1948, v172, 333.

16. Tsen, C. C., Tappel, A. L. *ibid.*, 1958, v233, 1230.

17. Zalokar, M., *Arch. Biochem. Biophys.*, 1953, v44, 330.

18. Challenger, F., *Adv. Enzym.*, 1951, v12, 429.

19. Rosenfeld, I., Eppson, H. F., *Am. J. Vet. Res.*, 1957, v18, 693.

Received December 11, 1961. P.S.E.B.M., 1962, v109.

Effect of Colchicine on Antibody Response in Hamsters. (27290)

ARTHUR A. HIRATA AND MARTIN REDLICH* (Introduced by Maurice Landy)

Laboratory of Chemical Pharmacology, National Cancer Institute†, Bethesda, Md.

Several substances are known to enhance antibody response. Among them colchicine, which has been reported to enhance antibody response in the rabbit(1,2,3), is of particular interest since the action of the drug on cells has been studied extensively. Most laboratory animals are highly susceptible to the toxic effects of colchicine. The hamster, however, is distinctive in that it can tolerate this drug even in massive dosage(4,5,6). It was therefore determined whether high dosage of colchicine would provide greater adjuvant action in hamsters than in rabbits.

Material and methods. Golden hamsters, weighing 100 ± 2 g, were injected intraperitoneally with an anesthetic (0.1 ml of a 60 mg/ml solution of Nembutal) prior to immunization and to bleeding. A single dose of the antigen, 0.1 ml of 5% rabbit erythrocytes in saline, was administered to each animal *via* the great saphenous vein. Immediately thereafter the hamsters were given, intraperitoneally, colchicine‡ dissolved in distilled water in the amounts indicated in Table I. On the seventh day after administration of the antigen, the animals were bled by cardiac puncture, and the sera separated and stored at −20°C.

Hemagglutination tests were performed by serial 2-fold dilution of sera with 0.9% saline. For the direct hemagglutination tests, 0.2 ml aliquots of 2% rabbit erythrocyte suspensions were added to each reaction mixture containing 0.2 ml of diluted serum. The reaction mixtures were incubated for one hour at 37°C and degree of hemagglutination was determined microscopically. The sera were also tested by the indirect (Coombs) test. As before, 0.2 ml aliquots of 2% rabbit erythrocyte suspensions were added to each reaction mixture containing 0.2 ml of diluted serum. After incubation of the reaction mixtures for one-half hour, 0.1 ml aliquots of rabbit antiserum, prepared against hamster serum, were added to each reaction mixture and then incubated for an additional hour. All sera were heat-inactivated (one-half hour at 56°C) before use.

Results. The toxic syndrome caused by high dose (300 to 500 mg/kg body wt.) of

TABLE I. Antibody Response of Hamsters to Rabbit Erythrocytes Following Administration of Colchicine.

Colchicine dosage mg/kg	Toxicity Dead/ total	Hemagglutination titer (reciprocal) Direct Avg	Direct Range	Indirect Avg	Indirect Range
		Non-immunized controls			
30	0/5	<2	<2	<2	<2
		Immunized Animals			
None	2/10	20	4–32	1392	128–4096
.9	1/5	88	16–256	2816	1024–4096
1.7	1/5	64	64	1664	512–4096
5.1	0/5	70	32–128	563	256–1024
25	0/5	70	32–128	1178	256–4096
50	1/5	96	64–128	576	256–1024
100	1/5	46	8–128	299	128–512
300	4/7*	5	4–8	75	32–128
500	7/9	<2	<2	2	<2–4

* LD_{50} for hamsters, determined separately in 16 animals, was 300 mg/kg (7 of 16 survived for 7 days).

* Present address: Univ. of Miami, School of Medicine, Coral Gables, Fla.

† Nat. Inst. of Health, P.H.S., U. S. Dept. of H.E.W.

‡ Colchicine, USP, was obtained from S. B. Penick & Co., New York City.

the drug such as drowsiness, partial paralysis, loss of weight(6), and increased sensitivity to Nembutal(5), was confirmed. A lower dose (50 to 100 mg/kg) increased irritability and frequent scratching of the nasal region was observed. Below this dosage no gross effects of the drug were noted. Orsini and Pansky(4) reported that administration of 20 mg of colchicine/kg body wt. did not alter the mitotic index. On the other hand, Cardinali *et al.*(7) observed a moderate increase in the mitotic index of the bone marrow cells with administration of 2.4 mg of colchicine/kg body wt. There was no apparent increase in the mitotic index when dosage was increased from 2.4 to 7.2 mg/kg.

As determined by direct hemagglutination tests, enhancement of hemagglutinin response occurred when low and moderate dosages of colchicine were used. However, at high dosages the antibody response was reduced. Hemagglutinin responses were 3 to 5 times that of the controls when 0.9 to 50 mg of colchicine/kg body wt. were injected. Administration of 300 and 500 mg/kg of the drug resulted in considerable reduction of the agglutinin response.

With the Coombs test for incomplete antibody, the titers obtained did not follow the pattern obtained with the above test for complete antibody, *i.e.*, no appreciable elevation of antibody response was discernible. Here, too, administration of high doses of colchicine resulted in reduction of the antibody response.

Normal hamster serum obtained from untreated animals and from those injected with colchicine alone (30 mg/kg body wt) did not agglutinate rabbit erythrocytes, as tested by both direct and indirect (Coombs) hemagglutination tests.

