liberated from the damaged liver cells. Such progressive thrombosis assists in producing a more widespread necrosis of the partly damaged liver tissue, even involving several neighboring lobules.

75 (892)

The effect of gentian violet on protozoa and on growing adult tissue.

By JOHN W. CHURCHMAN, M.D., and D. G. RUSSELL.

[*From the Laboratory of Surgery, Yale University.*]

This study was primarily undertaken to settle two questions raised by the observations made during the last two years on the effect of gentian violet on bacteria.[1] It was noticed early in the gentian violet studies that motile organisms not killed by the stain (violet negative organisms) retained their motility even though deeply stained; and that these stained violet negative organisms when transplanted to agar slants grew equally well with the control smears of unstained bacteria. The retention of motility by the stained organisms might in these experiments be explained as a survival phenomenon; and the growth of transplants made from the stained specimens might be regarded as arising, not from the organisms in the smear which had taken the stain, but from the few in the smear which had escaped it. It seemed altogether likely, from other observations that these explanations were not the correct ones; and that the violet negative organisms actually took the stain during life. Still, definite proof was lacking that gentian violet in these experiments was acting as a true intra-vital stain. To furnish this proof and to investigate the further problem (raised but not solved by the experiments with bacteria) as to whether the vital dye stained the nucleus or the protoplasm, two series of experiments have been done; one with a protozoon (paramecium) and another with living tissues.

EFFECT OF GENTIAN VIOLET ON PROTOZOA.

The paramecium used for this purpose came from a pedigreed race kindly furnished by Professor Woodruff. The effect of the

[1] Churchman, *Journ. Exp. Med.*, Vol. XVI, No. 2, 1912; Vol. XVI, No. 6, 1912; Vol. XVII, No. 4, 1913.

dye was investigated in two ways: 1st, by applying it directly to the organism and transplanting to another media and, 2d, by growing the organism in media containing the dye. In the first series of experiments the paramecium was found to be readily stained and promptly killed. When placed in media containing gentian violet, the effect of the dye could readily be studied on a single organism placed in a watch glass for this purpose. To the fluid in which the organism was swimming gentian violet in a dilution of 1 to 100,000 was added. The effect of the dye was sharp and constant. The nucleus soon became distinctly stained and the cell outline sharply marked out by the staining of the protoplasm. This took place while active motility of the organism was still retained (both rotatory and progressive) and while the cilia were still whipping violently. Before long, however, motility diminished and finally came to a standstill. The cilia continued for some time to wave. After a further interval the movement of the cilia stopped and the organism gradually swelled. Then the cell membrane ruptured, allowing the protoplasm to escape; and the organism either appeared as a deeply stained, motionless and structureless mass, or else persisted only as unrecognizable debris. The effect of the dye was, of course, varied by varying the dilution used. In strengths of 1 to 100,000, cessation of motility occurred in a few moments. In 1 to 500,000, some of the organisms were still motile 48 hours after immersion, but none of them survived. In strengths of 1 to 1,000,000, fission was always delayed and usually prevented, though some of the organisms reproduced slowly, and one which was thought to be faintly stained was seen in the dividing stage.

There was no question that the nucleus of the paramecium was in these experiments deeply stained, while both the organism and its cilia were still actively motile; and, if motility be regarded as a certain indication of life, the observations warrant the conclusion that gentian violet is a true, vital, nuclear stain. We were unable, however, to observe cell division in a *definitely* stained organism; and the motility in these experiments might well be interpreted as a survival phenomenon in a dead or dying organism. The nature of the experiments was, therefore, changed and the effect of the dye on growing animal tissue investigated.

EFFECT OF GENTIAN VIOLET ON GROWING ADULT TISSUE.

For this purpose transplants of pericardium from the adult frog were used; and the media employed was frog's plasma, obtained after the usual technique (Harrison, 1910). The effect of the gentian violet was studied by adding it to the plasma into which the transplants were made. In the first experiments the plasma contained gentian violet in a dilution of 1 to 2,000. Definite growth of tissue occurred in this dilution; but there seemed to be some retardation of growth, and in all subsequent experiments a weaker dilution of the dye was used (1 to 20,000). Here growth was active, keeping pace with the controls. Indeed, in some series the growth in stained media definitely outstripped the controls, and the possibility of a stimulation of tissue growth by weak solutions of the dye should be borne in mind. This growth occurred in spite of the fact that the tissue plant when placed in plasma containing dye became intensely stained.

