Relative Susceptibility of Young Mice and Hamsters to Coxsackie B-3 Virus. (26092)

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Mice and hamsters are considered to be the experimental animals of choice for study of Coxsackie viruses. Much information has been published on the effects of these viruses on suckling mice. It is well known that for most strains of Coxsackie viruses the susceptibility of the mouse is inversely related to the age of the animal. Thus for isolation of these viruses from clinical specimens it is recommended that mice be less than 24 hours old(1).

The utility of the hamster in studies on Coxsackie viruses has received little attention. The purpose of the study reported here was to compare age susceptibility of suckling hamsters with that of mice to a strain of Coxsackie Group B-3 virus.

Methods. Virus. The 4560 strain of Coxsackie B-3 virus was obtained from Dr. R. H. Heubner of the N. I. H. with a history of 9 passages in mice. A stock pool of a 10% virus suspension in physiological saline was prepared in the usual manner by injecting suckling mice intraperitoneally, harvesting and processing carcasses at the time animals were moribund. Virus was stored in a dry-ice chest. Aliquots of this pool which represent 10th serial passage virus in mice were used for all titrations in Experiment I. Another pool was prepared about a year later and this 11th passage virus was employed in all titrations in Exp. II. *Mice and hamsters.* The ICR strain of mice was used throughout. It had been previously determined that the susceptibility of these mice to the above strain of virus was about the same as that of CFW mice. Pregnant hamsters were obtained from Lakeview Hamster Colony, Newfield, N. J. and allowed to parturate in our laboratories. Ages of all suckling animals were accurate to within ± 15 hours. *Experimental procedure.* Age susceptibility of the hamsters and mice was compared by titrating stock virus in the various age groups of both species. At the time of titration animals of a given age were randomized by pooling and redistributing them back to dams. Usually at least 8 animals per dilution of virus were inoculated, but in a few titrations 2 unrandomized litters per dilution were used. Groups of hamsters and mice were injected intraperitoneally with 0.1 ml amounts of the various dilutions of virus. Titrations were scheduled so that several different age groups of each species were tested on the same day. A titration in younger animals was included each day to ascertain that maximum infectivity of the stock virus sample had been retained. Following inoculation animals were examined daily for 21 days. Criteria for infection were death or signs of neurological disturbance. Hamsters once developing signs usually succumbed. A few mice in the "older" age groups showed only mild signs followed by recovery. Titers are thus expressed as 50% infectious doses, but since most sick animals eventually succumbed, the infectious dose approximated the lethal dose.

Results. Both hamsters and mice showed essentially similar clinical responses to the infection. Few signs were noted prior to death of young animals receiving higher concentrations of virus. Young animals receiving higher dilutions of virus and older animals exhibited various manifestations of central nervous involvement including tremors, incoordination, hyperaesthesia in the early stages followed by depression, paralysis, coma and death. A few animals, especially mice, showed front leg paralysis involving one or both limbs. In hamsters the hind quarters were frequently paralyzed.

Incubation periods were shorter in the hamster. Day-old animals receiving 10% virus suspension showed first signs 24 hours following injection while day-old mice similarly inoculated appeared normal until the 3rd or 4th day after inoculation.

Table I, which presents typical titrations

TABLE I. Titration of Coxsackie B-3 Virus in Mice and Hamsters.

	Virus log dilution	−1	−2	−3	−4	−5	−6	−7	−8
Mice—	No. infected*	8	8	8	5	0			
	Incubation period (days)	4	5	7	8	—			
Hamsters—	No. infected*	8	8	8	8	8	7	4	1
	Incubation period (days)	1	2	2	3	5	7	7	8

* Eight animals were inoculated with each dilution of virus.

in each species, illustrates the differences in incubation periods. Also shown is the greater sensitivity of the hamster. The titer in the 3 day-old hamster was $10^{-7.0}$ compared to $10^{-4.1}$ obtained in the 2-day-old mouse.

Age-susceptibility comparisons are shown graphically in Fig. 1. The straight lines are drawn according to the method of least squares. Exp. I was completed about a year before Exp. II. Both experiments show the greater sensitivity of the hamster. Mice became refractory when they reached approximately one week of age. In hamsters of the same age the virus titer was still quite high. Hamsters became refractory at about 12 days.

Discussion. The indicator for infection in these studies was clinical response. It is probable that the susceptible period would be extended if subclinical infection were measured as shown in studies by Howes(2).

The strain of mice employed in these studies was possibly relatively resistant to the virus. However, in a few preliminary tests, the CFW strain of mice appeared to be of about equal susceptibility. Thus, it seems important to note that the hamster was the more susceptible species despite the fact that the strain of virus was adapted to the mouse

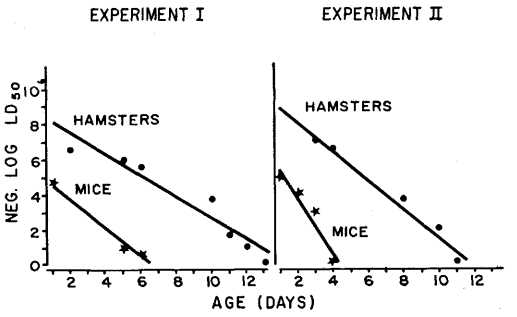

FIG. 1. Age-susceptibility of young hamsters and mice to Coxsackie B-3 virus. Points indicate infectivity titer of stock virus in the 2 species as influenced by age.

and furthermore, that the inoculum employed was grown in mice.

