

changes in erythrocyte K°_{LGG} occurred in this group of patients during ACTH or Cortisone therapy.

Summary and conclusions. 1. In a small control series the normal range of human plasma L-leucylglycylglycine-splitting (LGGase) activity has been established.

2. In 2 patients who responded to ACTH and Cortisone therapy, the pre-treatment LGGase activities were abnormally *high* and, concurrent with clinical remissions, plasma LGGase activities fell to normal levels only to rise again with onset of clinical relapses after cessation of therapy.

3. In 4 patients who did not respond to hormonal therapy, no change in plasma LGGase activity occurred irrespective of

whether pre-treatment levels were high or normal.

4. Plasma LGGase activity was normal in a patient with Cushing's syndrome and in a patient with Addison's disease but was significantly elevated in a second patient with Addison's disease complicated by arthritis.

5. These studies lend credence to the hypothesis that changes in plasma LGGase activity reflect changes in clinical status and are not necessarily mediated through the adrenal cortex.

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Application of the Feulgen Reaction to the Laboratory Diagnosis of Smallpox.* (17817)

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The elementary bodies of smallpox were first described by Buist, and then independently by Paschen(1). Methods for microscopical diagnosis of these bodies have been proposed by several investigators. Van Rooyen and Illingworth(2) were able to differentiate variola from varicella by staining the elementary bodies with basic fuchsin as proposed by Paschen. Herzberg(3) recommended victoria-blue for the same purpose. Recently Nagler and Rake(4) reported on

the use of electron microscopy for the diagnosis of smallpox. Phase contrast microscopy has also been suggested for this purpose (Barer)(5). None of these methods provides means of distinguishing elementary virus bodies from other small particles. Bland and Robinow(6) have shown that the elementary bodies of vaccinia, and the corresponding inclusion bodies react positively to Feulgen's method of staining desoxyribonucleoproteins. In view of the great similarity, or possible identity, of the viruses of vaccinia and variola, we applied the Feulgen reaction to the study of the variola virus. The present report deals with the use of the Feulgen reaction as a quick diagnostic test for smallpox.

Technic. Skin lesions, preferably fresh vesicles or papules are opened with a sharp instrument, laying free the base of the lesion.

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2. VanRooyen, C. E., and Illingworth, R. S., *Brit. Med. J.*, 1944, v2, 526.

3. Herzberg, K., *Klin. Wchnschr.*, 1936, v15, 1385.

4. Nagler, F. P. O., and Rake, G., *J. Bact.*, 1948, v55, 45.

5. Barer, R., *Nature*, 1948, v162, 251.

6. Bland, J. O. W., and Robinow, C. F., *J. Path. and Bact.*, 1939, v48, 381.

Scrapings of the base are then rubbed on the surface of a clean and smooth glass slide. The slide is fixed in ether-alcohol (1:1) mixture for a few minutes (in a Coplin jar), and then left to dry. The smears are stained according to Feulgen's method as described by Lillie(7), and counterstained with fast green FCF. They may be either dried in the air after having been washed in water, or else dehydrated in alcohols, cleared in xylene and mounted, if permanent preparations are required. Fast green FCF was found very useful in providing contrast to the light purple or red staining produced by the Schiff reagent. The study was performed with an ordinary laboratory microscope with a magnification of x 1000 diameters.

Results and discussion. In the scrapings of smallpox cases multiple Feulgen positive granules were found free, as well as within epithelial cells. Not all the cells contained the granules. Those which did, contained them in great numbers and density. The granules were sometimes found lying free in great numbers in the neighborhood of burst epithelial cells. The average size of the granules as determined by microphotography, was found to be about 0.3 μ . (Fig. 1). In the scrapings of varicella cases no such granules were found. Some cells contained a few minute granules, which could hardly be seen. The granules appeared smaller than those of smallpox, and were never found in great numbers or lying close to each other. As

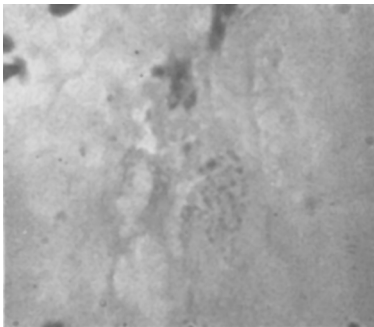


FIG. 1.
Elementary bodies of smallpox in a smear, stained with Feulgen's method. \times 1200.

was noted by van Rooyen and Illingworth the elementary bodies of varicella are relatively scanty and hard to detect.

