THE TOXICITY OF GENTIAN VIOLET AND ITS FATE IN THE ANIMAL BODY.*

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PLATES 47 AND 48.

The observation of bactericidal properties possessed by gentian violet, and particularly of its affinity for pyogenic organisms, made it important to determine the toxicity of this substance and its fate in the animal body. A series of about seventy-five experiments has been done on dogs and rabbits with these points in view and the more important results will be reported in this communication.

INTRAVENOUS INJECTIONS.

For the study of intravenous injections of gentian violet rabbits were used. The dye in varying concentrations was injected into the ear vein. The cornea, conjunctiva, and mucous membranes of both mouth and lips immediately became a deep blue. The animals in some instances remained perfectly passive during the injection; but more often they became restive after a portion of the dye had entered the vein, their struggles suggesting that the material was irritating. This was, however, a transient manifestation, the animals becoming perfectly quiet and normal as soon as the needle was withdrawn. We were unable to discover why this symptom of irritation was present in some cases and absent in others; possibly the rate of injection was the determining factor.

The results of intravenous injections were of two kinds. In one group of animals the dye seemed to be absolutely without effect. The rabbits either remained quiet during the experiment or struggled somewhat towards its close. They usually sat about quite still for a short time after the experiment and then resumed their normal habits. Several of the animals in this group received enormous doses (ten cubic centimeters of a 1 to 200 solution), so that gentian

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violet in a dilution of about 1 to 2,500 was circulating in the vessels. Yet these animals remained alive and are still perfectly well a number of months after the experiment. The experiments proved conclusively that large amounts of gentian violet in the circulation may be borne by rabbits without any apparent harm whatever.

In another group of experiments, however, the animals died within a minute or so after the injection. In these cases the rabbits went into violent convulsions as soon as the injection was completed and died rapidly, often in opisthotonus. The autopsy in one or two cases showed acute edema of the lungs, and occasionally frothy sputum was discharged from the mouth before death. But these features were not constant; the autopsy was usually negative, aside from the staining of the tissues; and we are unable to explain death in these animals. That it was not due to the toxicity of the drug, in the ordinary sense of that word, seems likely from the rapidity with which death occurred and from the survival, without any symptoms, of a number of rabbits which received a dose as large or larger. That it was immediately dependent on the injection is equally evident. Possibly the strain on the circulation by the injection of such a large amount of fluid was one of the factors, though this would hardly account for the convulsions.

THE FATE OF THE DYE IN THE BLOOD.

The fate of the dye in the blood was studied by means of divided blood plates made from animals which had previously received intravenous injections of gentian violet. These experiments showed conclusively that blood withdrawn from animals so injected possesses the selective bactericidal power of the dye, but that this power disappears completely in about two hours. Figures 1 to 5 show the results of such an experiment. This animal received ten cubic centimeters of a 1 to 200 solution of gentian violet into the ear vein. Blood was then withdrawn from the heart immediately, and at the end of three quarters of an hour, one and three quarters hours, two and one quarter hours, and one week. With this blood, divided plates were so made that the upper half should have contained (provided the gentian violet remained unchanged in the blood) the dye in a dilution of 1 to 10,000,—a strength far in excess
of that needed for the selective bactericidal action. It will be seen that in the plate made immediately (figure 1) the usual selective action of the dye has manifested itself; and this is also true of the plate made after three quarters of an hour (figure 2), though here the reaction is not quite so sharp. In the later plates, however, the blood has lost entirely its selective bactericidal property (figures 3, 4, and 5).

It was thought that this disappearance of the dye might be due to a chemical reaction between the blood itself and the gentian violet. Two sets of experiments were done to test this point. In the first set, blood was withdrawn and gentian violet added to it in a test-tube. With this blood divided plates were made immediately, at the end of twenty-four hours, and at the end of one week. The selective power of the dye was uninfluenced by the presence of the blood, as can be seen in figures 6, 7, and 8. A similar set of experiments was also done with blood drawn into sodium oxalate, to prevent clotting, and then dialyzed against normal sodium chloride. The selective bactericidal power was still present at the end of a week (figures 9, 10, and 11). It is evident therefore that gentian violet injected intravenously into rabbits disappears from the blood in a short time, and that there is no similar loss of selective bactericidal power when the dye is simply allowed to remain in contact with blood in vitro. It is to be remembered that in speaking of the disappearance of the dye we refer to the disappearance of the selective bactericidal power, for it was by bacteriological and not by chemical tests that the presence or absence of the dye was determined.

