mm; 18.2 mm; 18.3 mm; 18.4 mm; 18.5 mm; 18.6 mm; 18.7 mm; 18.8 mm; 18.9 mm; 19.0 mm; 19.1 mm; 19.2 mm; 19.3 mm; 19.4 mm; 19.5 mm; 19.6 mm; 19.7 mm; 19.8 mm; 19.9 mm; 20.0 mm; 20.1 mm; 20.2 mm; 20.3 mm; 20.4 mm; 20.5 mm; 20.6 mm; 20.7 mm; 20.8 mm; 20.9 mm; 21.0 mm; 21.1 mm; 21.2 mm; 21.3 mm; 21.4 mm; 21.5 mm; 21.6 mm; 21.7 mm; 21.8 mm; 21.9 mm; 22.0 mm; 22.1 mm; 22.2 mm; 22.3 mm; 22.4 mm; 22.5 mm; 22.6 mm; 22.7 mm; 22.8 mm; 22.9 mm; 23.0 mm; 23.1 mm; 23.2 mm; 23.3 mm; 23.4 mm; 23.5 mm; 23.6 mm; 23.7 mm; 23.8 mm; 23.9 mm; 24.0 mm; 24.1 mm; 24.2 mm; 24.3 mm; 24.4 mm; 24.5 mm; 24.6 mm; 24.7 mm; 24.8 mm; 24.9 mm; 25.0 mm; 25.1 mm; 25.2 mm; 25.3 mm; 25.4 mm; 25.5 mm; 25.6 mm; 25.7 mm; 25.8 mm; 25.9 mm; 26.0 mm; 26.1 mm; 26.2 mm; 26.3 mm; 26.4 mm; 26.5 mm; 26.6 mm; 26.7 mm; 26.8 mm; 26.9 mm; 27.0 mm; 27.1 mm; 27.2 mm; 27.3 mm; 27.4 mm; 27.5 mm; 27.6 mm; 27.7 mm; 27.8 mm; 27.9 mm; 28.0 mm; 28.1 mm; 28.2 mm; 28.3 mm; 28.4 mm; 28.5 mm; 28.6 mm; 28.7 mm; 28.8 mm; 28.9 mm; 29.0 mm; 29.1 mm; 29.2 mm; 29.3 mm; 29.4 mm; 29.5 mm; 29.6 mm; 29.7 mm; 29.8 mm; 29.9 mm; 30.0 mm; 30.1 mm; 30.2 mm; 30.3 mm; 30.4 mm; 30.5 mm; 30.6 mm; 30.7 mm; 30.8 mm; 30.9 mm; 31.0 mm; 31.1 mm; 31.2 mm; 31.3 mm; 31.4 mm; 31.5 mm; 31.6 mm; 31.7 mm; 31.8 mm; 31.9 mm; 32.0 mm; 32.1 mm; 32.2 mm; 32.3 mm; 32.4 mm; 32.5 mm; 32.6 mm; 32.7 mm; 32.8 mm; 32.9 mm; 33.0 mm; 33.1 mm; 33.2 mm; 33.3 mm; 33.4 mm; 33.5 mm; 33.6 mm; 33.7 mm; 33.8 mm; 33.9 mm; 34.0 mm; 34.1 mm; 34.2 mm; 34.3 mm; 34.4 mm; 34.5 mm; 34.6 mm; 34.7 mm; 34.8 mm; 34.9 mm; 35.0 mm; 35.1 mm; 35.2 mm; 35.3 mm; 35.4 mm; 35.5 mm; 35.6 mm; 35.7 mm; 35.8 mm; 35.9 mm; 36.0 mm; 36.1 mm; 36.2 mm; 36.3 mm; 36.4 mm; 36.5 mm; 36.6 mm; 36.7 mm; 36.8 mm; 36.9 mm; 37.0 mm; 37.1 mm; 37.2 mm; 37.3 mm; 37.4 mm; 37.5 mm; 37.6 mm; 37.7 mm; 37.8 mm; 37.9 mm; 38.0 mm; 38.1 mm; 38.2 mm; 38.3 mm; 38.4 mm; 38.5 mm; 38.6 mm; 38.7 mm; 38.8 mm; 38.9 mm; 39.0 mm; 39.1 mm; 39.2 mm; 39.3 mm; 39.4 mm; 39.5 mm; 39.6 mm; 39.7 mm; 39.8 mm; 39.9 mm; 40.0 mm; 40.1 mm; 40.2 mm; 40.3 mm; 40.4 mm; 40.5 mm; 40.6 mm; 40.7 mm; 40.8 mm; 40.9 mm; 41.0 mm; 41.1 mm; 41.2 mm; 41.3 mm; 41.4 mm; 41.5 mm; 41.6 mm; 41.7 mm; 41.8 mm; 41.9 mm; 42.0 mm; 42.1 mm; 42.2 mm; 42.3 mm; 42.4 mm; 42.5 mm; 42.6 mm; 42.7 mm; 42.8 mm; 42.9 mm; 43.0 mm; 43.1 mm; 43.2 mm; 43.3 mm; 43.4 mm; 43.5 mm; 43.6 mm; 43.7 mm; 43.8 mm; 43.9 mm; 44.0 mm; 44.1 mm; 44.2 mm; 44.3 mm; 44.4 mm; 44.5 mm; 44.6 mm; 44.7 mm; 44.8 mm; 44.9 mm; 45.0 mm; 45.1 mm; 45.2 mm; 45.3 mm; 45.4 mm; 45.5 mm; 45.6 mm; 45.7 mm; 45.8 mm; 45.9 mm; 46.0 mm; 46.1 mm; 46.2 mm; 46.3 mm; 46.4 mm; 46.5 mm; 46.6 mm; 46.7 mm; 46.8 mm; 46.9 mm; 47.0 mm; 47.1 mm; 47.2 mm; 47.3 mm; 47.4 mm; 47.5 mm; 47.6 mm; 