have been isolated from children with occult infection, by means of *in vitro* cultivation of cells from various organs, mainly adenoids[4] and kidneys[2]. This communication deals with accidental recovery of an agent causing cytomegalovirus type cytopathic effect from cell cultures derived from human embryonic tissues.

Cultures of embryonic skin and muscle cells were prepared initially for other purposes, and kept free of infection for 4 weeks at + 37° C. The tissues originated from a 12 week old human embryo, which was brought to the laboratory within 2 h of surgical abortion. Trypsin-dispersed stationary tube cultures were prepared and grown on 0·5 per cent lactalbumin hydrolysate in Hanks's balanced salt solution with 10 per cent calf serum. For the maintenance of cellular outgrowth 5 per cent calf serum was used. The nutrient fluids contained antibiotics and were replaced once a week.

Cytomegalovirus was identified by its distinctive cytopathology. The collodion membrane technique was employed, using haematoxylin and eosin to confirm the nature of the cellular lesions.

After 14–17 days in culture, focal areas of morphological changes developed in the fibroblast sheet in two out of ten tube cultures. The foci consisted of sharply demarcated, smoothly outlined oval or round cells (Fig. 1). On stained preparations the enlarged, sometimes multinucleated cells showed eosinophilic nuclear inclusions surrounded by clear spaces and separated by the prominent, basophilic nucleoli in two or more masses. In many affected cells there was a round eosinophilic area in the cytoplasm which displaced the nuclei towards the periphery of the cell. Vesicles and acidophilic granules were diffusely distributed in the cytoplasm (Fig. 2). The progression of the cellular lesions was extremely slow; no change was noted during a period of 14 days. The observed cytopathic effect was identical in all respects to that produced by previously isolated cytomegalovirus strains[3].

Serial passages of the virus in human embryonic fibroblast cultures could not be performed.

At the Centre of Normal and Pathological Embryology in Timisoara more than a hundred 1–3 month old human embryos were examined histopathologically, but no characteristic cytomegalic inclusion bodies were observed in them (personal communication from Professor Menkes). Few of the well documented instances of intra-uterine viral infections have been confirmed by *in vitro* isolation of the virus strain concerned. The results presented here indicate, however, that human embryos may harbour infective virus strains. The problem of viruses in human embryonic tissues should be investigated, therefore, systematically, and adequate tests devised for detection of minimal quantities of free virus.

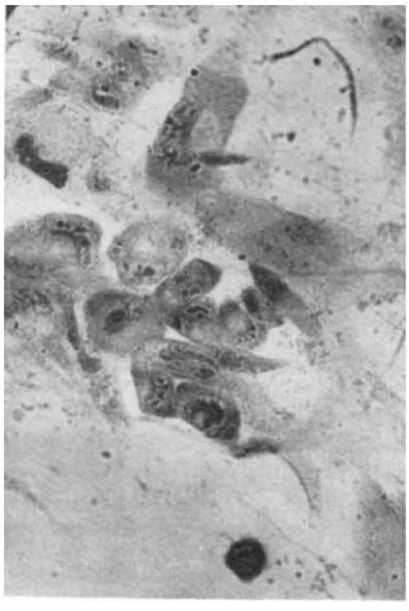

Fig. 1. Focal area of cellular lesions in human embryonic cell culture, haematoxylin–eosin stain. (× 120.)

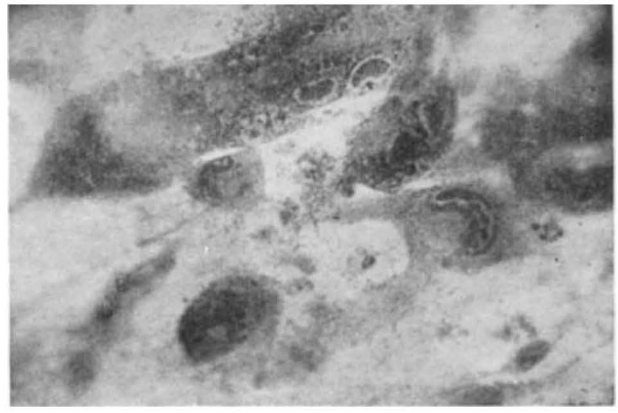

Fig. 2. High magnification of enlarged cells showing intra-nuclear inclusions, haematoxylin–eosin stain. (× 480.)

Employing the method of cell cultivation, used to unmask latent viruses in the cell, we succeeded in demonstrating the existence of a cytomegalovirus type intranuclear agent which produces an inclusion body in a 12 week old human embryo. Propagation of the virus could not be achieved, and cytopathology may have resulted from an abortive infectious process.

