white domestic ducks were used. Ducks were injected twice a week for 10 weeks with 5 ml of a 10% suspension of homogenized rabbit kidney. Pooled sera had a precipitin titer of 1/16 against rabbit kidney.

Fourteen rabbits received 6.6 ml of NTS I.V., 7 were treated with N.N. dimethyl N benzyl N. (alpha pyridyl) ethylene diamine (Pyribenzamine) and 7 served as controls. Five rabbits received 6.6 ml normal duck serum. The treated group received 15 mg/kg Pyribenzamine subcutaneously every 6 hours for 4 days, followed by 10 mg/kg every 4 hours until they were killed 14 days after the initial injection.

A second series of 28 rabbits received 5 ml of NTS. Fourteen received 0.8 mg Pyribenzamine subcutaneously every 3 hours starting 2 hours before the injection of NTS. They were sacrificed at the end of 8 days.

Urine was collected and examined for protein and formed elements each day. Terminally, the kidneys were examined histologically.

Results. Heavy albuminuria, hematuria, cylindruria appeared in all rabbits receiving NTS. The onset of albuminuria was delayed 1-3 days in those receiving Pyribenzamine in both series. Histologically, severe glomerular and tubular damage was present in all animals receiving NTS. No difference in severity was noted between those treated with Pyribenzamine and the untreated controls.

These results are in contrast with those of Reubi(6,7) who reported modification of nephritis using "Antistine" 50 mg subcutaneously twice daily and 75 mg 4 times daily, with diminution of albuminuria and striking improvement histologically. He concluded that histamine or "H substance" was of primary importance in the pathogenesis of nephritis, both experimental and in man. No evidence for a role of histamine in this tissue-damaging, antigen-antibody reaction was obtained in this experiment.

Summary. Acute nephritis was produced in rabbits by the injection of duck anti-rabbit-kidney serum. Attempt to modify the reaction by the intensive administration of Pyribenzamine was ineffective as judged by the histological picture, although it was accompanied by a slight delay in the onset of albuminuria.

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Simultaneous Occurrence of Two Immunological Types of Group A "Coxsackie" Virus in a Case of Herpangina. (19053)

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(Introduced by Karl Habel.)

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We are reporting the isolation of 2 Coxsackie viruses present simultaneously in the same individual because it represents an unique situation arising during our investigations of these agents(1,2). It has occurred in only one of the 106 persons from whom we have isolated Group A Coxsackie viruses during the past 2 years.

Experimental. The 2 viruses were isolated from L.B., a 2-year-old Negro male, ill with herpangina, who was seen at the outpatient department of Children's Hospital, Washington, D.C., in August 1950. He was first seen 3 days following his onset of illness, at which time an acute stage blood specimen was drawn. A fecal specimen was obtained 4 days later and a convalescent blood specimen was drawn 15 days following the onset of illness.

1. Huebner, R. J., Armstrong, C., Beeman, E. A., and Cole, R. M., *J.A.M.A.*, 1950, v144, 609.

2. Huebner, R. J., Cole, R. M., Beeman, E. A., Bell, J. A., Peers, J. M., *J.A.M.A.*, 1951, v145, 628.

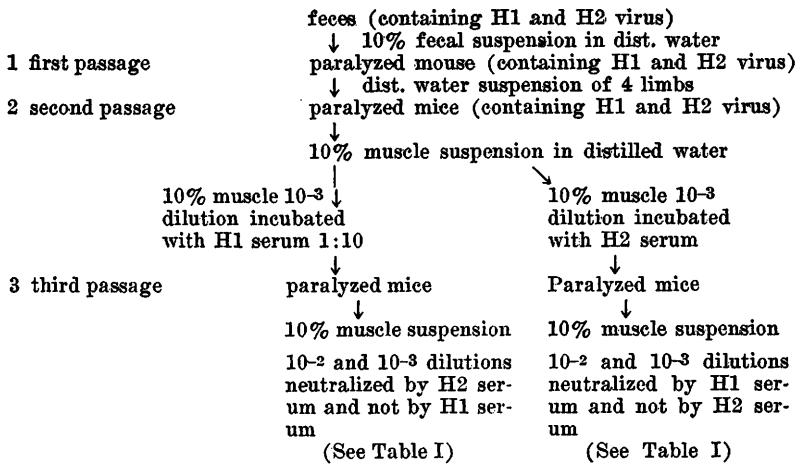

FIG. 1. Flow chart demonstrating steps in the separation of H1 and H2 viruses from the fecal specimens of patient L. B.

