Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 81(4) :389-393, out./dez. 1986

A COMPARISON OF SIMIAN ROTAVIRUS SA11 PREPARATIONS MAINTAINED IN DIFFERENT LABORATORIES

H.G. PEREIRA, VERA S. GOUVEA & A.M. FIALHO

Preparations of simian rotavirus SA11 maintained in different laboratories were compared with each other by polyacrylamide gel electrophoresis of genomic RNA. Differences in the migration of genome segments 4, 5 and 7 allowed the classification of eight virus preparations into four electrophoretic types.

Key words: rotavirus – electrophoresis – variation

Heterogeneity of the SA11 strain of simian rotavirus has been demonstrated by analysis of both the viral genomic RNA segments (Sabara et al., 1982; Pereira et al., 1984; Lopez et al., 1985) and gene products (Estes et al., 1982; Lopez et al., 1985). These findings suggest that although SA11 preparations available in different laboratories are all derived from a single isolate obtained by Malherbe and co-workers (Malherbe & Harwin, 1963; Malherbe & Strickland-Cholmley, 1967) they may not be identical to each other. In the present paper we compare SA11 preparations derived from 11 separate seeds received from five independent laboratories.

MATERIAL AND METHODS

Virus preparations – The following SA11 seeds were included in this study:

1. SA11-CDC: Received from Dr. G.W. Gary, Center for Disease Control, Atlanta, GA, USA in December 1979 labelled MA104, 11/20-23/79.

2. SA11-HBG: Received from Dr. Harry B. Greenberg, Stanford University, California, USA in September 1984.

3. SA11-TA277 (7/9/79), TA460 (11/12/81) and TA581 (6/2/82) received from Dr. H. Malherbe, Gull Laboratories, Utah, USA in September 1984.

4. SA11, clones 3, 28, 37 and 43 (Estes et al., 1982) received from Dr. Mary, K. Estes, Baylor College of Medicine, Houston, Texas, USA in October 1984.

5. SA11 4S and 4F derived in this laboratory from SA11-CDC (Pereira et al., 1984).

All preparations used in the present study were propagated in this laboratory through no more than three passages in M4104 cells except the variant 4F which was cloned by limit dilution passages as described by Pereira et al. (1984). Virus purification by sucrose and caesium chloride gradient centrifugation was performed as described by Beards (1982).

Aliquots of 1 to 2μl of purified virus preparations were added to 5 to 10μl of dissociation mixture consisting of 62.5mM Tris/Hcl pH 6.8, 5M urea, 5% (v/v) 2-mercaptoethanol, 3% (w/v) sodium dodecyl sulphate and 0.01% (w/v) bromophenol blue, heated at 56°C for 15 minutes and run on 12% (w/v) acrylamide, 0.3% (w/v) bis-acrylamide separating gel overlaid by a 3% (w/v) acrylamide, 0.08% (w/v) bis-acrylamide stacking gel in Laemmli's (1979) buffers. After electrophoresis at 15 to 20mA for 14 to 18 hours, gels were stained with silver nitrate by the technique of Sammons et al. (1981) modified by Herring et al. (1982).

RESULTS

Electrophoresis of the viral genome: RNAs extracted from early passages in MA104 cells of all the seeds listed in Material and Methods were compared with each other by co-electrophoresis. A preliminary comparison of the three preparations received from Dr. H. Malherbe (TA277, TA460 and TA581), failed to reveal differences between any of the genomic segments and TA277 was taken as representative of the three and used in all subsequent comparisons under the designation of HM.

This work was supported in part by CNPq and FINEP.

Instituto Oswaldo Cruz, Departamento de Virologia, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil.

Received for publication February 14th and accepted March 14th, 1986.