We thank Miss Elisabeth Kecskés for preparing the cell cultures.

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IMMUNOLOGY

Demonstration of IgA Polioantibody in Saliva, Duodenal Fluid and Urine

We[1] have reported that IgA polioantibody is present in the serum of adult human beings, and have also shown that nearly all of the polioantibody in human colostrum is IgA (ref. 2). Bellanti[3] has shown that IgA polioantibody is also present in nasal secretions.

In the experiments reported here, samples of saliva, duodenal juice and urine were investigated for polioantibody by a technique of radioimmunodiffusion which we have described before[1]. Briefly, this method identifies in the following manner immunoglobulins which have antibody activity. Microscope slides are covered with a layer of agar, and specimens are placed in antigen wells which are cut in the agar. An antiserum specific for one class of immunoglobulin is diffused from an antibody trough. After 24 h, unprecipitated protein is removed by

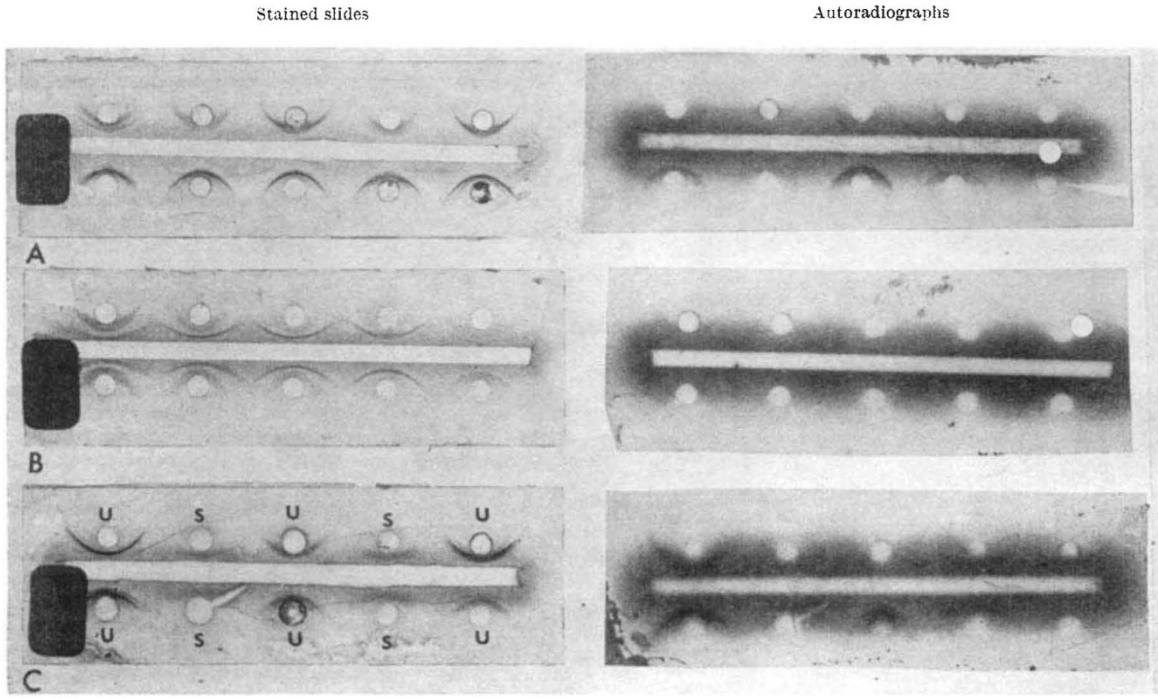

Fig. 1. Radioimmunodiffusion of saliva, duodenal fluid and urine using the rabbit anti-colostral IgA and labelled polio to develop stained precipitates and autoradiographs. (*A*) Ten samples of unconcentrated saliva. Binding of virus is seen in all samples. (*B*) Two samples of duodenal fluid. The samples had been concentrated approximately five times before dilutions were prepared. Binding of virus is seen in both undiluted samples. (*C*) Six samples of urine (*U*) concentrated 500 times and four samples of serum (*S*). Binding is visible in five of the six samples of urine.