The detailed methods used for virus isolations, neutralization tests, and complement fixation technics will appear later(3) and only a brief description of methods will be given here. A centrifuged, ether treated, distilled water suspension of the fecal specimen inoculated into a litter of 4-day-old albino suckling mice produced the characteristic paralysis associated with the histopathological lesions of Group A Coxsackie virus infection in all the mice within 4 days. A suspension made in distilled water from the limb muscles of one of the above mice was used to infect several litters of 4-day-old mice. Following the onset of paralysis in the latter, muscle tissue was harvested from the limbs and an ether treated 10% suspension in distilled water was prepared representing second passage virus. A preliminary examination of second passage virus by complement fixation with a group of reference immune serums indicated that the suspension probably contained the H_2 immunological type of Group A Coxsackie virus(1-3). For definitive immunological classification by the neutralization technic, serial 10-fold dilutions of the second passage suspension were incubated with H1, H2, and H3 Group A serums diluted 1:10 and the mixtures were inoculated into litters of suckling mice. The mice inoculated with the virus-H_2 serum mixtures survived several days longer than mice infected with either the comparable dilutions of the virus titration, or the virus mixed with the H1 and H3 serum; however, the mice inoculated with the virus-H2 serum mixtures eventually developed paralysis and died within the test period. A repetition of the neutralization test with the same materials yielded identical results. It then seemed probable that the second passage suspension contained more than one virus. A 10^{-3} dilution of this suspension was incubated with H2 serum diluted 1:10 and the mixture was inoculated into several litters of suckling mice. Limbs were harvested from the mice that became paralyzed from this inoculum and a 10% ether treated muscle suspension was prepared. Complement fixation testing of the latter suspension with a battery of Group A reference immune serums indicated the probable presence of H1 virus.

As a check, aliquots of a 10^{-3} dilution of the second passage virus were incubated separately with H1 and H2 immune serums and several litters of mice were inoculated with each mixture. Ten per cent muscle suspensions representing third passage virus were subsequently made from the mice paralyzed as a result of inoculation with these mixtures. Both third passage virus suspensions were tested by neutralization with H1 and H2 immune serums in suckling mice and each sus-

3. Beeman, E. A., Huebner, R. J., and Cole, R. M., *Am. J. Hygiene,* in press.

TABLE I. Results of Neutralization Tests in Suckling Mice of 2 Series of Third Passage Muscle Virus Suspensions Demonstrating Separation of Viruses Originally Present in Feces of L. B.

Virus	Virus dilutions										Log serum neutralization index vs. homologous virus	Log serum neutralization index vs. heterologous virus
	H1 serum 10^{-2}	H1 serum 10^{-3}	LD_{50}†	H2 serum 10^{-2}	H2 serum 10^{-3}	LD_{50}	Virus titration 10^{-5}	Virus titration 10^{-6}	Virus titration 10^{-7}	LD_{50}		
1st series												
Third passage suspension derived from second passage virus—incubated with H1 serum	8/8*	8/8	>3.5	0/8	0/8	<1.5	7/7	8/8	5/8	>7.2	>5.7	<3.7
Third passage suspension derived from second passage virus—incubated with H2 serum	0/7	0/8	<1.5	8/8	7/7	>3.5	8/8	5/8	1/8	6.3	>4.8	<2.8
2nd series												
Third passage suspension derived from second passage virus—incubated with H1 serum	8/8	8/8	>3.5	0/8	0/8	<1.5	8/8	8/8	3/8	>6.8	>5.3	<3.3
Third passage suspension derived from second passage virus—incubated with H2 serum	1/8	0/7	1.6	8/8	8/8	>3.5	8/8	8/8	4/8	7	5.4	<3.5

Serial 10-fold dilutions of virus suspension incubated 2 hr at 37°C with equal amounts of immune serum diluted 1:10. Following incubation, mixtures placed in cold water bath at 4°C and each mixture inoculated into litter of 4-day-old suckling mice. Inoculum consisted of .04 ml of mixture given by the intraperitoneal route.

* No. died/No. inoculated.

† 50% infectious doses causing death or paralysis. Reed-Muench method.

pension showed evidence of only one virus corresponding to the type not neutralized by the immune serum incubated with the second passage virus inoculum. The separation of the viruses is shown in the flow chart in Fig. 1.