The composite result of all the co-electrophoretic comparisons is shown in Table I and Fig. 1 where it is seen that the different preparations fall into four distinct electrophoretic types heareafter referred to as types I to IV. Type I included the preparations HM, HBG and CDC-4S and is presumed to represent the original wild type isolate. Type II, represented by clones 28 and 37 (M.K. Estes) differs from type I in only a minor shift of segment 5. Type III, including clones 3 and 43 (M.K. Estes) differs from type I by shifts in both segments 5 and 7. Type IV represented by the variant 4F previously described by Pereira et al. (1984) differs from type I in segments 4 and 5, from type II only in segment 4 and from type III in segments 4, 5 and 7. Differences in segments other than 4, 5 and 7 were not observed except for a slightly faster migration of segment 6 of variant 4F seen inconsistently.

TABLE I

Comparison of genome segments of different SA11 preparations by co-electrophoresis

SA11	HM	HBG	CDC 4S	C1.28	C1.37	C1.3	C1.43	CDC 4F
HM								
HBG	=							
CDC-4S	=	=						
C1.28	5	5	5					
C1.37	5	5	5	=				
C1.3	5-7	5-7	5-7	5-7	5-7			
C1.43	5-7	5-7	5-7	5-7	5-7	=		
CDC-4F	4-5	4-5	4-5	4	4	4-5-7	4-5-7	
Type		I		II		III		IV

Figures denote genome segments with different mobilities in each pair of strains.