47.7 mm; 47.8 mm; 47.9 mm; 48.0 mm; 48.1 mm; 48.2 mm; 48.3 mm; 48.4 mm; 48.5 mm; 48.6 mm; 48.7 mm; 48.8 mm; 48.9 mm; 49.0 mm; 49.1 mm; 49.2 mm; 49.3 mm; 49.4 mm; 49.5 mm; 49.6 mm; 49.7 mm; 49.8 mm; 49.9 mm; 50.0 mm; 50.1 mm; 50.2 mm; 50.3 mm; 50.4 mm; 50.5 mm; 50.6 mm; 50.7 mm; 50.8 mm; 50.9 mm; 51.0 mm; 51.1 mm; 51.2 mm; 51.3 mm; 51.4 mm; 51.5 mm; 51.6 mm; 51.7 mm; 51.8 mm; 51.9 mm; 52.0 mm; 52.1 mm; 52.2 mm; 52.3 mm; 52.4 mm; 52.5 mm; 52.6 mm; 52.7 mm; 52.8 mm; 52.9 mm; 53.0 mm; 53.1 mm; 53.2 mm; 53.3 mm; 53.4 mm; 53.5 mm; 53.6 mm; 53.7 mm; 53.8 mm; 53.9 mm; 54.0 mm; 54.1 mm; 54.2 mm; 54.3 mm; 54.4 mm; 54.5 mm; 54.6 mm; 54.7 mm; 54.8 mm; 54.9 mm; 55.0 mm; 55.1 mm; 55.2 mm; 55.3 mm; 55.4 mm; 55.5 mm; 55.6 mm; 55.7 mm; 55.8 mm; 55.9 mm; 56.0 mm; 56.1 mm; 56.2 mm; 56.3 mm; 56.4 mm; 56.5 mm; 56.6 mm; 56.7 mm; 56.8 mm; 56.9 mm; 57.0 mm; 57.1 mm; 57.2 mm; 57.3 mm; 57.4 mm; 57.5 mm; 57.6 mm; 57.7 mm; 57.8 mm; 57.9 mm; 58.0 mm; 58.1 mm; 58.2 mm; 58.3 mm; 58.4 mm; 58.5 mm; 58.6 mm; 58.7 mm; 58.8 mm; 58.9 mm; 59.0 mm; 59.1 mm; 59.2 mm; 59.3 mm; 59.4 mm; 59.5 mm; 59.6 mm; 59.7 mm; 59.8 mm; 59.9 mm; 60.0 mm; 60.1 mm; 60.2 mm; 60.3 mm; 60.4 mm; 60.5 mm; 60.6 mm; 60.7 mm; 60.8 mm; 60.9 mm; 61.0 mm; 61.1 mm; 61.2 mm; 61.3 mm; 61.4 mm; 61.5 mm; 61.6 mm; 61.7 mm; 61.8 mm; 61.9 mm; 62.0 mm; 62.1 mm; 62.2 mm; 62.3 mm; 62.4 mm; 62.5 mm; 62.6 mm; 62.7 mm; 62.8 mm; 62.9 mm; 63.0 mm; 63.1 mm; 63.2 mm; 63.3 mm; 63.4 mm; 63.5 mm; 63.6 mm; 63.7 mm; 63.8 mm; 63.9 mm; 64.0 mm; 64.1 mm; 64.2 mm; 64.3 mm; 64.4 mm; 64.5 mm; 64.6 mm; 64.7 mm; 64.8 mm; 64.9 mm; 65.0 mm; 65.1 mm; 65.2 mm; 65.3 mm; 65.4 mm; 65.5 mm; 65.6 mm; 65.7 mm; 65.8 mm; 65.9 mm; 66.0 mm; 66.1 mm; 66.2 mm; 66.3 mm; 66.4 mm; 66.5 mm; 66.6 mm; 66.7 mm; 66.8 mm; 66.9 mm; 67.0 mm; 67.1 mm; 67.2 mm; 67.3 mm; 67.4 mm; 67.5 mm; 67.6 mm; 67.7 mm; 67.8 mm; 67.9 mm; 68.0 mm; 68.1 mm; 68.2 mm; 68.3 mm; 68.4 mm; 68.5 mm; 68.6 mm; 68.7 mm; 68.8 mm; 68.9 mm; 69.0 mm; 69.1 mm; 69.2 mm; 69.3 mm; 69.4 mm; 69.5 mm; 69.6 mm; 69.7 mm; 69.8 mm; 69.9 mm; 70.0 mm; 70.1 mm; 70.2 mm; 70.3 mm; 70.4 mm; 70.5 mm; 70.6 mm; 70.7 mm; 70.8 mm; 70.9 mm; 71.0 mm; 71.1 mm; 71.2 mm; 71.3 mm; 71.4 mm; 71.5 mm; 71.6 mm; 71.7 mm; 71.8 mm; 71.9 mm; 72.0 mm; 72.1 mm; 72.2 mm; 72.3 mm; 72.4 mm; 72.5 mm; 72.6 mm; 72.7 mm; 72.8 mm; 72.9 mm; 73.0 mm; 73.1 mm; 73.2 mm; 73.3 mm; 73.4 mm; 73.5 mm; 73.6 mm; 73.7 mm; 73.8 mm; 73.9 mm; 74.0 mm; 74.1 mm; 74.2 mm; 74.3 mm; 74.4 mm; 74.5 mm; 74.6 mm; 74.7 mm; 74.8 mm; 74.9 mm; 75.0 mm; 75.1 mm; 75.2 mm; 75.3 mm; 75.4 mm; 75.5 mm; 75.6 mm; 75.7 mm; 75.8 mm; 75.9 mm; 76.0 mm; 76.1 mm; 76.2 mm; 76.3 mm; 76.4 mm; 76.5 mm; 76.6 mm; 76.7 mm; 76.8 mm; 76.9 mm; 77.0 mm; 77.1 mm; 77.2 