Experiments were performed to determine whether humoral factors in hamster blood inactivated colchicine toxicity. An aqueous solution of colchicine (3 mg/ml) was incubated for 30 minutes at 37°C with an equal volume of heparinized normal hamster blood. Approximately 3.5 ml of the mixture, which represented 3 mg of colchicine/kg body weight (LD_{50}), was injected intraperitoneally into 8 rabbits. There was no indication that colchicine toxicity had been affected by this treatment.

Discussion. The object of the present work was to determine whether a toxic agent known to have adjuvant properties would be more effective in an animal species capable of tolerating extremely high dosages of the agent. Hamsters are considerably more resistant to the toxic effects of colchicine than other laboratory animals such as rabbits, rats, and mice. Although the drug did enhance antibody response in hamsters as it has been reported to do in rabbits, the enhancement was obtained not only with toxic doses but also in the non-toxic range (0.9 to 25 mg/kg body wt.). Increase of dosage above the LD_{50} resulted in suppression of the antibody response. It is noteworthy that in the rabbit, the highest adjuvant effect was obtained with the use of near-lethal dosage of colchicine(1,2,8). Observations made by Johnson, Gaines, and Landy(9) on the adjuvant effects of endotoxin are pertinent to this issue: 1) Enhancement of antibody response in the rabbit was associated with administration of endotoxin at obviously toxic levels. 2) Likewise, in mice, a species much less susceptible to endotoxin, the adjuvant effect was not discernible unless toxic doses were given. The mechanism by which these and other agents act as adjuvants is still unresolved. There is, however, agreement that results obtained by use of these agents may differ considerably depending on the antigen and the species of animals used. The present study indicates that toxicity, *per se*, is not likely to be a prerequisite for enhancement of antibody response.

Test for incomplete antibody failed to disclose any enhancement of antibody response. This shows that the production of incomplete antibody is not necessarily affected by colchicine treatment even when production of complete antibody is altered.

There have been previous reports that colchicine can suppress antibody response. Forman *et al.*(10) injected 1.25 mg colchicine/kg body wt., subcutaneously, in rabbits for 11 to 14 days daily. The animals were immunized on the second day of colchicine treatment. Precipitin levels were depressed in

sera obtained after colchicine treatment. Since the present experiment showed that administration of a high dose of colchicine suppresses antibody response, the reduction in antibody response reported by Forman *et al.* may have been due to the action of the large amount of colchicine accumulated over the 11- to 14-day period. Fagraeus and Gormsen (11) injected 0.5 mg of colchicine/kg body wt. into rats 3 days after immunization and noted that agglutinin response was significantly reduced. Since this is a toxic dose for the rat(12), the reduction in antibody response may, here too, have been the result of the high dosage of colchicine.

Summary. The adjuvant effect of colchicine was investigated in the hamster primarily because this species is unique in tolerating high dosage of colchicine. Administration of non-toxic doses of colchicine enhanced hemagglutinin response, but the increase in antibody response was no greater than that reported for the rabbit, a species far more susceptible to the toxicity of colchicine. The increased level of hemagglutinin was for complete antibody; levels of incomplete antibody were not elevated. Increase in the dose of colchicine to near-lethal levels resulted in considerable reduction in antibody response.

We wish to thank Miss A. Spraggins for technical assistance.

1. Tanaka, N., Coons, A. H., *J. Histochem. and Cytochem.*, 1954, v2, 460.
2. ———, *Bull. N. Y. Acad. Med.*, 1956, v32, 171.
3. Taliferro, W. H., Jarowslow, B. N., *J. Infect. Dis.*, 1960, v107, 341.
4. Orsini, W. M., Pansky, B., *Science,* 1952, v115, 88.
5. Eigsti, O. J., Dustin, P., *Colchicine,* Iowa State College Press, Ames, 1955, p179.
6. Turbyfill, C. L., Soderwall, A. L., *Science,* 1957, v126, 749.
7. Cardinali, G., Cardinali, G., Handler, A. H., Agrifoglio, M. F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 891.
8. Jarowslow, B. N., *Radiation Research Suppl.,* 1961, in press.
9. Johnson, A. G., Gaines, S., Landy, M., *J. Exp. Med.,* 1956, v103, 225.
10. Forman, C., Seifter, J., Ehrich, W. E., *J. Allergy,* 1949, v20, 273.
11. Fagraeus, A., Gormsen, H., *Acta path. microbiol. scand.,* 1953, v33, 421.
12. Poe, C. F., Johnson, C. C., *Acta Pharmacol. et Toxicol.,* 1949, v5, 110.

Received December 11, 1961. P.S.E.B.M., 1962, v109.

Production of All-dystrophic Litters of Mice by Artificial Insemination.* (27291)

H. G. WOLFE AND JANICE L. SOUTHARD (Introduced by N. Kaliss)
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Dystrophia muscularis (gene symbol *dy*) is a primary myopathy in mice(1) that occurred as a spontaneous mutation in strain 129/Re, maintained by Dr. E. S. Russell of the Roscoe B. Jackson Memorial Laboratory. It is transmitted as an autosomal recessive, and its expression is remarkably constant on a large number of genetic backgrounds(2). A characteristic feature of the disease is pronounced weakness of the hind legs, and early death.

* This study was supported by a grant to Roscoe B. Jackson Memorial Laboratory from Muscular Dystrophy Assns. of America.

It is highly desirable to have available dystrophic tissue collected at prenatal and early postnatal stages, when clinical diagnosis is not possible. It may then be possible by retrograde histological and biochemical analysis to determine the primary cause of the myopathy. The earliest possible positive identification of dystrophic mice has been approximately 2 weeks after birth. Since dystrophic males rarely breed, it is impractical to use natural mating to obtain 100% dystrophic litters; neither are there any linked genetic characters known which could assist