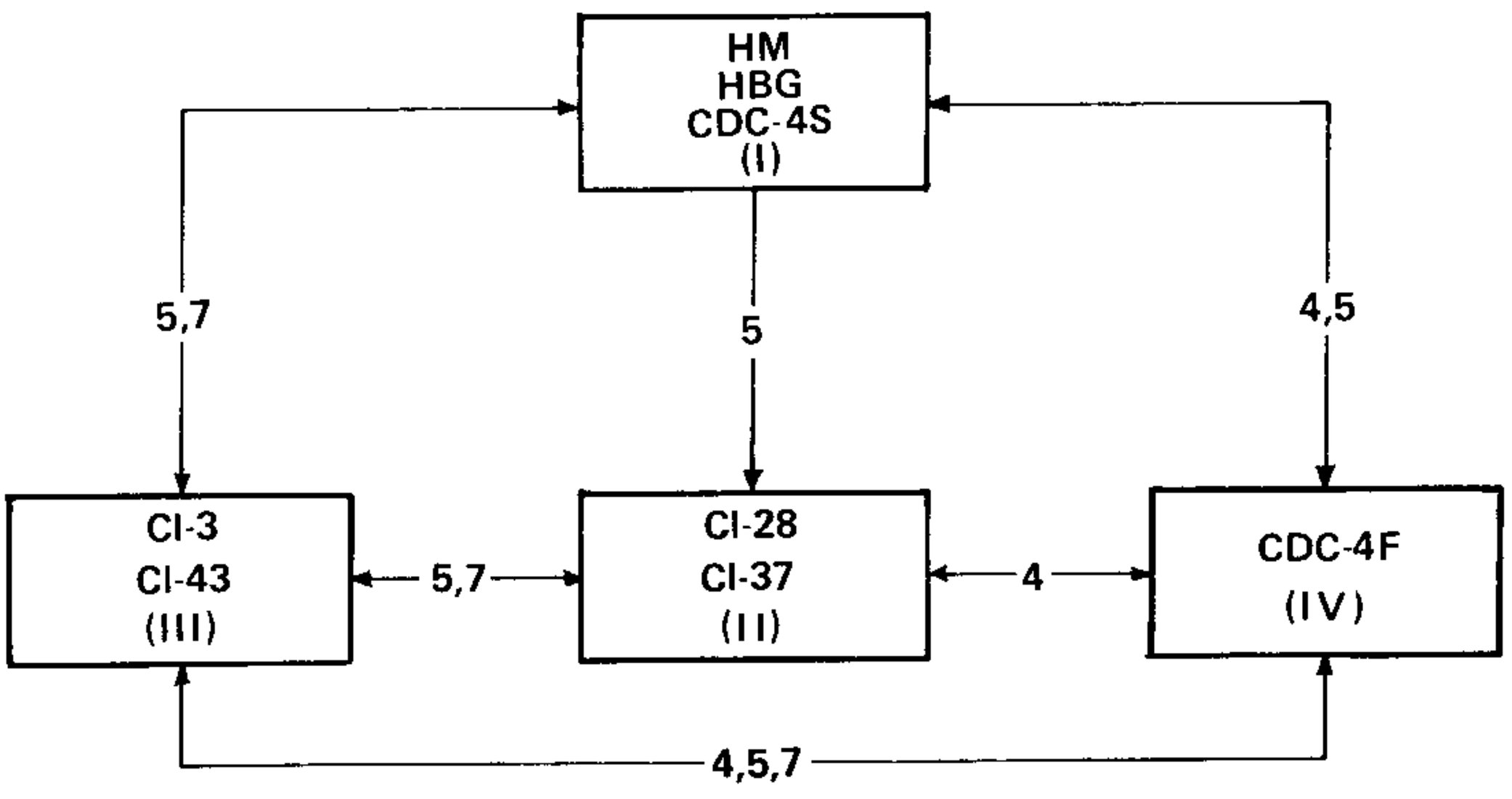

Fig. 1: relationships between electrophoretic types of SA11. (Types given as Roman figures in brackets. Figures on arrows denote genomic segments differeing between types).

An example of a co-electrophoretic comparison of representative preparations of each of the four types is shown in Fig. 2 where only segments 1 to 9 appear. The most obvious shift is seen in segment 4 of the variant 4F. Second in magnitude is the shift of segment 7 observed in clone 3 (and 47, not shown in the figure). The shift in segment 5 is most obviously seen between the variants 4S and 4F and was often difficult to demonstrate in other combinations where the small differences although seen during development of the silver stain were not obvious in photographs. Our results suggest that the shifts in segment 5 were not identical in all the pairs of preparations compared with each other but may have occurred as a gradual drift with several intermediary steps.

