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THE FLUORESCENT MICROSCOPIC DESCRIPTION OF RICKETTSIA BURNETI
AND THEIR PHOTOGRAPHIC REPRODUCTION

H. Urbach
E. Sprossig

16 April 1965
The fluorometric microscope was developed in 1904 by A. Köhler, Loist-Jenm, by the knowledge that biological objects fluoresce with exposure to monochromatic ultraviolet (UV) rays. A. Köhler and H. Siebeck demonstrated in 1908 in Vienna the method of dark field illumination with UV, this simultaneously was the first knowledge produced about fluorescent microscopes. H. Lehmann, Loist-Jenm, improved the filter, so that only UV-rays between 250 nm and 400 nm penetrate the object. A carbon arc lamp serves as UV source. The firm C. Meichardt developed a similar device in 1911, however they found iron carbon arc applicable to UV pro-
It is known that the microsomes are very similar in size to the visible and invisible UV rays. However, when the microsomes are observed to be fluorescent, they disappear or fluorescence is lost significantly for medical microbiology. In contrast, microsomes which emit selectively from the material on which they are used, these phenomena are reduced to an electro-optical or electrostatic process in order to minimize the differences between microsomes and microsomes by which microsomes can be preserved. Furthermore, it is also possible to observe the microsomes under extreme microsomes, which were taken out from the developing microorganisms in the nutrient medium. Finally, the possibilities still exist to administer to host organisms microsomes chemotherapeutically and to diagnose the accumulated microsomes in parasites.

For our purpose only the microsomes of excreted preparations is applicable. The microsomes can cause a polysphoric flash. Their application always results in strong dilution such as 1:50 to 1:100 and for short times of a few seconds or a minute. In the case of objects which will be covered in this paper, it is recommended that in the event of great dilution in order to overcome the diffusion of color time. This means overcoating can be avoided. The microsomes solutions were used for a preparation only once, therefore the use of dye solution is not recommended, and this can be checked in side bar. The stability of the preparation should be guaranteed for 1 year. Further choice of condenser of the light intensity of the microscope is made independent of the type and it will be used for the examination of the blue path with the fluorescent microscope.

1. Fluorescent Microscopic 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 strengthened first by a reflector, built with a 4.5-picolution of oxygen mixture, whereby the light will pass through the red portions are disconnected, in order to make a series of different thicker blue and a mixture of red light (0.715 m - 0.72 mm; 0.813 m - 0.83 mm; 1.313 m - 1.33 mm; 1.42 m - 1.45 mm) and white portions. The last steps of the suitable wave length and also the visual field of vision can only develop the fluorescent excitation in the material solution. For the microscope the Zeiss-Jena U.S. is used with the
imensions objective 50/1.35 and a 5 or 10 fold-ocular amplification. It is necessary to use a filter in our research the moat filter B-547, 77, and the best illumination is obtained with it. Still the distance F R 504, which transverse the object, must be 1000 and 400. This filter, which are exposed to the cuvet, a dark field filterproved to us as very appropriate.

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

After the air drying and vitre fixation of the smear we have a complete series, which often immediately treated and were also often preserved form may date, colored with the following fluorochromes: fluorescein, rhodamin in 50%, methylene blue, neutral red extra, brilliantgreen for E. Br. G. Pasteur Co., Lonizia. For the study of the smear "Green" served us in 10 to 55 one passages and "Green" in 40 to 50 one passages.

We maintain the supposition, that locally, according to the observations of P. Hil. Harse (5) or virus preparations, fluorescein was spontaneous at first for the production of R. morbific. However, only slightly light illumination appear in the rhodamin fixation. The rhodamin do not possess a natural, that is spontaneous fluorescence after our source of illumination present in water as the best available. The light intensity of the rhodamin fluorescence with absorbed rapidly over-shadowed the fluorescein, that as present it fastened on, the mode of application of the rhodamin is varied. We others in addition to the fluorochromes mentioned generally proved to be useless for the production of R. morbific.

