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Comparative Efficacy of Intravenous and Intraperitoneal Administration of Antiserum in Passive Anaphylaxis.* (26231)

E. J. COULSON AND HENRY STEVENS
Allergens Laboratory, Eastern Utilization Research and Development Division, U. S. Dept. of Agriculture, Washington, D. C.

Early workers(1) observed that the latent period in passive anaphylactic sensitization in guinea pigs varied with different routes of administration of the antiserum and was shortest following intravenous injection. For example, after intraperitoneal administration of antiserum, Doerr and Russ(2) obtained the best results by permitting 24 hours to elapse before challenging. This interval was shortened to 4 hours by injecting the antiserum intravenously. From these and similar observations, the natural deduction was made that the intravenous route of passive sensitization was more uniformly effective than other routes (3, cf4, p143). In their quantitative studies of passive anaphylaxis, Kabat and coworkers injected antiserum by the intravenous method and challenged after an interval of 48 hours. Under these conditions, they showed that about 0.03 mg of antibody nitrogen from either rabbit or guinea pig antiserum produced uniformly fatal sensitization when the animals were challenged with adequate doses of antigen (cf4, p144). However, because of its greater convenience, many contemporary investigators continue to use the intraperitoneal route for passive sensitization with the interval between injection of antiserum and challenge ranging between 24 to 48 hours(5,6,7). The present communication deals with a quantitative comparison of the effectiveness of the intravenous and intraperitoneal routes of passive sensitization when the interval between sensitization and challenge was maintained at 48 hours.

Comparison of effectiveness of the 2 routes of sensitization was accomplished by determining the median sensitizing dose (SD_{50}) of antibody nitrogen by the 2 methods and by determining the median lethal dose (LD_{50}) of antigen in animals passively sensitized with uniform doses of antibody by both routes.

Materials and methods. Rabbit antibodies to dextran were used to produce passive anaphylaxis in guinea pigs. The antiserum was produced by injecting rabbits with saline suspensions of formalin-killed *Leuconostoc mesenteroides*† (Strain NRRL B-1424(8)) that had been cultured in sucrose broth. Such antiserums give strong precipitin reactions to purified dextrans prepared from sucrose broth cultures(9). The serum was analyzed by the quantitative precipitin method of Heidelberg and Kendall (cf 4, p59) and found to contain 1.45 mg of antibody-N per ml.

For determination of the SD_{50} of antibody-N, 2 groups of 60 guinea pigs each, weighing between 280 and 320 g, were sensi-

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TABLE I. Comparison of Sensitizing Dose of Dextran Antibody after Intraperitoneal and Intravenous Administration.

Route of sensitization	Mortality ratios: Sensitizing dose in μg antibody-N 2.2	3.6	5.1	7.3	11	15
Intraper.	0/10	5/10	2/10	7/10	9/10	10/10
Intrav.	0/10	2/10	3/10	6/10	6/10	9/10

Guinea pigs challenged with 1 mg dextran intrav. 48 hr after sensitizing dose.

tized with dosage levels of antiserum ranging from 2.2 to 15 γ of antibody-N. One group was sensitized by the intraperitoneal route and the second group by the intravenous route. Forty-eight hours later, all the animals were challenged, intravenously, with 1 mg of dextran.‡

For determination of LD_{50} of dextran, 2 groups of 36 guinea pigs each were sensitized with 116 γ of antibody-N from the same antiserum. One group was sensitized by the intraperitoneal route and the second group by the intravenous route. Forty-eight hours later animals in both groups were challenged, intravenously, with dosage levels ranging from 10 to 34 γ of dextran.

Data collected for determinations of the median sensitizing and median lethal doses were used to construct dosage-response curves by the system of computation developed by Bliss and described earlier(10). SD_{50} and LD_{50} were then computed from the equations of the respective dosage-response curves.

Results. Data showing mortality ratios for the 2 groups of guinea pigs that had been passively sensitized with different doses of antidextran serum and challenged, intravenously, 48 hours later with 1 mg of dextran are recorded in Table I. With the exception of 2 animals, one in each group, all of the survivors showed slight to severe symptoms of anaphylaxis with complete recovery within a few hours. The fatal reactions terminated in death within 2 to 8 minutes. The calculated SD_{50} of antibody-N was 5.5 ± 0.6 γ for the intraperitoneal route of sensitization and 7.1 ± 0.9 γ for the intravenous route. The difference was not statistically significant ($P = 0.10$). The SD_{50} of antibody-N, calculated from the combined data, was 6.2 ± 0.6 γ.

