cepted. In the light of the data presented, it is conceivable that the depressor action of thiocyanate may be mediated by this mechanism either directly or as a sequel to its conversion to cyanide. A direct effect of thiocyanate or cyanide on the vascular system, however, cannot be excluded. In either case, it is apparent that the influence of derived cyanide throughout the body as well as upon the kidney must be included in any consideration of the mechanism of action of thiocyanate in hypertensive disease.

Summary. A small but highly significant sodium diuresis accompanied short-term thiocyanate administration to uncomplicated hypertensive subjects on constant dietary regimens. Although slight but insignificant differences were observed between the mean responses of normotensives as compared to hypertensives, it was suggested that the similarity was attributable to the presence of a few hyperreactive and perhaps "pre-hypertensive" individuals in the normotensive series. The conversion of thiocyanate to cyanide *in vivo* was confirmed. Thiocyanate-induced sodium depletion was suggested as a possible factor in the depressor action of this agent, mediated either directly or through derived cyanide.

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Effect of Ether on Certain Coxsackie Viruses.* (19807)

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Soon after the discovery of the Coxsackie viruses by Dalldorf and his associates(1), numerous similarities between these agents and the poliomyelitis viruses were noted(2). It has been emphasized that both are hardy viruses, highly resistant to physical and chemical agents; a notable similarity being their resistance to diethyl ether.

In other studies(3,4) we have been concerned with the *in vitro* effect of diethyl ether on a number of viral agents. Because of the accumulating evidence that the individual members of the Coxsackie family of viruses

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TABLE I. Effect of Ether upon the Ohio-R (Group B, Type 2) Strain of Coxsackie Virus after Contact for 2 Hours at 37°C.

Dilution of virus	Control	Final % ether by volume* 5%	10%	20%	30%	50%
10^{-1}			8/8	8/8	16/16	8/8
10^{-2}		8/8†	5/6	6/8	5/16§	6/19
10^{-3}	10/10	8/8	0/6	0/8	0/10	0/16
10^{-4}	9/9	3/8	0/6	0/8	0/6	0/14
10^{-5}	5/11	0/8	0/6		0/5	
10^{-6}	3/14					
10^{-7}	1/13					
LD_{50} titer of virus	$10^{-5.2}$	$10^{-3.4}$	$10^{-2.4}$	$10^{-2.3}$	$10^{-1.7}$	$10^{-1.9}$
Virucidal capacity‡ of ether		1.8	2.8	2.9	3.5	3.3

* Virus-ether mixtures shaken slowly in 37°C water bath for 2 hr before subcut. inj. into 2-day-old mice (inoculum –.05 ml).

† No. of mice which died from C virus infection/No. inoculated (not including those which died from non-specific causes).

§ Three additional mice showed symptoms and recovered.

‡ Value obtained by subtracting log of the LD_{50} of virus in the control from the log of the LD_{50} of virus in the presence of the concentration of ether indicated.

(C viruses) differ among themselves in many respects, it seemed worthwhile to determine the effect of diethyl ether on members of this group to see whether differences might be found among the various serologic types that would allow further classification.

Materials and methods. All C virus strains used in these experiments have been maintained by passage in 2-day-old Swiss albino mice. Three group A strains, type 1 (T.T.), type 2 (Fl.) and type 3 (J.Ol.), were supplied by Dr. Gilbert Dalldorf. The group A, type 4, Dallas-M.B. strain which is serologically similar if not identical to the Texas-1 strain, was isolated in this laboratory(5). The Texas-1 strain and the 3 group B strains, type 1 (Conn.-5), type 2 (Ohio-R), and type 3 (Nancy), were provided by Dr. Joseph L. Melnick. Randomized 2-day-old white Swiss mice (CFl strain) were used throughout these experiments and the Reed-Muench method was used to calculate end-points in the virus titrations. The method of determining the virucidal capacity of ether was similar to that described previously(3). Appropriate serial dilutions of each virus were mixed with varying amounts of diethyl ether† to give total volumes of 2.0 ml per tube. The dilution of the virus and the per cent ether indicated in each experiment represent the final concentration of each in the total volume. A control series of virus dilutions without ether was included for determining the LD_{50} under the conditions of the experiment. The tubes were tightly stoppered immediately after adding the ether and were placed in a 37°C water bath or in the refrigerator together with the controls. At the end of the period of incubation during which time the tubes were inverted from time to time the ether was removed by vacuum distillation or by evaporation prior to subcutaneous inoculation into 2-day-old mice. The inoculum for each animal was 0.05 ml. Ether-treated suspensions were injected first and controls last in order to minimize rather than emphasize any *in vitro* effect of ether. The virucidal capacity of ether was determined by subtracting the log of the LD_{50} of virus in the control from the log of the LD_{50} of virus in the presence of the different concentrations of ether. This difference is actually the log of the amount of virus inactivated.

Results. The group B, type 2, Ohio-R strain was used for the initial experiments because it was found to be more labile than other C viruses(6). Also, this strain seems to differ in other respects from other C viruses (7). Ether was mixed with virus to give final concentrations of 5.0, 10.0, 20.0, 30.0 and 50.0 per cent ether, and final tenfold dilutions of virus ranging from $10^{-1.0}$ to $10^{-7.0}$ and the

† Diethyl ether, Squibb, USP, known to be free of peroxides and aldehydes, was used exclusively.

mixtures were incubated for 2 hours at 37°C. The remaining procedure was the same as described above. Animals were observed frequently during the observation period of 2 weeks for symptoms of C virus infection and death.