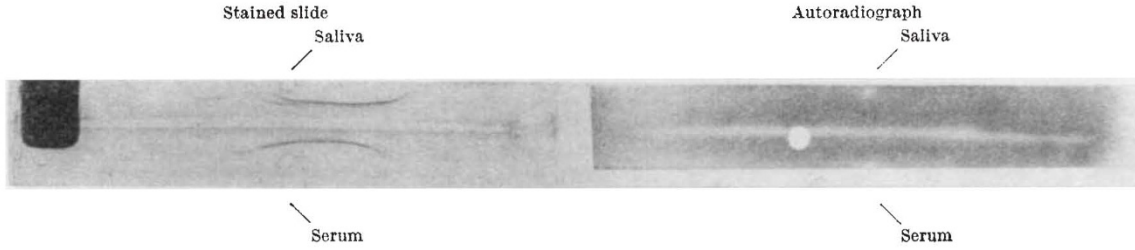

Fig. 2. Radioimmunoelectrophoresis of 5X saliva and serum. The antiserum was prepared against human colostral IgA and absorbed with human serum that contained no IgA. On the stained slide the precipitate arc of IgA is evident in both samples. Two other proteins are precipitated from the saliva. The autoradiograph, however, demonstrates that binding of labelled polio is seen only in the IgA and not in the other proteins.

washing with saline. Purified poliovirus labelled with phosphorus-32 is then diffused from the trough for 24 h, and after this the slides are washed to remove all unbound virus. The union of virus with the precipitated immunoglobulin is then demonstrated by autoradiography.

The specific rabbit anti-IgA serum used in the experiment described in this communication was prepared against human colostral IgA and was absorbed with human serum that did not contain any IgA. When this antiserum is diffused against human serum, only IgA is precipitated. When this antiserum is directed against colostrum, saliva, urine or duodenal secretions it also precipitates other proteins. These proteins, however, are not immunoglobulins; the only immunoglobulin precipitated is IgA.

The major immunoglobulin excreted in mixed saliva is of the IgA type[4]. Specimens of unconcentrated saliva from ten adult subjects were tested for poliovirus binding, and the presence of IgA polioantibody was demonstrated (Fig. 1*A*). No IgG or IgM polioantibody was found in the saliva of any of these subjects.

Specimens of duodenal fluid from twenty-two men and women were investigated in a similar manner. In most of the specimens, the major immunoglobulin present was IgA, although in some of the samples the concentration of IgG or IgM was equal to that of IgA. Radioimmunodiffusion showed that IgA polioantibody was present in five of the twenty-two specimens examined; Fig. 1*B* shows binding in two of the five specimens. In contrast, no polioantibody of the IgG or IgM type was found.

It has been reported that a microglobulin polioantibody is present in urine[5]. We have examined the urine of six subjects for the presence of IgA polioantibody and IgG polioantibody. Radioimmunodiffusion of urine concentrated 500 times by ultrafiltration revealed the presence of IgA polioantibody as well as IgG polioantibody in the urine of five of the six persons studied (Fig. 1*C*).

Radioimmunoelectrophoresis of the specific rabbit anti-IgA serum is shown in Fig. 2. The stained slide shows that only IgA is precipitated from the serum. IgA and two other proteins, however, are precipitated when the antiserum is directed against saliva. The autoradiograph of this slide shows binding of poliovirus labelled with phosphorus-32 only by the IgA and not by the other precipitated proteins of saliva.

It is clear that IgA polioantibody is present in saliva, nasal secretions, duodenal fluid, colostrum and urine. It

seems likely that antibodies of this type may play a part in the defence against invasion of micro-organisms.

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Polyaminostyrene Purified Chicken Antibodies

SEVERAL workers have used polyaminostyrene (PAS)-antigen complexes for the purification of mammalian sera[1]. We have attempted to use this method to purify chicken antibodies to either beef serum albumen (BSA) or Bryan strain Rous sarcoma virus (BS-RSV) for use in the reversed bisdiazobenzidine technique[2], a method of passive haemagglutination which detects antigen.

The general technique of Webb and Lapresle[3] was used for the preparation and use of the PAS-antigen complexes.

It was found that PAS–BSA adsorbed antibody specifically from hyperimmune chicken anti-BSA serum. The immunoadsorbant could be washed free of contaminating serum proteins and the attached antibodies eluted by reducing the *p*H with 0·5 molar acetate buffer (*p*H 3) followed by 0·1 molar hydrochloric acid containing 1 per cent sodium chloride. The elution pattern from an immunoadsorbant column is shown in Fig. 1. The proteinaceous material was eluted in two major fractions corresponding to the two buffers. The shoulder seen in the first peak sometimes formed another peak of activity. Precipitating activity against BSA, shown by gel diffusion[4], could only be found in material eluted by reducing the *p*H to 3. Two areas of anti-BSA activity were shown by a passive haemagglutination technique[5,6] both of which closely followed the protein curves eluted by the two buffers. Rabbit-antichicken plasma showed that the material eluted by *p*H 3 buffer, even after concentration, had only three components which migrated in the globulin region on electrophoresis (Fig. 2). The material eluted by the second buffer had one component only which also

