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Counter-electrophoresis as a possible method for typing ECHO and Coxsackie B viruses

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SUMMARY

A method for typing ECHO and Coxsackie B viruses using counter-electrophoresis has been developed using stock strains of 15 different ECHO types and 5 Coxsackie B viruses. Work is continuing to see whether the method is a practical one to use for typing of strains from patients infected with ECHO or Coxsackie B viruses.

INTRODUCTION

The realization that acute carditis due to enteroviruses may be difficult to differentiate from mycocardial infarction, and the possibility that enteroviruses may also be involved in chronic cardiac disease has led to an increasing demand for laboratory diagnosis of infection due to these viruses.

The usual method for typing Coxsackie and ECHO virus strains is the neutralization test, which even with a system of pools (Schmidt, Guenther & Lennette, 1961) as used by the Public Health Laboratory Service remains a tedious and time consuming procedure.

Work by Schmidt & Lennette (1962) suggests that sera prepared in animals against the enteroviruses are relatively specific when used in gel diffusion, but this method needs a very concentrated antigen.

Counter-electrophoresis has been widely used for the detection of Australia antigen in hepatitis and it is a sensitive and rapid technique. It therefore seemed worth testing antigen-antibody reactions of some of the ECHO and Coxsackie B viruses by this method.

METHODS

Viruses and antisera

Fifteen of some of the more commonly isolated ECHO types were studied. These were types 1, 2, 3, 4 (Pesacek and Dutoit) 6, 7, 9, 11, 13, 14, 15, 18, 19, 24 and 25. The Coxsackie B types 1–5 were studied. Type B6 was omitted as it occurs rarely.

The ECHO viruses and their appropriate sera were obtained from the Colindale Standards Laboratory where they had been stored frozen. The sera used were routine neutralizing sera. The Coxsackie B sera were freeze dried from the same source. The Coxsackie B3 virus was isolated in the Diagnostic Laboratory, St Mary's Hospital Medical School, while types 1, 2, 4 and 5 were stock strains

obtained from the Enterovirus Reference Laboratory of the Colindale Public Health Laboratory.

ECHO sera were stored in small volumes, undiluted and without preservative at −20° C. Coxsackie B sera were diluted immediately before use. Serum dilutions were made in phosphate buffered saline with a pH of 7·3.

Preparation of antigen

Primary rhesus monkey kidney cells were grown in 4 oz. medical flats. The growth medium was 12 ml. of MEM with 8% calf serum. In the maintenance medium for virus production the calf serum was replaced by 2% fetal calf serum.

From 0·5 to 1 ml. of a stock strain of undiluted virus was added to each bottle and the bottles were incubated at 36° C. until maximum cytopathic effect was obtained. The bottles were then frozen, thawed, treated in an ultrasonic bath for 2½ min. and the resultant suspension was spun at 3400 rev./min. in an M.S.E. Minor centrifuge for 10 min. The supernatant was concentrated 50 times in a B-15 Minicon concentrator (Amicon) and used without inactivation. Uninoculated bottles of monkey kidney cells were treated in the same way and used as controls. Each 4 oz. bottle thus produced just over 0·2 ml. of antigen, sufficient for over 20 tests.

Coxsackie B antigens were available from another investigation. Virus was grown in HEp2 cells in 12 oz. bottles and treated initially by the same method as the ECHO antigens. Concentration to approximately 50 times the strength of the original tissue fluid was effected by evaporation in visking tubing (8/32) in a litre Buchner flask containing approximately 100 ml. of water. The visking tubing was attached through a rubber bung to a filter funnel and could thus be refilled as necessary. The flask was evacuated by intermittent attachment to a Hy-flo piston pump, and the apparatus was kept at 4° C. for the 1 or 2 days required for concentration.

Prepared antigen was stored when necessary at −35° C.

Counter-electrophoresis

Electrophoresis equipment consisted of a Baird & Tatlock power pack and electrophoresis bath.