mm; 77.3 mm; 77.4 mm; 77.5 mm; 77.6 mm; 77.7 mm; 77.8 mm; 77.9 mm; 78.0 mm; 78.1 mm; 78.2 mm; 78.3 mm; 78.4 mm; 78.5 mm; 78.6 mm; 78.7 mm; 78.8 mm; 78.9 mm; 79.0 mm; 79.1 mm; 79.2 mm; 79.3 mm; 79.4 mm; 79.5 mm; 79.6 mm; 79.7 mm; 79.8 mm; 79.9 mm; 80.0 mm; 80.1 mm; 80.2 mm; 80.3 mm; 80.4 mm; 80.5 mm; 80.6 mm; 80.7 mm; 80.8 mm; 80.9 mm; 81.0 mm; 81.1 mm; 81.2 mm; 81.3 mm; 81.4 mm; 81.5 mm; 81.6 mm; 81.7 mm; 81.8 mm; 81.9 mm; 82.0 mm; 82.1 mm; 82.2 mm; 82.3 mm; 82.4 mm; 82.5 mm; 82.6 mm; 82.7 mm; 82.8 mm; 82.9 mm; 83.0 mm; 83.1 mm; 83.2 mm; 83.3 mm; 83.4 mm; 83.5 mm; 83.6 mm; 83.7 mm; 83.8 mm; 83.9 mm; 84.0 mm; 84.1 mm; 84.2 mm; 84.3 mm; 84.4 mm; 84.5 mm; 84.6 mm; 84.7 mm; 84.8 mm; 84.9 mm; 85.0 mm; 85.1 mm; 85.2 mm; 85.3 mm; 85.4 mm; 85.5 mm; 85.6 mm; 85.7 mm; 85.8 mm; 85.9 mm; 86.0 mm; 86.1 mm; 86.2 mm; 86.3 mm; 86.4 mm; 86.5 mm; 86.6 mm; 86.7 mm; 86.8 mm; 86.9 mm; 87.0 mm; 87.1 mm; 87.2 mm; 87.3 mm; 87.4 mm; 87.5 mm; 87.6 mm; 87.7 mm; 87.8 mm; 87.9 mm; 88.0 mm; 88.1 mm; 88.2 mm; 88.3 mm; 88.4 mm; 88.5 mm; 88.6 mm; 88.7 mm; 88.8 mm; 88.9 mm; 89.0 mm; 89.1 mm; 89.2 mm; 89.3 mm; 89.4 mm; 89.5 mm; 89.6 mm; 89.7 mm; 89.8 mm; 89.9 mm; 90.0 mm; 90.1 mm; 90.2 mm; 90.3 mm; 90.4 mm; 90.5 mm; 90.6 mm; 90.7 mm; 90.8 mm; 90.9 mm; 91.0 mm; 91.1 mm; 91.2 mm; 91.3 mm; 91.4 mm; 91.5 mm; 91.6 mm; 91.7 mm; 91.8 mm; 91.9 mm; 92.0 mm; 92.1 mm; 92.2 mm; 92.3 mm; 92.4 mm; 92.5 mm; 92.6 mm; 92.7 mm; 92.8 mm; 92.9 mm; 93.0 mm; 93.1 mm; 93.2 mm; 93.3 mm; 93.4 mm; 93.5 mm; 93.6 mm; 93.7 mm; 93.8 mm; 93.9 mm; 94.0 mm; 94.1 mm; 94.2 mm; 94.3 mm; 94.4 mm; 94.5 mm; 94.6 mm; 94.7 mm; 94.8 mm; 94.9 mm; 95.0 mm; 95.1 mm; 95.2 mm; 95.3 mm; 95.4 mm; 95.5 mm; 95.6 mm; 95.7 mm; 95.8 mm; 95.9 mm; 96.0 mm; 96.1 mm; 96.2 mm; 96.3 mm; 96.4 mm; 96.5 mm; 96.6 mm; 96.7 mm; 96.8 mm; 96.9 mm; 97.0 mm; 97.1 mm; 97.2 mm; 97.3 mm; 97.4 mm; 97.5 mm; 97.6 mm; 97.7 mm; 97.8 mm; 97.9 mm; 98.0 mm; 98.1 mm; 98.2 mm; 98.3 mm; 98.4 mm; 98.5 mm; 98.6 mm; 98.7 mm; 98.8 mm; 98.9 mm; 99.0 mm; 99.1 mm; 99.2 mm; 99.3 mm; 99.4 mm; 99.5 mm; 99.6 mm; 99.7 mm; 99.8 mm; 99.9 mm; 100.0 mm; 100.1 mm; 100.2 mm; 100.3 mm; 100.4 mm; 100.5 mm; 100.6 mm; 100.7 mm; 100.8 mm; 100.9 mm; 101.0 mm; 101.1 mm; 101.2 mm; 101.3 mm; 101.4 mm; 101.5 mm; 101.6 mm; 101.7 mm; 101.8 mm; 101.9 mm; 102.0 mm; 102.1 mm; 102.2 mm; 102.3 mm; 102.4 mm; 102.5 mm; 102.6 mm; 102.7 mm; 102.8 mm; 102.9 mm; 103.0 mm; 103.1 mm; 103.2 mm; 103.3 mm; 103.4 mm; 103.5 mm; 103.6 mm; 103.7 mm; 103.8 mm; 103.9 mm; 104.0 mm; 104.1 mm; 104.2 mm; 104.3 mm; 104.4 mm; 104.5 mm; 104.6 mm; 104.7 mm; 104.8 mm; 104.9 mm; 105.0 mm; 105.1 mm; 105.2 mm; 105.3 mm; 105.4 mm; 105.5 mm; 105.6 mm; 105.7 mm; 105.8 mm; 105.9 mm; 106.0 mm; 