Although the present report considers only one type of Coxsackie virus, it suggests that similar comparisons be made with other types. In several preliminary titrations, it was noted that the hamster was more susceptible than the mouse to Coxsackie B-2 virus (Powers strain). For example, the titer in day-old mice for this virus was $10^{-6.0}$, while in 10-day-old hamsters a value of $10^{-8.3}$ was obtained.

Although tissue cultures are commonly employed by diagnostic laboratories for isolation of viruses, not all Coxsackie virsues grow in cell cultures. It thus becomes necessary to test specimens in experimental animals. Most laboratories using mice for diagnostic purposes experience difficulty in having a supply of newly-born mice on hand. If the present observations hold true for other Coxsackie viruses, the mouse problem would be obviated. Day-old hamsters could be obtained at the first of the week and used throughout the week. The increased period of susceptibility of the hamster might also be useful in another way. It has been found that hamsters injected at 5 days of age with inactivated preparations of 5 different types of Coxsackie B viruses were resistant to challenge at 10 days with virulent homologous strains but were susceptible to heterologous types. This suggests that a challenge test could be developed for typing Coxsackie viruses.

Summary. Hamsters were more susceptible than mice to a strain of Coxsackie B-3 virus. This was shown by comparing the response of these species to virus as influenced by age. At a given age a stock virus preparation showed a consistently higher titer in the hamster. Furthermore, the period of suscep-

tibility of hamsters was about twice that of mice.

1. Dalldorf, G., in *Diagnostic Procedures for Virus and Rickettsial Diseases*, 1956, Am. Public Health Assn., N. Y., p. 156.
2. Howes, D. W., *Austral. J. Exp. Biol. and Med. Sci.*, 1954, v32, 253.

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Effect of Intravenous Injection of Oxidized Methyl Esters of Unsaturated Fatty Acids on Chick Encephalomalacia.* (26093)

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Diets high in unsaturated fats and deficient in Vit. E are known to cause chick encephalomalacia(1,2,3) which can be prevented by addition to the diet of tocopherols or specific synthetic antioxidants(4,5,6) but not by selenium(7,8). As autoxidation of unsaturated fats can readily occur in an environment devoid of antioxidants, encephalomalacia may represent the consequence of consuming peroxidized fat or may represent the result of peroxidation *in vivo*. Furthermore, it has recently been observed that inclusion of various oxidized fatty acids(9) or fat(10) in the diet accelerates the incidence of the disease. The present study was designed to clarify the effect of oxidized fatty acids on development of encephalomalacia.

Methods. To compare the effect of various oxidized fatty acids on incidence of cerebellar disorders, 4 groups of 4 to 8 one-week-old male chicks (New Hampshire Columbian cross) were fed *ad libitum* for 2 weeks a purified diet(9) which contained 10% corn oil‡ and 4 groups the same diet supplemented with 8 mg % DL-α-tocopherol. The diets were prepared daily. The oxidized fatty acids consisted of the hydroperoxide of methyl linoleate(11), the reduced hydroperoxide of methyl linoleate(12), and methyl 12-oxo-*cis*-9-octadecenoate prepared from methyl ricinoleate(13). These preparations were emulsified in chick serum (10 mg/ml) at approximately 1000 rpm for 1 minute at 0°C with a Teflon pestle homogenizer and 1 ml injected intravenously. After injection, the birds were allowed access only to water. When symptoms of cerebellar disorders were observed, the birds were sacrificed for histological examination(9).

In another study, week-old chicks were divided into 12 groups of 6 to 12 birds each and fed *ad libitum* Vit. E deficient diets. These diets contained 35.30 or 11.77% soy (ADM) protein, with or without inositol, ascorbic acid and varied amounts of stripped corn oil§ substituted into the diet at the expense of glucose. The diets were prepared daily. Birds which had not shown symptoms of encephalomalacia after 1 week were injected intravenously with 10 mg of methyl linoleate hydroperoxide emulsified in 1 ml of serum. The effect of dietary pretreatment on the severity of cerebellar disorders induced by intravenous injection of the lipohydroperoxide could thus be compared.

Four groups of 16 chicks which had been kept on the high protein, Vit. E deficient diet (treatment 2) were also injected as follows: one, 1 ml of low density lipoproteins (S_f 20-400 class) obtained from chick serum(14); two, 1 ml of the low density lipoproteins which had been denatured by heating for 1 minute at 100°C; three, 1 ml emulsified methyl linoleate hydroperoxide (10 mg/ml serum); and four, 10 mg linoleic acid hydro-

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‡ Mazola, furnished through courtesy of Corn Products Refining Co., Argo, Ill.

§ Corn oil stripped of vit. E by high vacuum distillation, furnished through courtesy of Distillation Products, Inc., Rochester, N. Y.