‡ Provided by Dr. Allene Jeanes, U.S.D.A.

Table II records data collected for determination of the LD_{50} of dextran in 2 groups of guinea pigs passively sensitized 48 hours previously with antidextran serum containing 116 γ of antibody-N per animal. All of the survivors showed slight to severe symptoms of anaphylaxis and the fatal reactions terminated in death within 2½ to 5 minutes. The calculated LD_{50} of dextran for animals sensitized by the intraperitoneal route was 18.4 ± 2.1 γ, and for animals sensitized by the intravenous route the LD_{50} was 18.6 ± 1.3 γ.

Discussion. Our results show that the intraperitoneal and intravenous routes of injection of antiserum were equally effective in development of passive anaphylactic sensitization when the animals were challenged after an incubation period of 48 hours. Accordingly, the more convenient intraperitoneal method of passive sensitization described here may safely be used in preparation of uniformly sensitized guinea pigs for quantitative assay of antigens.

To compare our results with those of earlier workers, the data of Kabat and Landow (cf4, p 144) were treated by the Bliss statistical method for calculation of SD_{50} of antibody-N. The calculated SD_{50} was 13 ± 2 γ antibody-N for the antiovalbumin rabbit serum and 13 ± 3 γ antibody-N for type III antipneumococcal rabbit antibody. These results are somewhat higher than the value of 6.2 ± 0.6 γ antibody-N for the antidextran rabbit serum found in this report, but are probably within the limits to be ex-

TABLE II. Comparison of Lethal Doses of Dextran in Guinea Pigs Passively Sensitized by Intraperitoneal and Intravenous Routes.

Route of sensitization	Mortality ratios: Shock dose in μg 10	15	23	34
Intraper.	1/8	4/10	5/10	8/8
Intrav.	0/8	2/10	8/10	8/8

Animals were sensitized with dextran antiserum containing 116 γ antibody-N and challenged intrav. 48 hr later.

pected from different laboratories using animals from different colonies.

Summary. The intraperitoneal and intravenous routes of injection of antiserum were equally effective in development of passive anaphylactic sensitization when the guinea pigs were challenged after an incubation period of 48 hours.

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Protective Influence of Glucose Cycloacetoacetate on Glutathione Depletion in Dietary Liver Necrosis.* (26232)

K. VADALKAR AND M. C. NATH

Department of Biochemistry, Nagpur University, Nagpur, India

Leaf and Neuberger(1) observed that a low protein diet containing 100 mg of dry yeast per day per rat as the sole source of protein, produced an instantaneous fall in liver GSH, which after 14 days reached a constant level corresponding to 20 to 30% of the initial values. Further, this depletion of GSH was prevented by addition of cystine to the same diet. Similar results were also obtained by Lindon and Work(2) who showed that a necrogenic diet containing 18% Baker's yeast as protein source lowered liver GSH level in rats to one-fourth of its normal value in 13 days.

Recently, Nath and Behki(3) have shown that sodium salt of glucose cycloacetoacetate (GCA), a condensation product of glucose and acetoacetic ester(4) can prevent depletion of blood GSH in acetoacetate induced diabetes. The authors(5) have also observed the GSH-sparing effect of GCA in blood of scorbutic guinea pigs. It was, therefore, thought desirable to study the influence of GCA and its hydrolyzed product on depletion of GSH during feeding of necrogenic diet to rats.

Since the level of ascorbic acid in rats has also been found to be significantly depressed during development of liver necrosis (2) and since GCA has been shown to be the precursor of Vit. C in germinating legumes and in rats by Nath *et al.*(6,7), the influence of GCA and its hydrolysis product on depletion of liver ascorbic acid has also been studied. Further, in view of the protective influence of Vit. B_{12} in CCl_4 liver injury in Vit. E deficient rats(8), analogous data comprising the influence of Vit. B_{12} have been collected.

Experimental. Animals and diet: Thirty-five weanling male albino rats weighing 40-60 g were divided into 5 groups as follows: Animals in Group I were fed normal stock diet consisting of casein 12%, cane sugar 36%, arrowroot 40%, groundnut oil 6.4%, codliver oil 1.6%, salt mixture 3.5%(9), L. cystine 0.3%, choline chloride 0.2%. The following vitamins were added per kg of the

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