A 0·05 M sodium barbitone-HCl buffer with a pH of 8·2 was used. Nine ml. of 1% Agarose (BDH) in 0·05 M buffer was pipetted onto 3 in. × 3 in. glass slides and when set holes were punched using a punch of outside diameter 3 mm, with 6 mm. between the centres of the holes. Antigen was added to the cathode side and serum to the anode side. A fixed current of 7·5 mA. per slide was run for 3½ hr. Slides were read after approximately 2 hr., again at 3½ hr. and checked the following morning after storing at room temperature overnight. The majority of results were present at 2 hr., a few weak reactions appeared at 3½ hr. or overnight.

Table 1. *Reactions of ECHO antisera with ECHO antigens*

Antisera to ECHO type	Reciprocal of homotypic titres	Heterotypic reactions: Antigens reacting with undiluted sera	Heterotypic reactions: Antigens reacting with dilutions of 1/8
1	64	13, 15, 18, 19, 24	None
2	64	15	None
3	64	6, 7, 9, 13, 14, 15, 18, 19, 24, 25, C	None
4 (Pesacek)	64	1, 2, 14, 15	None
4 (Dutoit)	64	1, 2	None
6	32	None	None
7	16	None	None
9	128	None	None
11	128	1, 2, 3, 7, 9, 13, 18, 19, C	2
13	128	18	None
14	8	None	None
15	64	2, C	None
18	32	3, 6, 13, 24, C	None
19	128	None	None
24	64	None	None
25	64	3, 6, 13, 14, 15, 18, 19, 24, C	None

C = Monkey kidney control.

RESULTS

The ECHO sera were titrated against their own antigens and tested undiluted against the 16 other ECHO antigens and the monkey kidney control. If a positive result was obtained against a heterotypic antigen the test was repeated using serum dilutions of 1/8 and 1/32.

The antisera to Coxsackie B types 1–5 which included 2 different batches against type 4 were titrated against the 5 Coxsackie B antigens. One pool of antisera to the Coxsackie B types 1–6 was tested against the 5 Coxsackie B types and a HEp2 control, and a second pool was tested against the 5 Coxsackie B types, a HEp2 control, the 16 ECHO antigens and a monkey kidney control.

Pools of ECHO sera were tested against the 16 ECHO antigens and a monkey kidney control, and against the Coxsackie B antigens types 1–5 and HEp2 control.

For the titrations, the end-point was taken as the highest dilution showing a definite line of precipitation (Pl. 1, fig. 1). The titres of the ECHO sera to their own antigens, and the presence of positive reactions with the other ECHO antigens are shown in Table 1. All sera reacted with their own antigens.

Two types of non-specific reaction were present, the first being a line which resembled the specific reactions (Pl. 1, fig. 1). These lines were often very faint and were not always reproducible with different batches of antigen. The second (Pl. 1, fig. 2) was around the serum cup, and consisted of diffuse precipitation sometimes combined with lines of varying character. It occurred using antisera to Coxsackie B2, ECHO type 6 and ECHO type 24, with the majority of antigens against which they were tested. This reaction was easy to differentiate from the

Table 2. *Reciprocals of titres of Coxsackie type B antisera with Coxsackie type B antigens*

Antisera to Coxsackie type	Antigens					
	B1	B2	B3	B4	B5	HEp2 control
B1	8	2	—	—	—	—
B2	—	16	—	—	—	—
B3	—	—	32	—	—	—
B4A	—	—	—	4	—	—
B4B	—	—	—	32	2	—
B5	—	—	—	—	16	—

—, No reaction with undiluted serum.
B4A and B4B were different batches of antiserum to type B4.

Table 3. *Composition of pools of antisera to ECHO viruses*

Pool 1		Pool 2		Pool 3	
Serum type	Reciprocal of serum concn.	Serum type	Reciprocal of serum concn.	Serum type	Reciprocal of serum concn.
1	10	9	10	3	20
2	10	11	33	4	10
6	10	14	5	13	50
7	10	15	10	18	20
19	10	24	10	25	20

specific reactions; it was present only in sera used at low dilutions and has not therefore been included in the Table.