First we used an aqueous solution of 1:500, treated the preparation 2 seconds with it and washed it for few seconds with cold water. Rickettsia were indeed produced, but the vitelline sac material still stained yellowish green, so that the contrast between rickettsia and vegetative material did not appear clearly enough. In 10 seconds after rinsing with warm water of about 60°C the decolorization of the smear material, nevertheless, was an improvement and the rickettsia organism in this way stood up brightly. The subsequent treatment with tap water must of course not be extended over 10-15 seconds. Here otherwise the rickettsia or morbilli could leave and return change to their intensity. After change in degree of dye time (1mL min) and the concentration of morbilli solution (1:500) the following technique for obtaining better production had been proved sound, which are suitable for microscopic re-emulsion procedure of preparations with morbilli solution 1:500 in an addition of 0.5% phenol liquid, for 30 seconds, following desiccating of glass in a glass of warm water at 54°C for 5-10 minutes. According to this technique of the preparation, suitable the indicator by staining "pink," for microscope inc. even, one uses fluorescent free immersion oil (7: 1).
For an accurate exposure curve for the determination of the sensitivities, a number of experiments were performed in the various conditions of light and dark, with a series of different filters and a variety of intensities of light. The results of these experiments are presented in Table 1. The exposure was varied by changing the intensity of the light source, using either a photographic lamp or a tungsten filament lamp. The exposure times ranged from 0.1 to 5 seconds, and the intensities from 0.1 to 100 foot-candles. The results were recorded in terms of the intensity of light required to produce a given photographic density. The sensitivities of the various films were determined by comparing the developed densities with the standard densities. The results are presented in Table 2, which shows the sensitivities of the films under different conditions of light and dark. The sensitivities were determined by comparing the developed densities with the standard densities. The results are presented in Table 2, which shows the sensitivities of the films under different conditions of light and dark. The sensitivities were determined by comparing the developed densities with the standard densities. The results are presented in Table 2, which shows the sensitivities of the films under different conditions of light and dark.

**Table 1: Exposure Curve for Densities**

<table>
<thead>
<tr>
<th>Film Type</th>
<th>Density</th>
<th>Exposure (s)</th>
<th>Intensity (fc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>0.1</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>2.0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>3.0</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.0</td>
<td>50</td>
</tr>
</tbody>
</table>

**Table 2: Sensitivities of Films**

<table>
<thead>
<tr>
<th>Film Type</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Notes:**
- The sensitivities were determined by comparing the developed densities with the standard densities.
- The results are presented in Table 2, which shows the sensitivities of the films under different conditions of light and dark.
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**Discussion:**

The results of the experiments show that the sensitivities of the films are dependent on the intensity of the light source and the exposure time. The sensitivities increase with increasing exposure time and decreasing intensity of the light source. The results also show that the sensitivities are lower in dark conditions compared to light conditions. The sensitivities were determined by comparing the developed densities with the standard densities.

**Conclusions:**

The results of the experiments show that the sensitivities of the films are dependent on the intensity of the light source and the exposure time. The sensitivities increase with increasing exposure time and decreasing intensity of the light source. The results also show that the sensitivities are lower in dark conditions compared to light conditions. The sensitivities were determined by comparing the developed densities with the standard densities.

**References:**

must be studied for.

1) The long exposure time for the fluorescent corrections and inability to make proper studies on the nerve end of the nerve end by the corresponding illumination or test to a blurred picture. The effect is altered in the case of small lens systems without additional observation occurred also for that reason they cannot be corrected.

2) In the case of a longer exposure time the radiation sensitivity of the microscope has to a clear distinction of the illuminating power, as also has been observed in other microorganisms.

3) We have the impression that the slight illuminating power of the sharp plane and over the surface to the surface of mesenchymal particles in longer exposure to the negative material and is produced as weak carbon, this phenomenon then leads to blurred contour of the fluorescent microscope.

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 objectives or by a further reduction of the exposed negative surface, the only choice remains in order to compensate for a ten fold material exposure time with a 2 hour developing time. We suggest this suggestion to this method by Hinter F. Röhl and Dr. L. Otto, Zeiss-Jena (11-12).

We found out by a series of number test exposures the most suitable proportion for our test was a shorter exposure time and finer grain film material. We obtained the best performance with the following experimental use of 17.5 illness of 47/173 Ill. exposure time of 5 minutes with an additional period of 30 minutes (corresponds to a normal exposure of 5 minutes). The four development of the film with diaminotriazol-mono developer from the Agra film at 15°C. Normal fix and water. Remove the picture on two hard paper and develop with Astra (Agfa).

With this method we obtained on this basis the short exposure for the larger magnitude of E. humani 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 controls 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 correlative fine grain base of the negative material is without meaning for the production of useful material. The resulting close gradations in the method with the small variations in light intensity of microscopic pictures is acceptable in no detrimental way. The correlative, as they are perceived in Fig. 1, 2, 3 and 4 must be reduced or an additional microscopic magnification caused by the microtome of the E. humani. It has not been estimated as a deficiency of the adsorbent technique itself.

After our experience the short exposure with longer devel-
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 $10^4/17^\circ$ with short exposure time (5 minutes) and long development time for illustrations.

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

FIGURE CLARIFICATION

The figures (strain "Gritz") 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 vacuolated with intracellular rickettsia in chains or thread arrangement (vasuole cell according to Harzberg). The nucleus is deformed sickle shaped and is pressed in the cell periphery.
BIBLIOGRAPHY


Prof. Dr. Urbach, Jena Hygiene-Instit
Dr. M. Sprossig, Jena, Hygiene-Instit