The microscopical appearance of the elementary bodies described above tallies with the description given by previous authors in all but one respect:

Our study of variola scrapings suggests that the elementary bodies are surrounded by a thin clear halo. The halo is reminiscent of that found around Guarnieri bodies in the cytoplasm of cells. It is not clear whether the halo is real, or a diffraction effect. Its possible relationship to the halo found around the Guarnieri bodies warrants further investigation.

The method has been tested so far in the following: 1. Two clinically certain cases of smallpox, whose diagnosis has been corroborated by various laboratory methods. In both these cases a positive result was obtained with the Feulgen procedure. 2. Nine cases in which there had been close contact with proved smallpox cases, and in which a mild disease, clinically similar to, but not identical with chickenpox had been observed. Eight of these gave a positive test for smallpox with the Feulgen procedure. The diagnosis of smallpox has been later confirmed by other laboratory methods (chorio-allantois cultures, and in some cases,—Paul's test). 3. Twenty-one clinically certain cases of chickenpox. Nineteen of these could be diagnosed with the Feulgen procedure as "smallpox negative". Two were doubtful. In 14 of the cases typical elementary bodies of chickenpox were found.

Comment. The Feulgen method affords means of visualising smallpox elementary bodies. The method has a distinct advantage over other methods of staining: It allows a clear differentiation of virus particles from dirt and extraneous material. Only virus particles and nuclear chromatin of cells are stained, while dirt, and cytoplasmic matter, which do not contain desoxyribonucleic acid give no positive reaction. The method allows a quick laboratory diagnosis of smallpox and does not require any complicated apparatus. The main points in the differential diagnosis of smallpox from chick-

7. Lillie, R. D., *Histopathologic Technic*, Blakiston, Philadelphia, 1948.

enpox remain those defined by previous observers: (1) Size of the elementary bodies, (2) their number inside cells and lying free, (3) their distribution. The method was found useful in the differential diagnosis of variola from varicella. Its application for establishing a positive diagnosis of chickenpox presents certain drawbacks: (a) Elementary bodies are not found in general in chickenpox vesicles 4-7 days old, and sometimes even in more recent lesions. (b) Vari-cella elementary bodies seem to lie near the limit of visibility, and their detection requires an effort on the part of the microscopist.

It should also be noted that the Guarnieri bodies in human skin and other tissues are Feulgen positive. Furthermore the Feulgen technic seems to be a method of choice for the study of Guarnieri bodies in tissue sections(8). The method was also applied with good results to smears and sections of chorio-

allantois cultures of smallpox virus. Rickettsiae could also be stained and differentiated from extraneous matter by the same technic.

Summary. A method for staining elementary bodies of smallpox is described. The method consists of the application of Feulgen's method for desoxyribonucleic acid to smears made of scrapings of the bottom of skin lesions. The procedure seems to afford the means for a quick diagnosis of smallpox, and allows differentiation of virus particles from extraneous material.

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Reversal of Aminopterin Inhibition in the Chick Embryo with Desoxyribosides.* (17818)

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Aminopterin (4 aminofolic acid, 4-aminopteroylglutamic acid) (1) is a highly toxic substance for certain microorganisms(1,2), mice (3), guinea pigs(4), chicks(5), rats(5,6), and man(7). In certain microorganisms (*e.g.*

Streptococcus faecalis) (1) and to a lesser extent in certain animals (*e.g.* mice)(3), its inhibitory effects are prevented by relatively high amounts of folic acid; for this reason, and because of its close structural similarity to the vitamin, aminopterin is considered almost universally to act *in vivo* as an antagonist to folic acid. Especially in animals, however, and at relatively high levels of administration in all organisms, it has not proved possible to counteract the toxic effects of aminopterin with folic acid(3,7), *i.e.* the antagonistic relationship is not fully competitive. If, under these circumstances, the inhibited reaction could be made non-essential by furnishing its product, alleviation of the

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