106.1 mm; 106.2 mm; 106.3 mm; 106.4 mm; 106.5 mm; 106.6 mm; 106.7 mm; 106.8 mm; 106.9 mm; 107.0 mm; 107.1 mm; 107.2 mm; 107.3 mm; 107.4 mm; 107.5 mm; 107.6 mm; 107.7 mm; 107.8 mm; 107.9 mm; 108.0 mm; 108.1 mm; 108.2 mm; 108.3 mm; 108.4 mm; 108.5 mm; 108.6 mm; 108.7 mm; 108.8 mm; 108.9 mm; 109.0 mm; 109.1 mm; 109.2 mm; 109.3 mm; 109.4 mm; 109.5 mm; 109.6 mm; 109.7 mm; 109.8 mm; 109.9 mm; 110.0 mm; 110.1 mm; 110.2 mm; 110.3 mm; 110.4 mm; 110.5 mm; 110.6 mm; 110.7 mm; 110.8 mm; 110.9 mm; 111.0 mm; 111.1 mm; 111.2 mm; 111.3 mm; 111.4 mm; 111.5 mm; 111.6 mm; 111.7 mm; 111.8 mm; 111.9 mm; 112.0 mm; 112.1 mm; 112.2 mm; 112.3 mm; 112.4 mm; 112.5 mm; 112.6 mm; 112.7 mm; 112.8 mm; 112.9 mm; 113.0 mm; 113.1 mm; 113.2 mm; 113.3 mm; 113.4 mm; 113.5 mm; 113.6 mm; 113.7 mm; 113.8 mm; 113.9 mm; 114.0 mm; 114.1 mm; 114.2 mm; 114.3 mm; 114.4 mm; 114.5 mm; 114.6 mm; 114.7 mm; 114.8 mm; 114.9 mm; 115.0 mm; 115.1 mm; 115.2 mm; 115.3 mm; 115.4 mm; 115.5 mm; 115.6 mm; 115.7 mm; 115.8 mm; 115.9 mm; 116.0 mm; 116.1 mm; 116.2 mm; 116.3 mm; 116.4 mm; 116.5 mm; 116.6 mm; 116.7 mm; 116.8 mm; 116.9 mm; 117.0 mm; 117.1 mm; 117.2 mm; 117.3 mm; 117.4 mm; 117.5 mm; 117.6 mm; 117.7 mm; 117.8 mm; 117.9 mm; 118.0 mm; 118.1 mm; 118.2 mm; 118.3 mm; 118.4 mm; 118.5 mm; 118.6 mm; 118.7 mm; 118.8 mm; 118.9 mm; 119.0 mm; 119.1 mm; 119.2 mm; 119.3 mm; 119.4 mm; 119.5 mm; 119.6 mm; 119.7 mm; 119.8 mm; 119.9 mm; 120.0 mm; 120.1 mm; 120.2 mm; 120.3 mm; 120.4 mm; 120.5 mm; 120.6 mm; 120.7 mm; 120.8 mm; 120.9 mm; 121.0 mm; 121.1 mm; 121.2 mm; 121.3 mm; 121.4 mm; 121.5 mm; 121.6 mm; 121.7 mm; 121.8 mm; 121.9 mm; 122.0 mm; 122.1 mm; 122.2 mm; 122.3 mm; 122.4 mm; 122.5 mm; 122.6 mm; 122.7 mm; 122.8 mm; 122.9 mm; 123.0 mm; 123.1 mm; 123.2 mm; 123.3 mm; 123.4 mm; 123.5 mm; 123.6 mm; 123.7 mm; 123.8 mm; 123.9 mm; 124.0 mm; 124.1 mm; 124.2 mm; 124.3 mm; 124.4 mm; 124.5 mm; 124.6 mm; 124.7 mm; 124.8 mm; 124.9 mm; 125.0 mm; 125.1 mm; 125.2 mm; 125.3 mm; 125.4 mm; 125.5 mm; 125.6 mm; 125.7 mm; 125.8 mm; 125.9 mm; 126.0 mm; 126.1 mm; 126.2 mm; 126.3 mm; 126.4 mm; 126.5 mm; 126.6 mm; 126.7 mm; 126.8 mm; 126.9 mm; 127.0 mm; 127.1 mm; 127.2 mm; 127.3 mm; 127.4 mm; 127.5 mm; 127.6 mm; 127.7 mm; 127.8 mm; 127.9 mm; 128.0 mm; 128.1 mm; 128.2 mm; 128.3 mm; 128.4 mm; 128.5 mm; 128.6 mm; 128.7 mm; 128.8 mm; 128.9 mm; 129.0 mm; 129.1 mm; 129.2 mm; 129.3 mm; 129.4 mm; 129.5 mm; 129.6 mm; 129.7 mm; 129.8 mm; 129.9 mm; 130.0 mm; 130.1 mm; 130.2 mm; 130.3 mm; 130.4 mm; 130.5 mm; 130.6 mm; 130.7 mm; 130.8 mm; 130.9 mm; 131.0 mm; 131.1 mm; 131.2 mm; 131.3 mm; 131.4 mm; 131.5 mm; 131.6 mm; 131.7 mm; 131.8 mm; 131.9 mm; 132.0 mm; 132.1 mm; 132.2 mm; 132.3 mm; 132.4 mm; 132.5 mm; 132.6 