at a relatively high level in the absence of anterior pituitary function.

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Demonstration of Antigen-Reagin Interaction by Free Boundary Electrophoresis Using Buffer Contaminated with Specific Antigen.* (26681)

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In vitro experiments showing the specific interaction between human reaginic serum and homologous antigen have not been demonstrated. We assume that such specific activity exists and that it can be observed through alterations in molecular behavior of reaginic serum as can be demonstrated with free boundary electrophoresis. Efforts to discover a difference between reaginic and non-reaginic serum by electrophoresis have not been clearly successful(1). Interaction of antigen with specific antibody in free boundary electrophoresis was first demonstrated by Campbell, *et al.*(2).

TABLE I. Calculation of Mobility and Percentage Composition.

				α				β					
		Alb.		α_1		α_2		β_1		β_2		γ	
Patient	Conc.	$\times 10^{-5}$	%	$\times 10^{-5}$	%	$\times 10^{-5}$	%	$\times 10^{-5}$	%	$\times 10^{-5}$	%	$\times 10^{-5}$	%
	WSR*												
DR	.0	7.08	58.19	6.01	3.86	4.45	8.77	3.56	5.20	3.19	13.53	1.25	10.45
	.02	7.29	50.13	6.19	5.85	4.43	9.97	3.23	20.21	—	—	1.15	13.81
	.04	6.95	52.48	5.88	4.95	4.37	9.05	3.28	22.46	—	—	1.26	11.02
	.06	7.09	57.78	6.25	3.80	4.40	9.77	3.60	3.26	3.20	15.98	1.29	9.38
	.10	6.82	59.18	5.73	4.23	3.87	8.39	3.41	5.34	3.04	14.82	1.07	8.01
	WSK†												
DR	.0	7.08	58.19	6.01	3.86	4.45	8.77	3.56	5.20	3.19	13.53	1.25	10.45
	.01	6.77	54.90	5.72	4.08	4.20	10.70	3.22	6.23	2.90	13.25	1.05	10.83
	.02	7.19	55.26	6.09	4.22	4.55	9.99	3.56	7.33	3.23	11.98	1.29	11.23
	.04	6.74	54.79	5.74	5.92	4.28	9.11	3.71	3.58	3.20	15.32	1.30	11.30
	.06	6.69	56.69	5.31	3.58	3.97	11.97	3.02	17.12	—	—	1.06	10.63
	WSR*												
UN	.0	6.85	65.80	4.40	6.34	—	—	3.06	13.32	—	—	1.09	14.52
	.01	6.87	65.88	4.53	7.12	—	—	3.09	13.14	—	—	1.10	13.83
	.02	6.82	64.66	4.46	8.18	—	—	3.09	13.56	—	—	1.23	13.58
	.04	6.96	62.05	4.55	10.13	—	—	3.11	13.30	—	—	1.19	14.52
	.06	6.90	65.27	4.53	8.06	—	—	3.30	13.50	—	—	1.14	13.18

α_1 and β_1 represent total α and β unless shown in respective columns.
* WSR = Water soluble ragweed.
† WSK = " " Koeleria.