Our impression after careful study of these growing transplants is that the cell nucleus itself is stained and that this nuclear staining is intra-vital and does not interfere with growth. This much is certain, that in those transplants resting in stained plasma the cell nuclei stand out with great clearness, a clearness by no means to be observed in the controls, and that these clearly seen nuclei appear violet. It is also certain that the cell outline, even when weak dilutions of the stain were used, was rendered very distinct; and in the experiments made with stronger dilutions the protoplasm was definitely stained.

In these observations we paid particular attention to the endothelial cells which grew out in definite sheets. Many of the specimens gave us the impression that the dye might be exercising a selective action; for when the tissue was grown in stained media the new growth took the form of a definite sheet of endothelial cells, not observed when the tissue was grown in unstained plasma. Further experiments are now under way to determine what is the explanation of this undoubted fact.

We have been able to follow the cell division of these stained cells and to observe the whole process of cell division, though the karyokinetic figures have not been clearly seen. We have also observed actively moving cilia in newly produced ciliated endo-

thelial cells from the peritoneum of the female frog, which had been lying in stained plasma for five days.

The growing cells seemed to have the power of gradually changing the stain so that its color fades. That a similar change takes place in the animal body was pointed out in an earlier communication.[1] It was there shown that large amounts of gentian violet injected into the ear vein of the rabbit soon disappeared from the blood and that the mucosa of the tongue and lips, though at first deeply stained, in a short time (about 48 hours) lost their violet color. Animals killed a few days after the injection showed no trace of the dye, nor did it appear in the urine. Attention was called in this paper to the care which must be used in speaking of the non-toxicity of this and other dyes; for, as was there suggested, the tolerance of the dye by the animal might have been due to the animal's ability to change the substance into another actually non-toxic one.

In the original communication on the selective bactericidal action of gentian violet the suggestion was made that similar studies should be carried out on the effect of the dye on growing tissue. This communication contains the first report of work undertaken in that direction, which is still being prosecuted in this laboratory. We have also under way an investigation of the effect of gentian violet and other dyes on growing embryonic tissue from the tadpole. The significance of the results thus far obtained seems to us to be:

1. The successful growth of tissue in stain-containing media suggests the possibility that stains may be found which have a selective action on tissue, similar to that possessed by gentian violet for bacteria; and that in this way pure cultures of tissue may be possible out of mixtures, just as pure cultures of bacteria may now be so obtained.

2. Certain animal tissues grow readily in gentian violet of a far stronger dilution than that necessary to kill many contaminating bacteria. In these experiments, for example, successful tissue growths were obtained when gentian violet, 1 to 20,000, was used; yet bacillus subtilis will not grow in 1 to 100,000 and grows very badly in 1 to 1,000,000. This fact may simplify the technique of tissue growth by eliminating the risk of bacterial contamination.

[1] Churchman and Herz, *Journ. Exp. Med.*, Vol. XVIII, No. 5, 1913.

3. If our impression that the nucleus of the growing cell is actually stained prove correct, the use of stains in the plasma in which tissue is grown should certainly facilitate the study of nuclear growth.

4. Certain observations made last year in this laboratory (too few to serve as more than a suggestion) seem to indicate that another dye (methylene blue) acted as a stimulant to the growth of connective tissue. This lead also should be followed out and the effect of all possible stains studied in the hope of discovering dyes which will have a sharp selective action on growing tissue.

5. The growth of animal cells in a strength of dye much more than sufficient to kill many pathogenic organisms lends encouragement to the efforts now being made in this laboratory to apply the observations on the bactericidal effect of gentian violet and allied stains to the treatment of disease.

76 (893)

On the hexosamine of chondroitin sulphuric acid.

By **P. A. LEVENE** and **F. B. LA FORGE.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*]

In a previous communication[1] the writers reached the conclusion that the nitrogenous component of chondroitin sulphuric acid was glucosamine. The conclusion was based on the analytical data of the hydrochloride of the amino sugar, and on the magnitude of its optical rotation.

However, recently it was discovered that the optical activity of the amino sugar differed considerably from that of glucosamine, if measured under very definite conditions. The conditions required are the following: low temperature of the solution, comparatively high concentration of the sugar solution, and measuring the initial rotation immediately after the solution of the sugar is accomplished. Under such conditions it was found that the specific rotation of the amino sugar of the chondroitin sulphuric acid was about 25 per cent. higher than that of glucosamine. Both

[1] *Jour. Biol. Chem.*, XV, p. 155, 1913.