mm; 132.7 mm; 132.8 mm; 132.9 mm; 133.0 mm; 133.1 mm; 133.2 mm; 133.3 mm; 133.4 mm; 133.5 mm; 133.6 mm; 133.7 mm; 133.8 mm; 133.9 mm; 134.0 mm; 134.1 mm; 134.2 mm; 134.3 mm; 134.4 mm; 134.5 mm; 134.6 mm; 134.7 mm; 134.8 mm; 134.9 mm; 135.0 mm; 135.1 mm; 135.2 mm; 135.3 mm; 135.4 mm; 135.5 mm; 135.6 mm; 135.7 mm; 135.8 mm; 135.9 mm; 136.0 mm; 136.1 mm; 136.2 mm; 136.3 mm; 136.4 mm; 136.5 mm; 136.6 mm; 136.7 mm; 136.8 mm; 136.9 mm; 137.0 mm; 137.1 mm; 137.2 mm; 137.3 mm; 137.4 mm; 137.5 mm; 137.6 mm; 137.7 mm; 137.8 mm; 137.9 mm; 138.0 mm; 138.1 mm; 138.2 mm; 138.3 mm; 138.4 mm; 138.5 mm; 138.6 mm; 138.7 mm; 138.8 mm; 138.9 mm; 139.0 mm; 139.1 mm; 139.2 mm; 139.3 mm; 139.4 mm; 139.5 mm; 139.6 mm; 139.7 mm; 139.8 mm; 139.9 mm; 140.0 mm; 140.1 mm; 140.2 mm; 140.3 mm; 140.4 mm; 140.5 mm; 140.6 mm; 140.7 mm; 140.8 mm; 140.9 mm; 141.0 mm; 141.1 mm; 141.2 mm; 141.3 mm; 141.4 mm; 141.5 mm; 141.6 mm; 141.7 mm; 141.8 mm; 141.9 mm; 142.0 mm; 142.1 mm; 142.2 mm; 142.3 mm; 142.4 mm; 142.5 mm; 142.6 mm; 142.7 mm; 142.8 mm; 142.9 mm; 143.0 mm; 143.1 mm; 143.2 mm; 143.3 mm; 143.4 mm; 143.5 mm; 143.6 mm; 143.7 mm; 143.8 mm; 143.9 mm; 144.0 mm; 144.1 mm; 144.2 mm; 144.3 mm; 144.4 mm; 144.5 mm; 144.6 mm; 144.7 mm; 144.8 mm; 144.9 mm; 145.0 mm; 145.1 mm; 145.2 mm; 145.3 mm; 145.4 mm; 145.5 mm; 145.6 mm; 145.7 mm; 145.8 mm; 145.9 mm; 146.0 mm; 146.1 mm; 146.2 mm; 146.3 mm; 146.4 mm; 146.5 mm; 146.6 mm; 146.7 mm; 146.8 mm; 146.9 mm; 147.0 mm; 147.1 mm; 147.2 mm; 147.3 mm; 147.4 mm; 147.5 mm; 147.6 mm; 147.7 mm; 147.8 mm; 147.9 mm; 148.0 mm; 148.1 mm; 148.2 mm; 148.3 mm; 148.4 mm; 148.5 mm; 148.6 mm; 148.7 mm; 148.8 mm; 148.9 mm; 149.0 mm; 149.1 mm; 149.2 mm; 149.3 mm; 149.4 mm; 149.5 mm; 149.6 mm; 149.7 mm; 149.8 mm; 149.9 mm; 150.0 mm; 150.1 mm; 150.2 mm; 150.3 mm; 150.4 mm; 150.5 mm; 150.6 mm; 150.7 mm; 150.8 mm; 150.9 mm; 151.0 mm; 151.1 mm; 151.2 mm; 151.3 mm; 151.4 mm; 151.5 mm; 151.6 mm; 151.7 mm; 151.8 mm; 151.9 mm; 152.0 mm; 152.1 mm; 152.2 mm; 152.3 mm; 152.4 mm; 152.5 mm; 152.6 mm; 152.7 mm; 152.8 mm; 152.9 mm; 153.0 mm; 153.1 mm; 153.2 mm; 153.3 mm; 153.4 mm; 153.5 mm; 153.6 mm; 153.7 mm; 153.8 mm; 153.9 mm; 154.0 mm; 154.1 mm; 154.2 mm; 154.3 mm; 154.4 mm; 154.5 mm; 154.6 mm; 154.7 mm; 154.8 mm; 154.9 mm; 155.0 mm; 155.1 mm; 155.2 mm; 155.3 mm; 155.4 mm; 155.5 mm; 155.6 mm; 155.7 mm; 155.8 mm; 155.9 mm; 156.0 mm; 156.1 mm; 156.2 mm; 156.3 mm; 156.4 mm; 156.5 mm; 156.6 mm; 156.7 mm; 156.8 mm; 156.9 mm; 157.0 mm; 157.1 mm; 157.2 mm; 157.3 mm; 157.4 mm; 157.5 mm; 157.6 mm; 157.7 mm; 157.8 mm; 157.9 mm; 158.0 mm; 158.1 mm; 158.2 mm; 158.3 mm; 158.4 mm; 158.5 mm; 158.6 mm; 158.7 mm; 158.8 mm; 158.9 mm; 159.0 mm; 159.1 mm; 159.