STAINING OF THE TISSUES.

We have already referred to the fact that intravenous injection of gentian violet results in prompt and fairly deep staining of the visible mucous membranes. The dye also appears in the mucous membranes lining the gastro-intestinal tract. The visible membranes remain stained for about forty-eight hours, the stain disappearing slowly during this time. We have not followed the fate of the dye in the other organs. That the staining of the organs accounts in part for the disappearance of the dye from the circulation is obvious; the phenomenon is, however, chiefly due to rapid oxidation or reduction.
Toxicity of Gentian Violet.

We have made a number of observations on the effect of the dye when applied directly to living tissues. The first studies were made by painting the tongue of dogs and rabbits with strong solutions of the dye. Frozen sections showed that penetration had occurred through the thickness of the mucosa down to the muscularis. Similar observations were made on the bladder, the mucosa of which is readily stained by injecting the dye into that viscus through the urethra. In like manner, the synovial membranes may be deeply stained by intra-articular injections. Just how irritant the dye is cannot at present be stated. On the tongue it seems to be without irritant effect; in the bladder, in the very strong solutions used, there was a good deal of inflammatory reaction.

An attempt was made to treat a diphtheria carrier by painting the pharynx and tonsils with gentian violet. In view of the fact that the diphtheria bacillus is constantly gentian-positive\(^1\) and that the mucous membranes may be stained to their depths by painting the dye on the surface, this seemed to be a rational procedure. The results were somewhat suggestive, but an unexpected obstacle was met in the secretion of the mucous glands of the pharynx. This material lay over the surface of the pharynx in a thin slimy layer, which was renewed by fresh secretion as rapidly as it was removed, and which prevented intimate contact between dye and mucous membrane as effectually as a layer of grease would have done. The observation was instructive as indicating some of the difficulties which will have to be met in attempting to treat infections of mucous membranes by local applications. This obstacle is being borne in mind in attempts which are now being made in the New Haven Hospital to apply some of the findings as to the bactericidal properties of gentian violet to the treatment of septic arthritis.

EXPLANATION OF PLATES.

Plate 47.

Fig. 1. Divided plate made with blood withdrawn from the heart of an animal which had received 10 c.c. of a 1 to 200 solution of gentian violet intravenously.

\(^1\) In the first communication on the selective bactericidal action of gentian violet the diphtheria bacillus was reported as gentian-positive (Churchman, J. W., Jour. Exper. Med., 1912, xvi, 221). This statement was based on the study of only a few strains. It has recently been found in a study of a large number of strains that the organism is constantly gentian-positive (Guthrie, C. C., personal communication).
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

(Churchman and Herz: Toxicity of Gentian Violet.)
Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

(Churchman and Herz: Toxicity of Gentian Violet.)
Blood withdrawn and plate made immediately after injection. Organisms used were *B. coli, B. subtilis, M. aureus*, and *B. typhosus*.

Fig. 2. The same as figure 1, except that blood was withdrawn and the plate made three quarters of an hour after injection.

Fig. 3. The same as figure 1, except that blood was withdrawn and the plate made one and three quarters hours after injection.

Fig. 4. The same as figure 1, except that blood was withdrawn and the plate made two and a quarter hours after injection.

Fig. 5. The same as figure 1, except that blood was withdrawn and the plate made one week after injection.

Fig. 6. Divided plate made with gentian violet and blood mixed in a test-tube. Plate stroked immediately with *B. coli, M. aureus, B. subtilis*, and *B. typhosus*.

Plate 48.

Fig. 7. Divided plate made with blood and gentian violet which had been allowed to stand in contact in a test-tube for twenty-four hours. Note that the gentian violet is present in strong enough dilution to inhibit *B. typhosus*, though still without effect on *B. coli*, illustrating the greater sensitiveness of the former organism to the dye.

Fig. 8. Divided plate made with blood and gentian violet which had been allowed to stand in contact in a test-tube for one week.

Fig. 9. Divided plate made with blood passed into sodium oxalate and then mixed with gentian violet. Plate made and stroked immediately.

Fig. 10. Divided plate with blood passed into sodium oxalate and then allowed to stand in contact with gentian violet in a test-tube for twenty-four hours.

Fig. 11. Divided plate made with blood drawn into sodium oxalate and then allowed to stand in contact with gentian violet in a test-tube for one week.