Having established the presence of 2 viruses in the second passage muscle suspension, it was of importance to determine whether accidental contamination had occurred in making the serial passage from the mouse paralyzed by the fecal inoculum. A fresh suspension made from a different portion of the original fecal specimen was inoculated into a litter of 4-day mice. When paralysis occurred a muscle suspension was made from one paralyzed mouse and was inoculated into several litters of 4-day mice. The muscle suspension from the latter, representing second passage virus was processed as above for the first series. Evidence was obtained again of two viruses, H1 and H2, by repetition of the procedures described for the first series of passages. Table I gives the results of neutralization tests performed with the third passage virus suspensions of both lines of virus derived from the same fecal specimen. The results indicate that the 2 viruses had been present in the original stool and had been separated in the 3rd passage by the reciprocal neutralization of each component in the 2nd passage suspensions with the appropriate immune serum.

An additional experiment was performed to demonstrate the presence of the H1 and H2 types in the feces. The original stool suspension and the second suspension used to recheck the results of the original were submitted to the following procedure: Aliquots of both suspensions were incubated with equal amounts of (1) normal mouse serum, (2) H1

TABLE II. Neutralization Tests* in Suckling Mice of Viruses Present in Original Fecal Suspension (10% in Distilled Water).

Fecal suspensions	Serums: Normal mouse serum	H1 serum	H2 serum	H1 and H2 serum
1st	6/6†	8/8	7/7	0/8
2nd	8/8	8/8	8/8	0/7

* See technic in Table I.

† Mice died/No. inoculated.

TABLE III. Immune Response of L. B. to Viruses Isolated From His Fecal Specimen and to Other Viruses.

Days serum obtained following onset of herpangina	Log of neutralization index of serum 1:10 against viruses isolated		Log of neutralization index of serum 1:10 against other Group A viruses	
	H1	H2	H3	H4
3	0	3.6	0	4.3
15	3.2	4.5	0	4.5

serum alone, (3) H2 serum alone, (4) H1 and H2 serum combined. The serums were used diluted 1:10. The mixtures were each inoculated into a litter of suckling mice. The results are shown in Table II. Paralysis and death occurred in all but the two litters inoculated with the fecal suspension-combined serum mixtures. It seems unlikely that the original fecal specimen could have been accidentally contaminated with another virus either in the laboratory or in the home.

Table III illustrates the serological response of the patient to the two viruses isolated from him as well as to 2 other Group A types. The results are expressed as the logarithm of the neutralization index of the serum diluted 1:10. The patient possessed neutralizing antibodies in his acute stage blood serum for the H2 virus followed by a slight rise in the convalescent serum. He possessed no detectable antibodies in the first serum against the H1 virus and his second serum showed the development of an appreciable level of antibodies against this type. It is probable that the patient's acute illness during which the fecal specimen was taken had been caused by the H1 virus. Since no history was obtained of a similar antecedent illness within one to 2 months prior to this episode, he may have been recovering from an inapparent infection produced by the H2 virus. The patient had antibodies against the H4 virus in both serums with approximately the same titer in each. This indicates prior infection with the H4 virus. It is not uncommon to find neutralizing antibodies in children against multiple types of Group A virus(3).

Discussion. In earlier reports(1,2), we have described 2 outbreaks of illness in a small Maryland community wherein 5 of the cases were infected with the Group A Coxsackie virus the first year followed by infection with a different immunologic type the succeeding year. Four of the 5 persons became ill with what is now regarded as herpangina caused by the Type 2 virus in 1949. The other was asymptomatic but harbored the virus in his feces. In 1950, 2 of the 5 had herpangina and the other 3 became infected without evidence of illness. The H3 virus was isolated from these persons at that time. In 1950 two viruses were present simultaneously within the same family group in the Washington, D.C area in different cases of herpangina(4). Based on 2 years study of isolations of Group A Coxsackie viruses from various population groups and illnesses (mainly herpangina) it is apparent that these viruses are widely prevalent during the summer and early fall months among the pediatric age group(4). There is no evidence at present to indicate that infection with one immunological type confers immunity to other known types of Group A viruses. It is therefore possible for a person to become infected in rapid succession with the virus types to which he is susceptible and to which he has had opportunity for exposure.

Summary. Two viruses occurring simultaneously were isolated from a case of herpangina. They were identified as the H1 and H2 immunologic types of Group A Coxsackie virus.

4. Cole, R. M., Bell, J. A., Beeman, E. A., and Huebner, R. J., *Am. J. Public Health,* in press.

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