immersions objective 90/1.25 and a 5 or 10 fold-ocular amplification. It is mostly used in the 4 filters in our research the UVette Filter B-3/4 mm, which the best illumination is obtained with it. Still the disturbing UV rays, which concentrate the object, must be inserted through intermediate filters, which are exposed to the center. A dark gold filter proved to us as very appropriate.

We prepared the preparations from antigen material, as it is obtained by us for the development of the complement fixation reaction, besides from vitelline sac cultures and in the form of testicle spot cultures from infected guinea pigs.

After the air drying and with fixation of the smear we have a complete series, which often immediately treated and were also often preserved for many days, colored with the following fluorochromes: Primulin, Auramin, Rickettsin 3 G0, methylviolet extra, neutral red extra, brilliant green 2 or 3. Dr. G. Grubler and Co., Leipzig. For the study of the strain "Grita" served us in 70 to 85 egg passages and "Schwaben" in 40 to 50 egg passages.

We maintain the supposition, that itself, according to the observations of P.K.H. Hagenbach (5) on virus preparations, Primulin was spontaneous at first for the production of *R. rickettsiae*. However, only slightly light illumination appears in the Primulin fluorochroming. The rickettsia do not possess a natural, that is spontaneous fluorescence after our research on unilateral preparations, so that a fluorochroming is necessary. Of all fluorochromes used in different smear preparations, the Auramin according to our source demonstrated to us, a light yellow soluble diphenylmethane pigment in water, as the best available. The light intensity of the rickettsia fluorochromed with Auramin plainly overshadowed the Primulin, that is present it is added on, the mode of application of the Auramin is varied. The others in addition to the fluorochromes mentioned generally proved to be useless for the production of *R. rickettsiae*.

