tested. Only 2 active compounds were found; the common sympathomimetic amines were inactive.

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Protection Afforded by Aqueous-Alcohol Solutions of Cortisone Against Dextran.* (20542)

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Attention has been called to the protection afforded by large daily doses of cortisone against the lethal anaphylactoid response of 24-hour adrenalectomized rats to intravenous infusions of the polysaccharide dextran(1). The animals were unequivocally shielded from shock but the procedure employed was slow and expensive since it required a total dosage of 60 mg of a microcrystalline suspension of cortisone (Merck's Cortone), administered over 5 days as a fore-treatment before giving the shock dose of dextran. It was considered desirable to devise a method whereby the dosage could be drastically reduced and the time necessary for adequate prophylaxis lessened without diminishing the effectiveness of the protection afforded. The following experiments demonstrate that, by using a dilute alcoholic solution of cortisone instead of the slowly absorbed microcrystalline suspension, the effective dose of the steroid which will give protection to more than 90% of animals can be reduced from 60 mg to 2 mg or less, and the time required for adequate fore-treatment shortened from 5 days to 6 hours. The simplicity of this modified procedure led the writers to adopt it as a test for screening compounds assumed to have cortisone-like activity.

Materials and methods. 138 virgin female rats averaging 133 g in weight were employed; when ready for use they were adrenalectomized and 24 hours later infused with dextran (Commercial Solvents Corp.; a sterile 6% solution in 0.9% saline); this material is used as a plasma expander for treatment of shock. The dextran was administered intravenously as a single injection, representing 7.5 mg/100 GBW (g of body weight), at the rate of one cc/minute. Rats with intact adrenals tolerate large doses of dextran by vein; however, the 24-hour adrenalectomized animal is extremely sensitive to the material and generally dies within 30 to 60 minutes after infusion(1). If the dextran is given intraperitoneally to intact rats in relatively huge doses, marked edema and erythema may appear(2,3). The striking

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TABLE I. Protection Afforded by Aqueous-Alcohol Solutions of Cortisone against Dextran.

No. of rats	Avg wt	Dosage, mg/rat	Survival No.	Survival %	No. cyanotic	No. edematous	No. erythematous	No. edematous and cyanotic	No. edematous and erythematous	No. symptom-free
A. Adrenalectomized—controls										
25	137	—	0	0	25	0	0	0	0	0
B. Adrenalectomized—experimentals										
1 34	129	10*	33	97	15	7	1	3	1	7
2 26	134	5†	25	96	14	1	4	2	4	1
3 29	124	2†	27	93	6	1	8	2	11	1
4 14	125	1†	10	71	10	0	1	2	1	0
5 10	125	0.5†	3	30	7	1	0	1	0	1

* 4 inj. (i.m.) over a 6 hr period prior to dextran infusion.
† 1 " " 6 hr prior to dextran infusion.

anaphylactogenic response of the intact rat to dextran seems to be species specific since other non-adrenalectomized mammalian types, *e.g.*, man or the dog, only occasionally exhibit mild reactions even when infused with large amounts. The free alcohol of cortisone was rendered suitable for parenteral injection by dissolving in 95% warm ethanol and then diluting dropwise with warm distilled water until the clear solution showed a faint haze. 10 mg/cc in 28-29% alcohol can be dissolved in this way. Smaller amounts such as 5, 2, 1 or 0.5 mg/cc were diluted to 10% ethanol or less. One cc was the maximum quantity of fluid given at an injection and the intramuscular route was used exclusively.

Results. The essential data obtained from the study of the soluble cortisone are shown in Table I. The controls (Series A) consisted of 25 rats adrenalectomized 24 hours previously and receiving no salt in the drinking water or prophylactic treatment. All animals succumbed when infused with 7.5 mg dextran per 100 GBW usually within the first hour following infusion. They exhibited profound cyanosis and weakness.

Thirty-three of 34 adrenalectomized rats (B-1) survived after receiving a total of 10 mg cortisone per cc of 28-29% alcohol given in divided doses over a 6-hour period previous to dextran infusion. Although symptoms, such as cyanosis, edema, and erythema, appeared, they were mild and disappeared within a very short time. Since 10 mg of cortisone in solution, administered over a 6-hour period had proven as efficacious in protecting the dextran-infused rats as had 60 mg of the microcrystalline suspension, given as a foretreatment for 5 days, it was considered worthwhile to study the effects of smaller amounts given as a single injection 6 hours before infusing the dextran.

Table I (Series B-2-5) summarizes data from experiments involving lower dosage. Twenty-six animals (B-2) received a single injection of 5 mg of cortisone in 1 cc of 10% ethanol 6 hours before infusion. Twenty-five animals survived; only mild symptoms appeared and were of brief duration. These positive results with lower dosage and but one injection led to further experimentation in

which 2 mg/cc of 10% ethanol were used for prophylaxis (B-3). Of 29 rats so treated, 27 or 93% survived in excellent condition; as in the earlier experiments, the animals displayed only evanescent signs of distress upon infusion. Another series of 14 animals was given a prophylactic dose of one mg cortisone in one cc of 10% alcohol 6 hours before administering dextran; 10 or 71% survived (B-4). A final test was made on 10 adrenalectomized rats (B-5) in which the cortisone dosage was reduced to 0.5 mg/cc in 10% alcohol and given in one injection 6 hours before the infusion. Three rats or 30% lived and these showed severe cyanosis and prostration. The low incidence of protection afforded by this dose of the steroid indicated that the *minimum* effective dose adequate for protection was one mg since this amount protected 71% of the rats.

Discussion. The data in Table I reveal the marked superiority of soluble cortisone as a protective agent over the microcrystalline suspension of the steroid now commercially available and also emphasize the waste of valuable material which evidently occurs when crystalline suspensions are used. Thus by employing a weak alcoholic solution, the effective dose required to insure adequate potection for at least 90% of the rats against dextran was approximately 30 times less than that previously found necessary with the crystalline suspension of cortisone acetate(1). The time required for prophylactic fore-treatment was reduced from 5 days to 6 hours. The best results are obtained when 5-6 hours are allowed to elapse between cortisone injection and dextran infusion. The reason for this is not clear; perhaps this interval represents the optimum time needed for cortisone to suppress or modify the action of immunological mechanisms involved in the lethal response to dextran. The deleterious action of this polysaccharide in the 24-hour adrenalectomized rat is apparently anaphylactoid in nature and essentially similar to that induced by the injection of globin(1) and egg white(4-6). The serological activity of dextrans has been commented upon(7,8). The protective action of small amounts of soluble cortisone upon the dextran-infused 24-hour adrenalectomized rat has been utilized by the writers as the basis for a simple screening test for compounds assumed to have cortisone-like action. Substances possessing small amounts of such activity afford some degree of protection.

Summary. Intramuscular injections of a soluble cortisone are much superior to the microcrystalline suspensions commercially available in affording protection to the 24-hour adrenalectomized rat infused with dextran. The amount of the soluble steroid required to give adequate protection is 30 times less than that found necessary when the suspension is used and the requisite time for prophylactic fore-treatment is reduced from 5 days to 6 hours. The procedure employed in these experiments can be utilized as the basis for a simple screening test for compounds with cortisone-like activity.

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