insulin was contained in the beta component. The results are reenforced by observation of a gradient of activity in which an optimal zone of interaction exists between antigen excess and reagin excess. Our data has repeatedly confirmed the illustrated results, and we have also noted that the optimum concentration of the contaminating antigen varies with the serum of different allergic individuals.

Summary. Disturbances caused by interaction of reaginic sera of ragweed sensitive patients and its specific antigen are observed during free boundary migrations. Their respective changes of composition and mobilities of fractions are calculated and studied. Alteration of beta and, to a lesser extent, gamma globulin occur at optimum concentrations of ragweed contaminated buffer.

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Propagation of Group A Coxsackie Viruses in Primary Human Amnion Cells. I. Cytopathic Changes Produced by 5 More Serotypes.* (26682)

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The Group A Coxsackie viruses previously reported cytopathic for tissue cultures include types 4, 6, 7, 9, 10, 11, 13, 14, 15, 16, 18, 20, 20a, 20b, 21 and 23(1,2,3,4,5,6,7). During the past 18 months we have attempted to obtain virus growth associated with cytopathic changes (CPE) in human amnionic cells with 24 serotypic Group A viruses. We were able to confirm the previous resports for all but 3 of the viruses listed above; we also obtained CPE for 5 additional serotypes heretofore unreported.

Materials and methods. Viruses. Twenty-four Group A serotypes and 2 subtypes (types 20a and 20b)† were obtained from Dr. Hildegard Plager, Division of Laboratories, New York State Dept. of Health, Albany. Virus stocks used for primary inoculation of tissue cultures were derived from suckling mice. *Antisera.* Type specific antisera were also provided by Dr. Plager. Other antisera prepared by Syverton and associates(8), and some prepared in this laboratory were available also.

Tissue cultures. Primary cultures of human amnion cells were prepared(9). The medium used for growth of cells consisted of medium 199 containing 20% human serum; for maintenance, washed cells were overlaid with medium 199 containing 0.5% lactalbumin enzyme hydrolysate, pH 7.3.

Measurements. Each mouse-adapted stock virus was inoculated into 4 cultures (0.1 ml per culture). If CPE was not noted by the 7th ± 1 day the fluid overlay was harvested and inoculated into another set of cultures. Four passages, at least were made with each serotype. The appearance of CPE was followed through additional passages until maximal CPE was obtained. At this level of passage fluid overlays were harvested and pooled for use in titration and specificity studies.

Virus titrations were done in human amnion cell cultures; successive 10-fold dilutions of virus were tested using 3 or 4 cultures per dilution. Type specificity was ascertained from serum dilution endpoints (SDE's) obtained with known serotypic antisera using approximately 100 tissue culture

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† Type 20, 20a and 20b are provisionally classified as type 20 viruses. Reference to 26 viruses in the text includes 20a and 20b among 24 serotypes.

TABLE I. Propagation of 5 Group A Coxsackie Viruses.

Group A type	No. serial passages	Extent of CPE at indicated level of passage						CPE (days)		Titer TCD_{50} per .1 ml	Serotypic neutralization	
		1	2	3	4	5	≥6*	First noted	Maximal		SDE	TCD_{50}
3	12	1+	1+	1+	1+	2+	4+	3	5	4.7	256	250
8	7	0	1+	4+	4+	4+	4+	"	6	3.8	1024	80
12	6	1+	4+	3+	3+	4+	4+	"	5	4.7	500	370
17	8	3+	0	0	4+	4+	4+	"	"	3.5	630	20
24	9	4+	1+	1+	0	2+	4+	"	6	4.5	64	270

CPE, cytopathic effect; TCD_{50}, 50% tissue culture infective dose; SDE, serum dilution endpoint.

Extent of CPE: 1+, focal signs of CPE involving <50%; 2+, 50%; 3+, 75%; and 4+, 100% of cell monolayer.

* Type of change observed at passage level entered in column 2. Data for onset and progression of CPE refer to virus stocks showing maximal CPE.

infective doses (TCD_{50}) of virus.

Results. Virus producing CPE. Eighteen of the 26 Group A Coxsackie viruses produced CPE initially, or after several passages in primary cultures of human amnion cells. Fourteen strains produced unequivocal CPE during first passage; 4 viruses (types 3, 8, 12 and 16) either failed initially to cause CPE, or the changes were indecisive. Eight serotypes (types 1, 2, 4, 5, 6, 7, 19 and 22) have thus far in the study failed visibly to affect human amnion cells.