The homotypic titres of the antisera to the ECHO viruses varied a little with different batches of antigen, but the titres were a sufficient guide to the choice of serum dilutions which gave homotypic reactions only. In practice it was found that most sera gave good homotypic reactions at a dilution of 1/10. One (type 14) with a low homotypic titre of 1/8 was used in a pool at 1/5 and one (type 11) with a heterotypic titre of 1/8 was used in a pool at 1/33. No serum gave heterotypic reactions at 1/32.

The type 4 antiserum (Pesacek) with a homotypic titre of 1/64 reacted with the Dutoit antigen at a titre of 1/64. The antiserum type 4 (Dutoit) with a homotypic titre of 1/64 reacted with the Pesacek antigen at a titre of 1/16. The Pesacek antiserum was therefore used in the pool at a dilution of 1/10.

Antiserum to type 13 exhibited a prozone phenomenon and did not react with the homotypic antigen at a dilution of $< 1/16$. Its homotypic titre was 1/128 and at a dilution of 1/32 showed no heterotypic reactions. It was used in the pool at a dilution of 1/50.

The reactions of the Coxsackie B sera with their own antigens and the other Coxsackie B antigens are shown in Table 2. Antisera to types 2–5 had reasonable levels of homotypic antibody, and little or no heterotypic antibody. The difference between the homotypic and heterotypic titres of the antiserum to type B1 was

rather small for practical use. Both pools of Coxsackie B sera to types 1–6 reacted with the 5 Coxsackie B antigens, and were negative with the HEp2 control. The pool tested against the ECHO antigens did not react with any of them or with the monkey kidney control.

Pools of ECHO sera were made up as shown in Table 3. Pools 1 and 2 reacted only with the antigens for which they contained antisera. Pool 3 contained sera with marked or numerous non-specific reactions. When the final dilution of the antisera to types 3, 18 and 25 was 1/10, the pool reacted with ECHO types 19 and 24 in addition, suggesting that non-specific reactions might summate. When the dilutions were changed to 1/20 the non-specific lines disappeared, leaving good specific lines. None of the pools reacted with any of the coxsackie B types studied, nor with the HEp2 control.

Schmidt & Lennette (1962) state that lines produced by animal sera in precipitation tests with the Coxsackie viruses are single and specific although there are exceptions. Some of the sera in the present study did show two lines, which were not always present, probably because of dependence on relative concentration of antibody and antigen. For practical purposes, no differentiation was made between single and double lines.

DISCUSSION

This work shows that it is possible to type ECHO or Coxsackie B viruses using counter-electrophoresis, even using sera not prepared specifically for this purpose. The use of Minicon concentrators is simple and rapid, and typing of a virus suspension can be carried out in one working day. The test is thus considerably less tedious and time consuming than the neutralization test.

Work is now continuing to see whether this method is applicable to wild strains of virus, and to other members of the enterovirus group. If these react satisfactorily it should be possible by using a system of pools to screen viruses with a large number of typing sera relatively easily.

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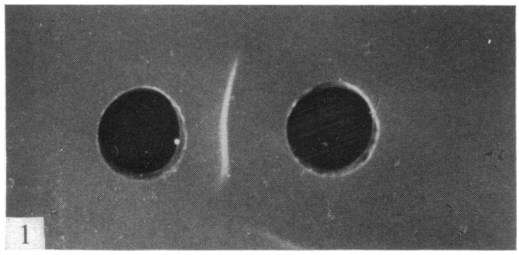
EXPLANATION OF PLATE

Fig. 1. Typical line of positive precipitation obtained on counter-electrophoresis. Antigen in left-hand well (cathode); serum in right-hand well (anode).

Fig. 2. Non-specific precipitation around serum well, present with a few concentrated sera. Antigen in left-hand well (cathode); serum in right-hand well (anode).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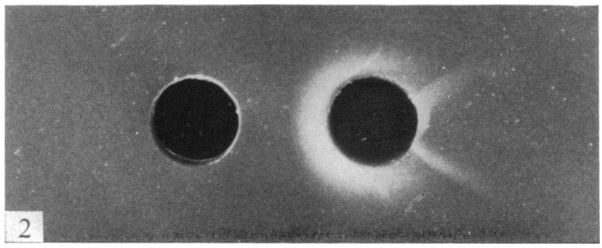
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