

Cross-Neutralization Tests with Coxsackie Viruses. (17675)

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The variable clinical features presented by the patients admitted to the Parkland City-County Hospital at Dallas with an initial diagnosis of poliomyelitis during the 1949 epidemic suggested multiple etiologic agents. Studies were undertaken on materials from selected patients to determine whether agents other than poliomyelitis virus were causative factors in this outbreak. This report is concerned with a study of the serological relationship between 2 strains of Coxsackie virus isolated in this laboratory and those recovered by other investigators(1-4). These 2 strains of Coxsackie virus (Dallas—M.B. and Bonham—N.V.), isolated from stools of patients with paralytic infection, produced paralysis and myositis in infant mice. The patients from whom they were obtained showed residual muscular weakness four months after onset of the illness, and neutralizing antibodies for the homologous strain have been demonstrated in their sera. A significant rise in neutralizing antibody titer was also demonstrated in serum specimens from a non-paralytic patient who had been in close contact with one of these patients prior to illness. Included in this serological analysis is another strain isolated in Texas, the B.J. strain, kindly provided by Dr. J. V. Irons. The Texas 1-2 strain, recovered from flies trapped in the Rio Grande Valley in 1947, and the Connecticut No. 5 strain were provided by Dr. J. L. Melnick. Coxsackie strains T.T. (type 1), Fl. (type 2) and J.Ol. (type 3) were supplied by Dr. Gilbert Dalldorf.

Immune sera were prepared in guinea pigs for the Dallas—M.B., Connecticut No. 5 and Coxsackie T.T.(type 1) strains by intra-

peritoneal injections of 10% infected mouse brain tissue. Neutralization tests were carried out by mixing equal amounts of immune serum with the supernatant fluid from a lightly centrifuged 10% suspension of brain tissue from infected mice. The mixture was allowed to stand at room temperature for one hour prior to intra-abdominal injection into 1- to 3-day-old suckling mice. Each mouse was inoculated with 0.03 ml of the mixture and all animals were observed for a period of 12 days. The results of these neutralization tests are shown in the accompanying table.

It is evident from the results presented in this table that the 2 strains isolated in this laboratory (Dallas—M.B. and Bonham—N.V.) are serologically identical and appear to be immunologically related to the Texas 1-2 strain. The Connecticut No. 5 strain was neutralized only by the homologous anti-serum and showed no relationship to any of the other strains studied. Failure of the animals to develop paralysis, rather than survival, was used as the criterion of neutralization in the case of this strain, as many of the animals showing paralysis survived longer than the observation period of 12 days. The Connecticut No. 5 strain should probably be classified among Dalldorf's recently designated Group B strains due to its peculiar behavior in the experimental animal(5,6). The two strains recovered in this laboratory differ also from the strains received from Dalldorf as shown by the fact that type 1 serum failed to neutralize either the Dallas—M.B. or Bonham—N.V. strains, and by the fact that the serum prepared against the Dallas—M.B. strain failed to neutralize the Coxsackie types 1, 2 and 3. In a previous report, Sickles and Dalldorf(6,7) have shown that the types

1. Dalldorf, G., and Sickles, G. M., *Science*, 1948, v108, 61.

2. Dalldorf, G., and Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.*, 1949, v89, 567.

3. Melnick, J. L., Shaw, E. W., and Curnen, E. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, v71, 344.

4. Irons, J. V., personal communication.

5. Dalldorf, G., *Bull. N. Y. Acad. Med.*, in press.

6. Dalldorf, G., personal communication.

7. Sickles, G. M., and Dalldorf, G., *Proc. Soc. Exp. Biol. and Med.*, 1949, v72, 30.

TABLE I.
Cross-Neutralization Tests with Coxsackie Viruses.

Virus strain*	Controls	Immune serum		
		Dallas—M.B.	Conn. No.5	Coxsackie—T.T. (type 1)
Dallas—M.B.	2,2,2,3,3†	S,S,S,S	4,4,4,4	3,3,3,4
Bonham—N.V.	2,2,2,2,3,3,3	S,S,S,S,S	3,4,4,4	4,4,4,5,5
Texas 1-2	3,3,3,3,4,4,4	S,S,S,S	—	3,4,4,4,4
Conn. No. 5	5,5,5,7,7,7,7	5,5,5,5	S,S,S,S	3,3,3,5,5,5,5
Coxsackie—T.T. (type 1)	3,3,3,3,3,3,3	3,3,3,3	3,3,3,3	S,S,S,S,S
" —Fl. (type 2)	2,2,2,2,3	3,3,3,3,3	3,3,3,3,3	—
" —J.Ol. (type 3)	2,2,2,2,3	2,2,2,2,3	2,2,2,3,3	—
B.J.	5,5,7,7,7	7,7,7,7	5,5,7,7	—

* 10% infected mouse brain suspension.

† Figures indicate days of death or paralysis.

S—Animal survived.

— Tests not done.

1, 2 and 3 are serologically distinct. Dall-dorf(6) has further indicated that the Texas 1-2 strain of Melnick differs serologically from the strains which he has designated types 1, 2 and 3. Melnick and associates(3) have reported that the Connecticut No. 5 and Texas 1-2 strains are unrelated.

Thus it appears that at least 5 serological types of Coxsackie virus have been encountered. Whether the B.J. strain represents another serological type or is a member of the Coxsackie types 1, 2 or 3 has not been determined.

The observation that 2 of the strains (Dallas—M.B. and Bonham—N.V.) isolated in this laboratory are similar to the strain isolated by Melnick from flies collected in the Rio Grande Valley in 1947(3) and the rela-

tionship of the latter to a strain isolated from sewage collected in North Carolina(3) may have epidemiological significance. To our knowledge human infection with this type has not been previously reported.

Summary. A serological comparison of 2 virus strains recently isolated from human paralytic infections and other viruses of the Coxsackie family is presented. The results indicate that at least 5 serological types of Coxsackie virus have been encountered. To our knowledge human infection with the type isolated in this laboratory has not been previously reported.

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Complement Fixation with a Coxsackie Virus. (17676)

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The need for a rapid and simple method of diagnosis in Coxsackie virus infections prompted an investigation of the complement-fixing capacity of virus suspensions and specific immune serums. Because of the widespread occurrence of neutralizing antibodies in presumably normal persons, the neutralization test as previously described(1,2) and as

done in this laboratory may prove to be of limited value in the diagnosis of Coxsackie virus infections. In addition, the procedure is time-consuming and costly. Immune guinea

1. Dalldorf, G., Sickles, G. M., Plager, H., and Gifford, R., *J. Exp. Med.*, 1949, v89, 567.

2. Melnick, J. L., Shaw, E. W., and Curnen, E. C., *Proc. Soc. Exp. Biol. and Med.*, 1949, v71, 344.