First we used an Auramin solution of 1:500, treated the preparation 2 seconds with it and washed it for a few seconds with cold water. Rickettsia were indeed produced, but the vitelline sac material still flashed as yellowish green, so that the contrast between rickettsia and background material did not appear clearly enough. In 10 seconds after washing with warm water of about 60°C the decolorization of the smear materials, nevertheless, was an improvement and the micro-organisms in this way show up brightly. The subsequent treatment with warm water must of course not be extended over 10-15 seconds, since otherwise the rickettsia or Auramin could leave and undergo damage to their intensity. After changes in degree of dye time (in 1 min.) and the concentration of Auramin solution (1:1000) the following technique for obtaining better production has been proved sound, which are suitable for microscopic reproduction: creation of preparations with Auramin solution 1:500 + an addition of 0.5% phenol liquefact. for 30 seconds, following centrifuging of the slide in a glass of warm water at 60°C for 3-15 seconds, according to the thickness of the preparation, consistent with the incubator by standing upright. For microscopic inspection, one uses fluorescent free immersion oil (1.5").

It also the fluorescent plate is a reflection of R. rickettsii in the culture. In order to reflect the light from the plate, it is necessary to use a glass slide which is not too thick. The slide must be thin enough to allow the light to pass through it. The fluorescent plate is a reflection of the fluorescent material which is on the slide. This is very useful for the valuation of the culture in the vitelline sac. The process is therefore used for valuation of rickettsia content of the vitelline sac, just as this is necessary for control in the case of infection production for the emulsion vaccine preparation. The color technique employed by the method which can appear as a reflection in the plate, which is not too thick, they indicate the diagnosis as well as not. The color is correspond in tone to the fluorescent material. A possibility of error with the bright yellow chlamydia rickettsia is therefore not given.

III.3.2 MICROPHOTOGRAPHIC METHOD

In the case of the film, the light is available for the microscopic exposure of a fluorescent material. When changing light with a clear light source, since a glass plate absorbs the light, the angle of the fluorescent rickettsia and for that reason the light source, the observation, a certain significance belongs to this method. Our small lens camera was attached to the intermediate microscope with an 11/100 microscope on the straight tube of the Zeiss Jena film. The optic system of the microscope is composed of an achromatic objective 20/1.30 and a combination ocular with 10 fold magnification. The same type and ocular interference filter was used for the restoration of the exposure as well as for the visual observation and the fluorescent observation oil film 1.520. A series of proof exposure, showed clearly, that an improvement of exposure conditions cannot be accomplished by an exchange of filter series. This is due to the fact that the camera is for the reason necessary, while the objective combination with the eye does not always coincide with the color sensitivity of different negative materials.