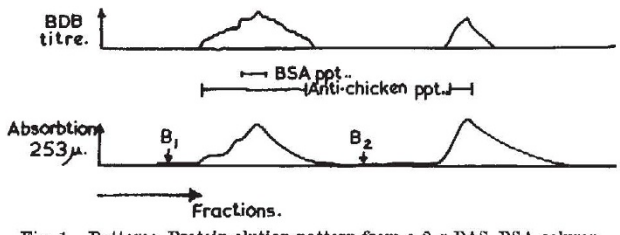

Fig. 1. Bottom: Protein elution pattern from a 2 g PAS–BSA column after non-adsorbed serum proteins had been washed off. B_1 is the point of application of the 0·5 molar acetate buffer, *p*H 3, B_2 the application point of 0·1 molar hydrochloric acid, 1 per cent sodium chloride buffer. Top: The agglutination titre of each fraction for bisdiazobenzidine (BDB)–BSA sensitized red cells. The fractions in which BSA precipitating antibody (BSA ppt) and chicken proteins (anti-chicken ppt) were found without further concentration are also shown.

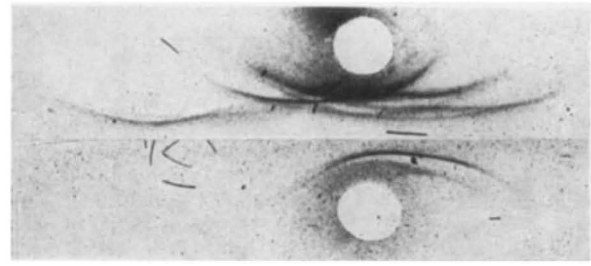

Fig. 2. Immunoelectrophoresis of normal chicken serum (top) and the concentrated antibody from a PAS–BSA column eluted by *p*H 3 0·5 molar acetate buffer (bottom). Precipitin lines developed with rabbit-antichicken serum.

migrated in the globulin region but appeared to be of high molecular weight. The finding of several peaks of activity indicates that the BSA antigen molecule probably has more than one antigenic determinant as has been shown for human serum albumen[3].

It was found that only the material from the *p*H 3 eluate could be coupled to red cells and used to detect antigen. Estimates of antibody yield from this fraction were from 12–20 per cent of the total adsorbed haemagglutinating antibody. The amount of material needed was 1–3 mg protein/0·1 ml. of 50 per cent cells. The sensitized cells could detect 1 μg/ml. to 10^{-4} μg/ml. BSA with maximal agglutination at 10^{-2} μg/ml. BSA. The range could be increased into the prozone region at concentrations greater than 1 μg/ml. BSA by removing the supernatant fluid after the cells had settled and resuspending them in diluent containing antibody. An added advantage of the latter step was the greater amount of agglutination that occurred over the whole detectable range.

Attempts were made to carry out similar procedures with Bryan standard strain Rous sarcoma virus (BS RSV). Moloney *T*2 preparations[7] were prepared in the normal way from 60 g of confluent chorio-allantoic membrane. The final pellet (containing 10^6–10^8 pock forming units (P.F.U.)) was taken up into 5 ml. of 1/15 molar *p*H 7 phosphate buffer which was added to 2 g of diazotized PAS and the coupling carried out as usual. Preliminary experiments had shown that neutralizing antibody could be regained from virus-antibody complexes by simple centrifugation after dissociation at *p*H 3 even though viral infectivity was destroyed at this *p*H. It was difficult, however, to remove contaminating serum proteins from the virus-antibody complex by this method. The washed PAS-virus complex adsorbed 70–80 per cent of the neutralizing activity from 5 ml. of serum. After exhaustive washing to remove serum proteins, neutralizing activity could be recovered by reducing the *p*H to 3. The eluted material had similar components to that seen in the BSA antibody eluted at this *p*H. The yield of antibody as measured by neutralization[8] was 1 per cent or less, which when concentrated to the original serum volume gave a protein concentration in the region of 0·5 mg/ml. Several eluates were concentrated and pooled to provide sufficient material for coupling over the range of 1–3 mg protein/0·1 ml. of 50 per cent red cells. No agglutination was observed when these cells were tested against 10^4 P.F.U. or less of BS–RSV.

Several factors may explain this last result. The antibody titre of the starting serum can affect the yield of suitable material[2]. The ratio of non-specific protein to actual antibody protein is probably high as a result of the low protein yield per serum volume[9]. The use of strong buffers may also have been deleterious to the antibody[8]. Improved yields of antibody may be obtained by using better virus preparations, either in virus content or purity, for coupling to the PAS columns.