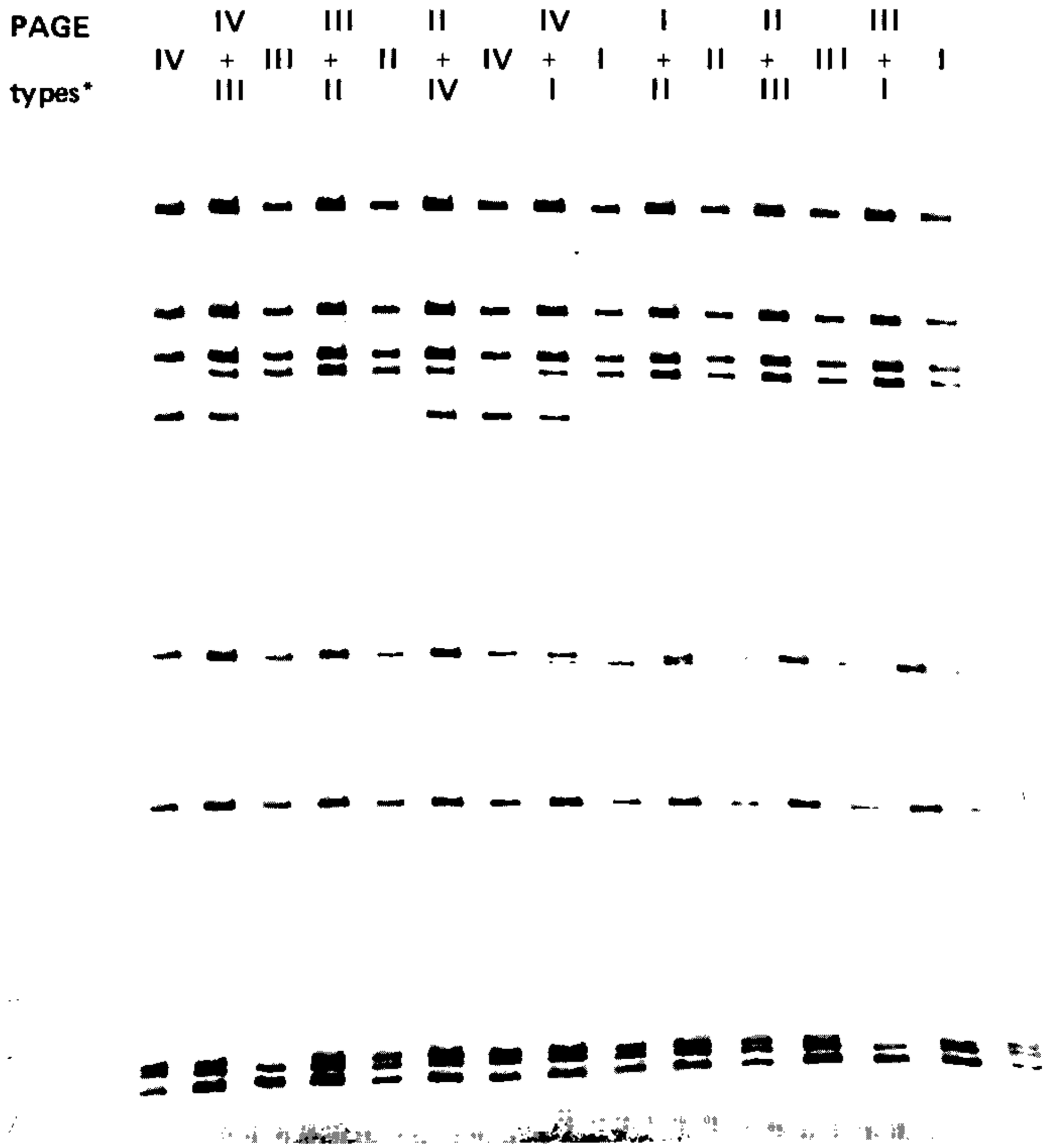

Fig. 2: comparison of representative preparations of types I to IV by co-electrophoresis. *- Representative strains used: Type I, CDC-4S; Type II, clone 37; Type III, clone 3; Type IV, CDC-4F. Electrophoresis in 12% acrylamide, 0.3% bis-acrylamide gel slab 1mm thick, 150mm wide, 120mm high run for 16 hours at 20mA.

The shift in segment 7 observed in clones 3 and 43 is illustrated in Fig. 2A where segments 7, 8 and 9 of clone 3 are compared with those of variant 4F. Two possible interpretations of the change in migration pattern of this group of bands are suggested in Fig. 2B and 2C, one involving a shift of both bands 7 and 8 (2B) and another a shift of only band 7 which would be transposed to position 8 in the variant clones (2C). The second alternative is simpler and considered more probable.

DISCUSSION

Polyacrylamide gel electrophoresis has been extensively used as a tool for the diagnosis and the epidemiological investigation of rotavirus infections (see review by Estes et al., 1984). The simian rotavirus SA11 is commonly used as a standard for a comparison with test strains. Variations of its electrophoretic profile must therefore be taken into account when results from different laboratories are compared. Variations described in the present study were limited to segments 4, 5 and 7 although analysis of gene products (to be published) demonstrated changes in segment 9 also as previously described by Estes et al. (1982). Genomic segment 4 showed the greatest migratory shift. This was observed only in variant 4F derived from the CDC inoculum as previously described (Pereira et al., 1984). A similar variant of SA11 has been described by Lopez et al. (1985). Variation in segment 7 was observed only in clones 3 and 43. It is possible that segment 8 may also have varied in these clones (see Results and Fig. 2) but we favour the alternative explanation of a transposition of segment 7 from the original strain to position 8 in clones 3 and 43. Attempts to substantiate his interpretation by Northern blot hybridization are in progress.

Variation of genome segment 5 was the most frequent but of smallest magnitude. This change was difficult to demonstrate between some of the paired preparations. Our results suggest that at least two different shifts occurred in this segment, one between type I and type III, and another between type I and types II or IV (Fig. 3).