For a series of proof exposures, the film was taken of the exposure time of the film with different sensitivity. Even with optical fluorescence of rickettsia, which in subjective observation presents a full picture, do not appear the light intensity of these small micro-organisms in order to obtain a useful exposure time also for highly sensitive negative materials. So it requires for the film exposure time of 1/1000 sec. an exposure time of 25 minutes, for the film 1/1000 sec. an exposure time of 50 minutes. The fact that the film is not so numerous details of other methods, can be derived from the substantial strong magnification which is necessary for the film in its order of magnitude which is not too thick. In the case of the film, the exposure time is 1/1000 sec. as it is applied in the Zeiss Jena microscope in the bright field microscope (11/100 sec.) exposure time is 1/1000 sec. of exposure corresponding to 1/1000 sec. in the film, as short as possible for fluorescent exposure on the film.

must be derived from.

1) The longer exposure time provides numerous corrections and finally an image of substance which was made and thereby by the corresponding vibration is also by its ability to a blurred picture. The cannot be checked in the case of a small lens camera without additional observation oculars and also for that reason they cannot be corrected.

2) In the case of a longer exposure time the radiation sensitivity of the rickettsia leads to a clear diminution of its illuminating power, as also has been observed in other micro-organisms.

3) We have the impression, that the slight illuminating power of the sharp plane adds over and beyond to the extended rickettsia particles in longer exposure to the negative material and is produced as a weak corona. This phenomenon then leads to blurred contours of the fluorescing rickettsia.

Since in the case of the optical tools used by us the shorter exposure time can be possible either by the insertion of a suitable aperture of the objective or by a shorter reduction of the exposed negative surface, the only choice remains in order to compensate for a ten fold shortened exposure time with a 2 hour developing time. We suggest the suggestion to this method by Mister F. Kroll and Dr. L. Otto, Zeiss-Jena (11-12).

We found out by a series of further test exposures the most suitable proportion for our test was a shorter exposure time and finer grained film materials. We obtained the best performance with the following operation: use of Agfa-Films of 17/170 Din., exposure time of 5 minutes with an illumination criterion 500:1 (corresponds to a normal exposure of 50 minutes). Two hour development of the film with ultrafine-grained -total developer from the Agfa firm at 15°C. Normal fix and water. Remove the picture on to extra hard paper and develop with Blautal (Agfa).

With this method we attained on this basis the short exposure for the larger magnitude of R. burnetii which shows in the fluorescent microscope on a very weak light source, a picture reproduction, as we can never obtain it of the same quality with the usual common methods even after extended tests. In their structure the larger nuclei can be produced with fine detail, which is not visible by observation with the naked eye. The regular corresponding fine grey haze on the negative material is without meaning for the production of useful positives. The resulting close granulations in the method with the small variations in light intensity of microscopic pictures is accomplished in no detrimental way. The correctness, as they are perceived in Fig. 1, 2, 3 and 4 must be reduced on an additional photographic supplemental magnification caused by the minuteness of the R. burnetii. It has not been estimated as a deficiency of the adsorptive technique itself.

After our experience the short exposure with longer develop-

ping time produced a useful method for photographic representation in fluorescent microscopy.

SUMMARY

With the help of the fluorescent microscope the Rickettsia burnetii can be produced by different materials (Antigen, vitelline sac, testicle smears). Auramin 1:500 is especially suited as a fluorochrome. The microscopic fluorescent picture can be best obtained with $W/17^\circ$ with short exposure time (5 minutes) and long development time for illustrations.

The med.-techn. assistant Frl. E. Hartmann-Heyn has afforded us valuable help with the making and photographic production of the preparation.

FIGURE CLARIFICATION

The figures (strain "Grita") show in the figure criterion of 900:1 and of a remagnification of 2000:1 in:

Figures 1 and 2. Smear of infected 8 day pre-incubated fowl eggs, 5-6 daily vitelline sac material.

Fig. 3 and 4. Test spot preparation of guinea pig abouton the fifth day of fever. Rickettsia near and between the histiozyt nucleus.

Fig. 5. Series of rickettsia suspensions as antigen for the complement fixation reaction.

Fig. 6. Bright field absorption of a test spot preparation. Histiozyt vacuolized with intracellular rickettsia in chains or thread arrangement (vacuole cell according to Herzberg). The nucleus is deformed sickle shaped and is pressed in the cell periphery.

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H. A. Spröbzig, H. A.
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Bd. 5, S. 973 (Jahrgang
1952). — 12. Otto
von Guericke-Verlag,
Mgdeburg.

Hygiene-Institut,
Mgdeburg.

Abbildungsmaßstab:

Untermaxillatorial,
etwa am 5. Fieber-
tag Komplement-
fixiert, vakuolisiert
und (Vakuolenzellen)
die Zellperipherie



GRAPHIC NOT REPRODUCIBLE

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