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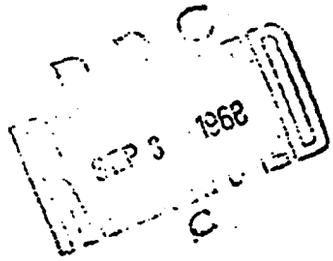

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THE FLUORESCENTMICROSCOPIC DESCRIPTION OF RICKETTSIA BURNETI AND THEIR PHOTOGRAPHIC REPRODUCTION

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16 April 1965

THE FLUORESCENT MICROSCOPIC DEMONSTRATION OF RICKETTSIA BURNETI AND OTHER PATHOGENIC MICROORGANISMS

Following is the translation of an article by H. Urbach and H. Impressin, published in Zentr. Bakt. I. Orig. 161: 39-44, 1954. Translated by S. MacDonald.

For the first time in German literature H. Herzberg (1) in 1946 and 1947 reported on the microscopic description of Rickettsia burneti (R. burneti) and by this opened an equilateral sphere of action, that by the study of these agitators 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 (3). 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 agitator producer or further treatments are necessary and so on. Especially in the production of antigen from vitelline sac cultures 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 material to the antigen. The work undergoes an objectionable interruption, 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 treatment of the same and yet guarantees a good general view in the microscope investigation. The advised requisition under consideration appears appropiate to us, for the valuation the flourescent microscopy is referred to.

The founiation for the development of the flourescent microscope took place in 1904 by A. Kohler, Zeiss-Jena, by the knowledge that biological objects flouresce with exposure to monochromatic Ultraviolet (UV) rays. A. Kohler and H. Siedentopf 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, Zeiss-Jena, improved the filter, so that only UV-rays between 320 mu and 400 mu 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-

duction. S.V. Provazek made in 1914 the first fluorescent coloring and S. Hammer [illegible] in 1936 colored animal material with fluorescent substances ([illegible]). It is the especial merit of Haitingers, [illegible] [illegible] fluorescent [illegible] (fluorochromes) have been reported on their [illegible] [illegible] [illegible] and new color effects, the so called secondary fluorescence. In 1937 [illegible] W.H. Hagemann had then referred to the fluorochromes in the production of protozoa bacteria and different virus breeds.

The principle of fluorescent microscopic [illegible] [illegible] known afterward that the fluorochrome [illegible] object [illegible] visible and invisible UV rays change to visible light, through which the fluorochromed substructure 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 deposit selectively as dye material on special elements must be used. These phenomena are reduced to an electrochemical tive or electrostatic process. In order to unite the fluorochrome with objects described, [illegible] [illegible] 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 substances 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:[illegible] and for short times of a few seconds or a minute. In the [illegible] of objects which still are not [illegible] in their behavior [illegible] 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 [illegible] for a preparation only once. Therefore the use of dye [illegible] is not recommended, the use can be [illegible] in slide [illegible]. The stability of the preparation should be guaranteed for 1 year. [illegible] [illegible] reduction of the light intensity of the [illegible] object itself [illegible] 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 [illegible] first by a cooling bulb with a 4% solution of copper sulfate, whereby the [illegible] [illegible] red portions are disconnected, in order to pass a series of different thicker blue [illegible] [illegible] filters (UG [illegible] [illegible] [illegible] 1:2 mm; UG 1:3.5 mm; [illegible] 3:4) [illegible] [illegible] [illegible] rays of the suitable wave length and also the visible [illegible] can develope the fluorescent stimulation in the [illegible] [illegible] tion. For the microscopic the [illegible] [illegible] is [illegible] [illegible] [illegible]

immersions objective 90/1.25 and a 5 or 10 fold-ocular amplification. We mostly use the violet filters in our research the Uvett filter B+ 3/4 mm, where the best illumination is obtained with it. Still the disturbing UV rays, which penetrate the object, must be absorbed through interference filters, which are erected to the ocular. 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 accomplishment of the complement fixation reaction, besides from vitelline sac cultures and in the form of testicle spot smears 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 form many days, colored withthe following fluorochromes: Primulin, Auramin, Rhodamin B 60, methylviolet extra, neutral red extra, brilliantdirekt green F or Fa. Dr. G. Grübler and Co., Leipzig. For the study of the strain "Grita" served us in 70 to 85 egg passages and "Schauben in 40 to 50 egg passages.

We maintain the supposition, that itself, according to the observations of P.H.H. Hegenkamp (5) on virus preparations, Primulin was spontaneous at first for the production of R. burnetii. However, only slightly light illuminat ons appear 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 overshadows the Primulin, that as present it depended 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. burnetii.

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 up yellowish green, so that the contrast between rickettsia and underground 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 re-production: coloration 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 5-10 seconds, according to the thickness of the preparation, decolorization the incubator by standin[illegible] right. For microscopic inspection, one uses fluorescent free immersion oil [illegible]).

[illegible] the fluorescent [illegible] of R. [illegible] is not [illegible] to [illegible]. [illegible] to this [illegible] with [illegible] film [illegible] the fluorescent [illegible], which [illegible] very useful for the valuation of rickettsia increase in the vitelline sac cultures. This process is therefore used for valuation of rickettsia content of the vitelline sac, just as this is necessary for [illegible] in the case of [illegible] production for the complement fixation reaction. With the color technique employed [illegible] which can appear as [illegible] in the [illegible], [illegible] to [illegible], they [illegible] are [illegible] as such [illegible] but correspond in tone to the background material. A possibility of [illegible] with the bright yellow shining Rickettsia is therefore not given.