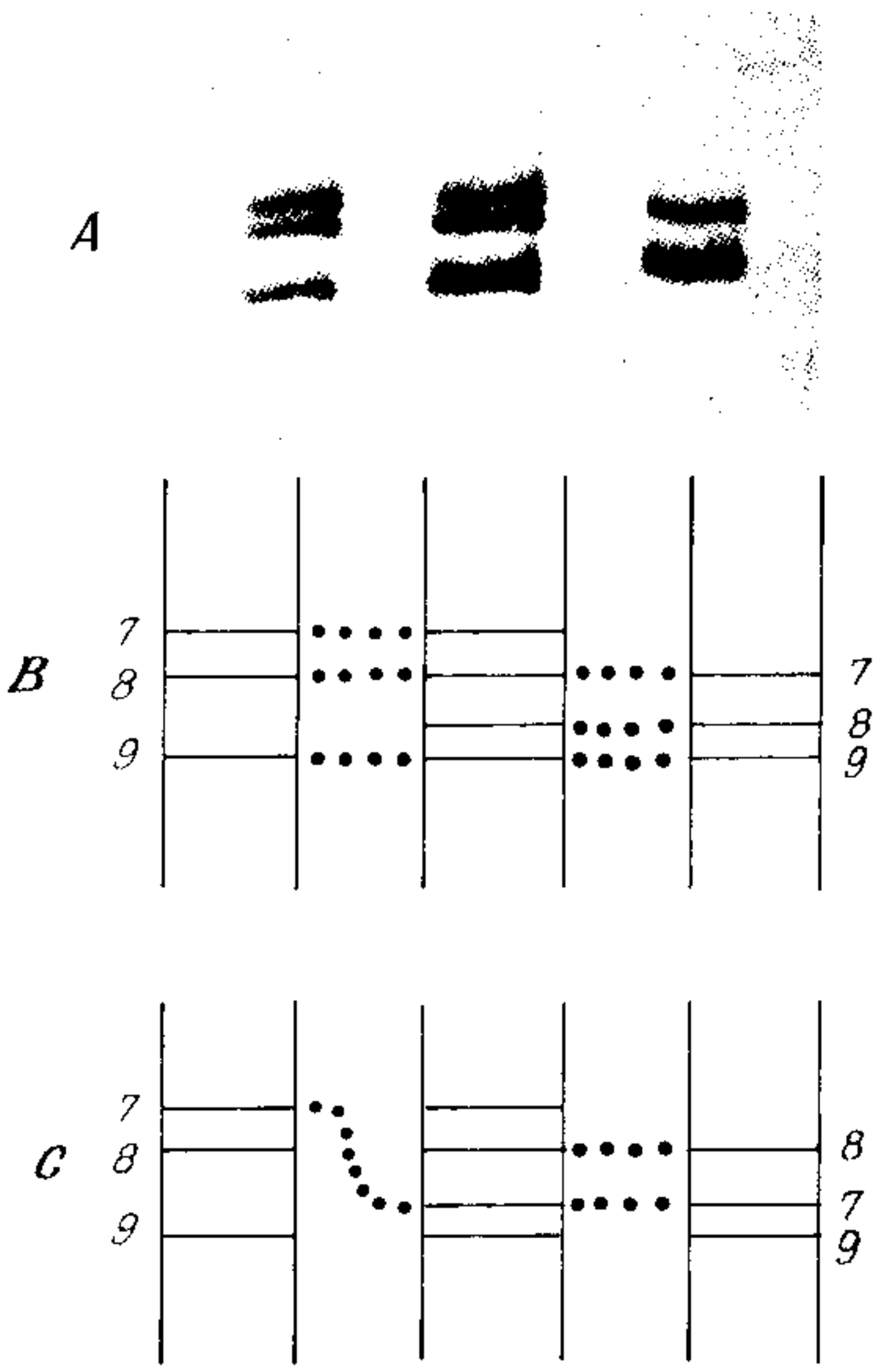

Fig 3: detail of electrophoretic comparison of genome segments 7, 8 and 9 of types I and III. (Left lane: Type I (CDS-4S) – Right lane: Type III (clone 3); Centre lane: co-electrophoresis).

