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EXTRACTION AND PARTIAL PURIFICATION OF COXSACKIE VIRUS FROM HUMAN STOOLS WITH THE USE OF ION-EXCHANGE RESINS

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Our present methods for virus diagnosis cannot be utilized by the physician in the diagnosis of virus infection during the acute phase. Virus diagnosis now rests on the demonstration of increased antibody titer during the course of the disease and convalescence. Thus diagnosis must be made in retrospect which is of little value to the clinician. If the virus, which is known to be present during the acute stage of illness, could be extracted from the excreta and identified by means of known sera, it would place the diagnosis of certain virus infections in the category of bacteriological procedures. The purpose of this communication is to briefly describe a new laboratory tool with which to explore this phase of the diagnostic problem.

A technic for the partial purification and concentration (Ref. 1-2) of the poliomyelitis virus from central nervous system tissue using a strong base anion exchange resin (Amberlite XE-67) has been reported². However, since complement fixing antibodies have not been satisfactorily demonstrated in poliomyelitis, this virus is not suitable for preliminary investigations. On the other hand, the Coxsackie virus, an agent to be considered in the differential diagnosis of poliomyelitis infection and which is also found in stools, is known to possess complement fixing properties. The agent is more suitable for the elaboration and study of this concept of early diagnosis of virus infections. For this reason the technic developed with the poliomyelitis virus was adapted to the extraction of the Coxsackie virus excreted in stools. This procedure is particularly applicable to the extraction, partial purification and concentration of virus found in the excreta of acutely ill patients. It has two major advantages: (1) its capacity for handling large volumes of crude infected material in a relatively short period of time and (2) its relative simplicity in that it can be conducted at room temperature without the use of the ultra-centrifuge.

The amount of Coxsackie virus for these studies was limited as only one positive stool was obtained out of 40 examined during the 1952 poliomyelitis season. The material obtained from this one specimen was sufficient to develop the extraction procedure given below.

METHOD

The procedure may be described briefly in three distinct steps. These are conducted at room temperature.

Step 1 involves removal of anions and cations from a 1% fecal suspension. Several liters of material can be processed in 3-4 hours or 5 gallons can be processed un-

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attended over night. The material flows through a glass column containing a mixture of two resins: one a strong base anion exchange resin in the hydroxyl form (Amberlite* IRA-410), the other a weak acid cation exchange resin in the hydrogen form (Amberlite IRC-50). IRC-50 had to be substituted in the procedure for processing of fecal material where a strong acid cation exchange resin was used for tissue material. The effluent from this column contains the virus. It flows directly into a large vessel containing the virus-adsorbing resin Amberlite XE-67.

Step 2 consists of adsorbing and eluting the virus from Amberlite XE-67. Virus adsorption to this resin is complete in 30 minutes. The resin is then recovered and the large volume of fluid discarded. The recovered resin-virus-protein complex is then treated with a small volume of 10% disodium acid phosphate which elutes the virus from the resin complex in approximately 30 minutes. This procedure leaves the bulk (88-94%) of the extraneous nitrogenous material on the resin. The purification and concentration is obtained from this step in the process.

In step 3 the excess disodium acid phosphate is removed from the virus-phosphate eluate. This can be accomplished either by dialysis against distilled water or by the use of another resin column. The resin column technic is simpler and less time consuming.

The Coxsackie virus obtained by this procedure showed a high degree of infectivity for suckling mice. However, it was first found unsatisfactory for serological work in the complement fixation test. This was due to minute traces of phosphate which proved to be anticomplementary but were subsequently removed by more thorough dialysis against distilled water. Further studies were not possible due to the lack of clinical material. However, three additional positive specimens were isolated during the 1953 poliomyelitis season, so that these investigations can now be completed.

It remains to be demonstrated that the Coxsackie virus extracted from the stools can be used as a complement fixing antigen in a serological test for an early presumptive diagnosis of Coxsackie infection. Confirmation of the disease, however, would still rest on the increase of antibody titer during convalescence. The principal value of this work is not so much its use in the early diagnosis of Coxsackie infection but rather the fact that a new "tool" has been developed which can be utilized in the search for a practical laboratory diagnosis of virus diseases in general.

*Amberlite resins were supplied through the courtesy of Rohm & Haas Co., Philadelphia, Pa.

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