II. [illegible] MICROPHOTOGRA[illegible]

[illegible] of the Fil[illegible] Dresden [illegible] available for the microscopic exposure of [illegible] fluorescent [illegible]. These [illegible] shafts of light with a [illegible] were supplied. Since [illegible] glass absorbed the small light [illegible] of the fluorescing rickettsia and for that reason brings about the reduction, a special significance belongs to this [illegible]. Our small [illegible] camera was attached thru the intermediate microscope part on [illegible] CE microscope on the straight tube of the Zeiss Jena firm. The optic system of the microscope is composed of an Apochromat objective 90/1.30 and a compensation ocular with 10 fold magnification. The same [illegible] and ocular interference filter was used for the [illegible] of the exposure as well as for the visual observation and the fluorescence-free immersion oil [illegible]= 1.520. A series of proof exposures [illegible] that an improvement of exposure conditions cannot be [illegible] by an exchange of filter [illegible]. This [illegible] camera is for the reason necessary, while the subjective observation with the eye does not always coincide with the color sensitivity of different negative materials ( ).

Further series of proof exposures served for the determination of the exposure time [illegible] of film with different sensitivity. Even with [illegible] fluorescence of rickettsia, which in subjective observation present a full picture, [illegible] the light intensity of these small micro-organisms [illegible] to attain a useful exposure time also for [illegible] negative material. So it requires for [illegible] film by [illegible]/10° Din. an exposure time of 25 minutes, [illegible] 17/10° an exposure time of 50 minutes. The fact that [illegible] in numerous details of other authors, can be traced back to the substantial strong magnification which [illegible] in its order of magnitude [illegible]. In the case of the use of the [illegible] 17/10° Din. as it is applied in [illegible] in the bright field microscope (see fig. 6) [illegible] of exposure corresponding to about 2[illegible] minutes. An exposure time as short as possible for fluorescent exposure on the basis

must be strived for.

1). The long exposure time [illegible] numerous corrections and finally an [illegible] of [illegible] [illegible] the [illegible] and thereby by the corresponding vibration leads by necessity to a blurred picture. This 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 further 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 support 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-F-films of 17/17° Din., exposure time of 5 minutes with an illustration criterion 900:1 (corresponds to a normal exposure of 50 minutes). Two hour development of the film with ultrafinegrained [illegible] developer from the Agfa firm at 13°C. Normal fix and water. Remove the picture on to extra hard paper and develop with Eukutol (Agfa).

With this method we attained on this basis the short exposure for the larger magnitude of R. burneti 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 gradations in the method with the small variations in light intensity of microscopic pictures is accomplished in no detrimental way. The coarseness, 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. burneti. It has not been estimated as a difficiency of the adsorptive technique itself.

After our experience the short exposure with longer develo-

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

SUMMARY

With the help of the fluorescent microscope the rickettsia burneti 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 17/17° 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:

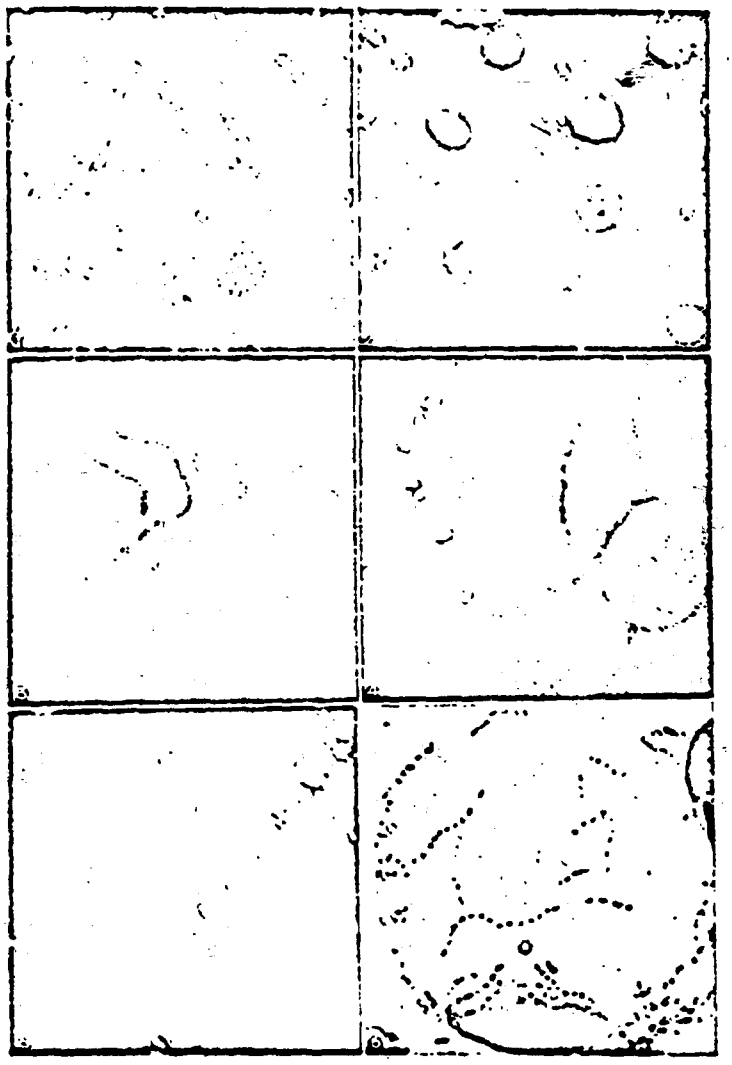
Figures 1 and 2. Smear of infected 8 day pre-incubated fowl eggs, 5-6 daily vitelline sac material.

Fig. 3 and 4. Test spot preparation of guinea pig abouton the fifth dey of fever. Rickettsia near and between the histiocyt 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 perifery.

Urbach u. Spröglg, Fluorescenzmikroskopische Darstellung der Rickettsia burneti



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