The 5 serotypes, heretofore unreported as propagating in tissue cultures, but which produced CPE in the present study were types 3, 8, 12, 17 and 24. Data obtained during studies with these viruses appear in Table I.

Kind of CPE. The time of first appearance of CPE, and the time required for maximal CPE differed for these viruses. After onset of CPE complete lysis of cells occurred within 24 hours for some (*e.g.*, type 14), whereas others required 2 or 3 days. The pattern of CPE was quite often related to the rapidity of cell destruction. For the viruses associated with rapid CPE cells were lysed completely. But some viruses, including those listed in Table I were associated with a slower CPE. The first changes were sequestration of cells from the intact monolayer; the separated cells became shrivelled (pyknotic) and granular in appearance. While many cells remained attached to the glass surface of the roller tube, many also sloughed off into the fluid overlay.

Titers. TCD_{50} endpoints ranged from $10^{3.5}$ to $10^{4.7}$ per 0.1 ml for the 5 viruses entered in Table I. A titer of $10^{6.5}$ was obtained for type 9, the highest yet obtained.

Serotypic specificity. The Group A Coxsackie viruses in the fluid overlays obtained from amnion cultures were neutralized by type specific antisera. The SDE's obtained for the 5 serotypes most recently propagated in tissue cultures are given also in Table I.

Discussion. Nineteen of the 24 serotypes currently listed in the Group A Coxsackie viruses have been propagated in tissue cultures. We have added 5 (types 3, 8, 12, 17 and 24) viruses to the 14 previously reported to propagate *in vitro*. The viruses not yet associated with CPE include types 1, 2, 5, 19 and 22. Undoubtedly these last 5 will yield CPE when proper environmental requirements are fulfilled.

We have also studied the 24 serotypes in other cultural systems (human skin epithelium, kidney, HeLa and KB cells; monkey kidney and testis; mouse embryonic cells). Although many human cell lines supported growth of these Group A viruses, the response was not all or none. Thus, types 3 and 12 produced CPE only in human amnion, whereas types 11, 13, 14, 15, 18, 20, 20a, 20b and 21 produced CPE in each human cell line entered in the study. Other viruses, at corresponding levels of passage had cytopathic affinities between these extremes. The type 7 virus produced CPE in renal cells from monkeys and in HeLa cells, but at the 4th level of passage CPE has not been observed in amnion cells. None of the Group A viruses was cytopathic for mouse embryonic cells.

We have not been able to obtain CPE with the Albany strains, types 4 and 6. Slater and Syverton(1) obtained propagation with a type 4 (Minnesota strain) virus for 24 consecutive passages in minced mouse embryonic cells. We have not obtained either CPE. or evidence of virus multiplication on repassage of tissue culture fluids in suckling mice with the Albany type 4 virus. Lehmann-Grube and Syverton(6) also reported that type 6 propagated in human amnion cells. We failed to obtain signs of virus growth in human amnionic cells. although at the 4th passage fluid overlays obtained from cultures inoculated with this serotype produced typical disease in mice. When cultural conditions are properly fulfilled types not yet associated with CPE will probably yield. Since the media used for growth and maintenance of cells in various laboratories often differ. measurements on susceptibility and resistance of cultured cells for Group A Coxsackie viruses in different laboratories will not always be the same.

In previous reports(6,7) several Group A viruses after serial propagation in human amnion cells were, in comparison with infected mouse tissues from which they were derived, associated with a significant reduction in virulence for mice. Our results support these earlier findings. Indeed. undiluted tissue culture fluids have been required to produce illness in a fraction of infected mice. These observations point up the unreliability of using suckling mice as indicator of virus multiplication in tissue cultures. Viral antigens present in infected cells may however be delineated by the fluorescent antibody staining method(11).

Summary. Seventeen serotypic Group A Coxsackie viruses, representing prototypes established by Dalldorf and Sickles(5,10) have been propagated in tissue cultures. Sixteen strains produced CPE in primary human amnion cells; another (type 7) which has failed thus far to affect amnion cells produced CPE in renal cells obtained from monkeys. Among these 17 Group A viruses were 5 serotypes heretofore not associated with CPE in tissue cultures.

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Electron Microscopy of the Arthus Reaction, Using Ferritin as Antigen. (26683)

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Although it is known that the Arthus phenomenon is dependent upon presence of circulating precipitating antibody(1,2,3) and that antigen is localized in blood vessel walls (4), the antigen-antibody complex has heretofore not been demonstrated in the reactive site.

The use of the electron-dense protein, ferritin, as antigen(5,6) has enabled direct visualization of the antigen-antibody precipi-