Our findings are consistent with reports reviewed by Reanney (1982) and Holland et al. (1982) demonstrating the rapid evolution of RNA viruses. Even with the limitation of the methods used in our work which only reveal gross alterations in electrophoretic mobility it was possible to demonstrate mutations in several genomic segments. More refined methods such as RNA fingerprinting or sequencing would certainly reveal more frequent mutants. The wild-type SA11 considered in this study to be represented by type I (HM, HBG and CDC-4S) may consist of a heterogeneous population in which a particular genomic composition predominates in an equilibrium pool as described by Domingo et al. (1978) in bacteriphage Q beta. Successive passages or cloning procedures would have selected spontaneous mutants represented by types II, III and IV. In fact, all the preparations differing electrophoretically from the original type I were derived from clones obtained either by plaquing (Estes et al., 1982) or by limit dilution passages (Pereira et al., 1984). Selection and characterization of mutants with altered phenotypic behaviour may prove useful in elucidating molecular aspects of virus growth and pathogenicity.

RESUMO

Preparações da amostra SA11 de rotavírus símio mantidas em diferentes laboratórios foram comparadas entre si por eletroforese do genoma viral em gel de poliacrilamida. Diferenças na migração dos segmentos genômicos 4, 5 e 7 permitiram a classificação de oito preparações virais em quatro tipos eletroforéticos.

ACKNOWLEDGEMENTS

We are grateful to Drs. G.W. Gary, H.B. Greenberg, H. Malherbe and M.K. Estes for the supply of virus seeds.

REFERENCES

BEARDS, G.M., 1982. Polymorphism of genomic RNA within rotavirus serotypes and subgroups. *Arch. Virol., 74* :65-70.

DOMINGO, E.; SABO, D.; TANIGUCHI, T. & WEISSMANN, C., 1978. Nucleotide sequence heterogeneity of an RNA phage population. *Cell., 13* :735-744.

ESTES, M.K.; GRAHAM, D.Y.; RAMIG, R.F. & ERIKSON, B.L., 1982. Heterogeneity in the structural glycoprotein (VP7) of simian rotavirus SA11. *Virology, 122* :8-14.

ESTES, M.K.; GRAHAM, D.Y. & DIMITROV, D.H., 1984. The molecular epidemiology of rotavirus gastroenteritis. *Prog. Med. Virol., 29* :1-22.

HERRING, A.J.; INGLIS, N.F.; OJEH, C.K.; SNODGRASS, D.R. & MENZIES, J.D., 1982. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. *J. Clin. Microbiol., 16* :473-477.

HOLLAND, J.; SPINDLER, K.; HORODYSKI, F.; GRABAU, E.; NICHOL, S. & VANDE POL, S., 1982. Rapid evolution of RNA genomes. *Science, 215* :1577-1585.

LAEMMLI, U.K., 1979. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227* :680-685.

LOPEZ, S.; ARIAS, C.F.; BELL, J.R.; STRAUSS, J.H. & ESPEJO, R.T., 1985. Primary structure of the cleavage site associated with trypsin enhancement of rotavirus SA11 infectivity. *Virology, 144* :11-19.

MALHERBE, H.H. & HARWIN, R., 1963. The cytopathic effect of vervet monkey viruses. *S. Afr. Med. J., 37* :407-441.

MALHERBE, H.H. & STRICKLAND-CHOLMLEY, M., 1967. Simian virus SA11 and the related O agent. *Arch. Ges. Virusforsch., 22* :235-245.

PEREIRA, H.G.; AZEREDO, R.S.; FIALHO, A.M. & VIDAL, M.N.P., 1984. Genomic heterogeneity of simian rotavirus SA11. *J. Gen. Virol., 65* :815-818.

REANNEY, D.C., 1982. The evolution of RNA viruses. *Ann. Rev. Microbiol., 36* :47-73.

SABARA, M.; DEREGT, D.; BABIUK, L.A. & MISRA, V., 1982. Genetic heterogeneity within individual bovine rotavirus isolates. *J. Virol., 44* :813-822.

SAMMONS, D.W.; ADAMS, L.D. & MISHIKAWA, E.E., 1981. Ultra sensitive silver base colour staining of polypeptides in polyacrilamide gels. *Electrophoresis, 2* :135-141.