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Effect of Rate of Injection of Alloxan on Development of Diabetes in Rabbits.* (21162)

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Alloxan has been a useful laboratory tool in the production of hyperglycemia and the study of various aspects of diabetes, hypoinsulinism and carbohydrate metabolism. Knowledge of its use has been summarized and reviewed(1). Early in the course of our observations of animals made diabetic by alloxan, we noted that the toxic dose was very close to the adequate dose for the production of diabetes and that there was considerable variation in the mortality rate and in the number of animals which developed hyperglycemia. On carefully evaluating our technic and after reviewing the available literature, it appeared that differences in the rate of injection might explain some of the variations which we obtained. Although some authors have made note of the rapidity of injection this factor had not been studied extensively. As a result the study to be reported was undertaken.

The relatively rapid destruction of alloxan in blood and the fact that clamping the pancreatic vessels for a period of 5 minutes after injection prevented the appearance of diabetes (2) suggested that the persistence of adequate blood levels is essential in producing the desired Beta cell destruction. The observation that abnormalities in carbohydrate metabolism may occur despite an absence of demonstrable histological change(3) suggested that variable numbers of cells in any islet might be affected at any one time, or that functional changes might occur in normal appearing cells.

Materials and methods. The rabbits employed in this study were housed in our animal quarters for 3 to 8 weeks before they were used. They were fed purina rabbit chow and allowed water *ad lib.* No preliminary starvation was employed. Alloxan monohydrate was injected intravenously in a 3.3 or 5% solution in distilled water made slightly acid so that the pH was approximately 4.5. Rate of injection was controlled by means of a constant-rate infusion pump. Alloxan was injected in doses of 75, 100 and 150 mg/kg into the marginal ear vein. The rates of injection fell into 3 groups. (1) less than one minute, generally 15-30 seconds, (2) 5-20 minutes, generally about 10 minutes, and (3) between 30 and 50 minutes. After 3-4 hours, the animals were allowed food and water and 10 cc of a 10 or 20% solution of glucose was injected subcutaneously at 2 hour intervals for approximately 12 hours, to prevent the early hypoglycemia. After 24-48 hours, observations including blood sugar, urine volume and glucose were made. Those animals dying before the above studies were made are classified as dead. Few animals died between 48 hours and one week after injections, most of these being diabetic. Those animals showing definite hyperglycemia and glycosuria were classified as diabetic. A group of animals which showed transient hyperglycemia or glycosuria, or had abnormal glucose tolerance tests were classified as questionably diabetic.

Results. The results are presented in Fig.

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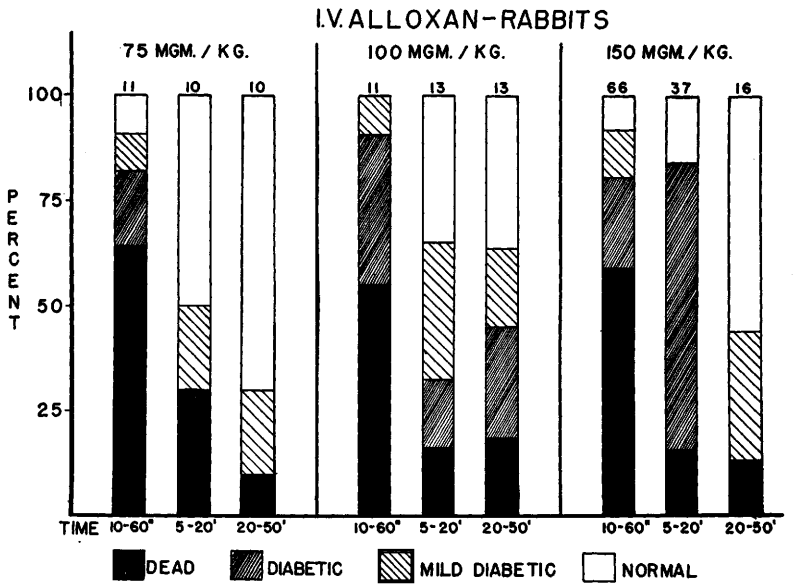

FIG. 1. Effect of rate of injection of alloxan and dose of alloxan on mortality rate and occurrence of diabetes in rabbits.

I. For each dose of alloxan the mortality rate and the number of animals with "diabetes" decreased as the rate of injection was slowed. When the alloxan in any of the doses employed was injected very rapidly, more than 50% of the animals died. When the rate was very slow, about 50% of the animals failed to show any evidence of diabetes. It would appear that a rate of injection between these extremes is most satisfactory, and when the alloxan was administered in a period of about 10 minutes, the largest number of surviving severely diabetic animals resulted.

Discussion. It would appear, from a survey of our data, that an adequate blood level of alloxan must be maintained for a certain period of time to produce regularly the desired effect on the pancreatic islets. Although we did not determine the blood levels of alloxan, it may be that the rate of destruction or inactivation of this material might differ in different individuals. Another possibility is that the blood supply to the individual islets may be intermittent, hence an adequate level of alloxan for a short time might destroy an insufficient number of Beta cells to produce diabetes.

This report was withheld for a period of almost 2 years because of the occurrence in our laboratory of a tremendous increase in mortality as a result of this treatment. We were unable to account for this by a change in diet, technic, or in the strain of animal used. For the past year, however, our results with moderately slow injection of 150 mg of alloxan per kg have again been similar to that shown in the chart. It seems obvious to us that factors other than those considered in this report must play an extremely important part in the susceptibility of the animal to this very toxic chemical. Our continued observations leave us with the distinct impression that the rate of injection as well as the dose of the drug is an important determinant in the occurrence of diabetes and survival of these animals following alloxan administration.

Conclusions. Our results indicate that the rate of injection of alloxan into rabbits is an important factor in the production of hyperglycemia and survival of these animals.

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