It is evident from the results summarized in Table I that ether even in a concentration of 5.0% had some virucidal activity, causing a reduction of 1.8 log units in the LD_{50} of this virus. Higher concentrations of ether ranging from 10.0 to 50.0% were markedly virucidal reducing the LD_{50} titer of the virus by 2.8 to 3.3 log units. It is significant, however, that the highest concentration of virus used (10.0% suspension) was still infective for all mice inoculated after contact with ether for 2 hours at 37°C. This would suggest that the large amount of tissue contained in this suspension may be a limiting factor. This has been noted previously with the St. Louis encephalitis and Eastern equine viruses(3).

There was little evidence of virucidal activity when the ether-virus mixtures were incubated for 2 hours at 4°C despite the greater solubility of ether at the lower temperature. Fifty per cent ether caused a reduction of only 0.6 log unit in the LD_{50} of this virus. However, when this concentration of ether was in contact with the virus for 18 hours at 4°C there was a 1.1 log decrease in the titer of the virus. None of the other C viruses studied, including four group A and two group B serological types, were affected by ether when tested at 37°C or 4°C.

Discussion. It is becoming increasingly apparent that the currently designated Coxsackie family of viruses is a heterogeneous group that requires further classification. The production in suckling mice of a systemic disease predominantly involving skeletal muscle is a feature which all members of this group have in common. Evidence accumulates, however, that individual members of this group differ among themselves in many respects. In addition to differing among themselves serologically(2) and in their pathogenesis in suckling mice(8,9) and in cortisone pretreated adult mice(7), differences in size(10), thermosusceptibility(11,12), effect of pH(11), and even in epidemiologic patterns(13-15) occur among the various types.

The present report calls attention to another feature by which certain C viruses might be differentiated. Of the eight strains of C viruses tested including 5 of group A representing 4 serological types and three of group B, only one strain, the group B, type 2, Ohio-R strain proved to be susceptible to ether. Concentrations of ether ranging from 10.0 to 50.0% were markedly virucidal after contact with the virus for 2 hours at 37°C. The effect of ether was considerably less when the mixtures were incubated at 4°C despite the greater solubility of the anesthetic agent at the lower temperature. This observation would place this strain of C virus, along with the influenza group, arthropod-viruses and lymphogranuloma-psittacosis group in a category of agents which are susceptible to diethyl ether. All other C viruses tested seem to fall in a category of hardy agents, along with the viruses of poliomyelitis, rabies, Col. SK, measles, foot-and-mouth disease, and others, which are notably resistant to ether(4,16).

Variations in the resistance of different strains of poliomyelitis and rabies virus have been noted(17,18). The same may be true of other group B, type 2, C viruses. Furthermore, the resistance of a given strain to the virucidal activity of ether may vary on animal passage(19,20). Rabies *virus fixé,* for example, is usually more susceptible than street virus to *in vitro* destruction by this agent. Whether or not early passages of the Ohio-R strain of C virus were less susceptible to the effects of ether could not be determined since this material was not available for study.

The Ohio-R strain was isolated originally from specimens which had been treated with penicillin-streptomycin mixtures to remove concomitant organisms. Many investigators, however, use ethyl ether and occasionally submit specimens to triple ether treatment to remove contaminating microorganisms. The type 2, group B Coxsackie virus has been isolated less frequently than other serologic types. Since clinical specimens presumably contain only small amounts of virus it is likely that ether treatment may have influenced the frequency with which this serologic type of C virus has been encountered

even though this agent is only weakly sensitive to ether at 4°C.

Summary. Of 8 strains of Coxsackie viruses tested including 5 of group A representing four serological types and 3 of group B, only one strain, the group B, type 2, Ohio-R strain proved to be susceptible to diethyl ether. In addition to serving as a means of differentiating the Ohio-R strain from other serological types of C viruses, the use of ether may be a limiting factor in the isolation of this agent.

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Interference Between Japanese B Encephalitis Virus and Western Equine Encephalomyelitis Virus in the Rat. (19808)

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It has recently been reported(1,2) that 3- to 4-week-old rats, which are highly susceptible to intranasally instilled Western equine encephalomyelitis (W.E.E.) virus, are much less susceptible if St. Louis encephalitis (S.L.E.) virus is instilled into their noses 72 hours prior to the inoculation of W.E.E. virus. The purpose of the studies reported here was to determine whether Japanese B encephalitis virus which, like S.L.E. virus, induces a fatal infection in 7-day-old but not in 21-day-old rats(3) would "interfere" with W.E.E. virus following intranasal inoculation of both viruses.

Materials and methods. Viruses and animals. The Japanese B encephalitis virus used was the Nakayama strain which had been carried through an unknown number of intracerebral passages in mice. The strain of W.E.E. virus used has already been described (1,2). The virus suspensions were prepared from virus-infected brains of 2- to 3-week-old Swiss mice. The infected brains were placed in a Waring blendor (semi-micro size jar) together with sufficient cold, inactivated, undiluted rabbit serum to make a 10% suspension of the infected brains. The blendor was

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