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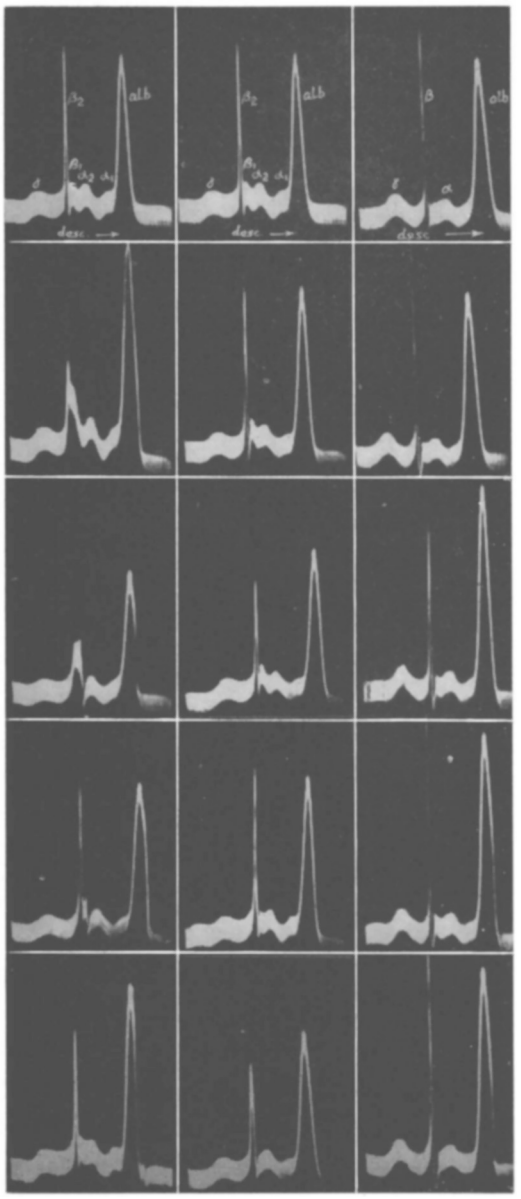

Fig. 1 Fig. 2 Fig. 3

All illustrations represent the descending boundary at end of 120 min.

FIG. 1a. Reaginic serum (DR)—buffer only.
FIG. 1b. Reaginic serum (DR); buffer contaminated with 0.02% WSR.
FIG. 1c. Reaginic serum (DR); buffer contaminated with 0.04% WSR.
FIG. 1d. Reaginic serum (DR); buffer contaminated with 0.06% WSR.
FIG. 1e. Reaginic serum (DR); buffer contaminated with 0.1% WSR.
FIG. 2a. Reaginic serum (DR)—buffer only.
FIG. 2b. Reaginic serum (DR); buffer contaminated with 0.01% nonspecific antigen (WSK).

Procedure. Specific reaginic serum was migrated in a Perkin-Elmer free boundary apparatus in presence of barbital buffer contaminated with serial concentrations of Ambrosia elatior extract (WSR) within a concentration range of .01 mg-0.10 mg/ml of buffer (pH 8.6, 0.10 μ). Controls consisted of (1) reaginic serum migrated in uncontaminated buffer, (2) reaginic serum migrated in buffer contaminated with an extract of a nonspecific pollen (*Koeleria cristata*) and, (3) nonreaginic serum migrated in buffer contaminated with specific antigen (Ambrosia elatior).

Results. Fig. 1 illustrates the visible beta globulin disturbance limited to a specific range of concentration when reaginic serum is migrated in presence of specific antigen. Fig. 2 shows the absence of this disturbance in reaginic serum in presence of a nonspecific antigen. In Fig. 3 the non-reaginic serum fails to demonstrate a change in the beta region in presence of the specific antigen. It should be noted that where specific changes in beta globulin occur (Fig. 1) similar but less conspicuous alterations may be observed in the gamma component. Calculation of the percentage composition in these findings also shows this alteration in both beta and gamma globulin (Table I).

Discussion. The results indicate interaction between reaginic serum and specific antigen which does not occur in the control experiments. The more impressive alteration of the beta as compared with the gamma component suggests that reaginic activity exists in this area, confirming the results of previous workers such as Cann and Loveless (3) who found that reaginic activity against

FIG. 2c. Reaginic serum (DR); buffer contaminated with 0.02% nonspecific antigen (WSK).
FIG. 2d. Reaginic serum (DR); buffer contaminated with 0.04% nonspecific antigen (WSK).
FIG. 2e. Reaginic serum (DR); buffer contaminated with 0.06% nonspecific antigen (WSK).
FIG. 3a. Buffer only.
FIG. 3b. Nonreaginic serum (UN); buffer contaminated with 0.01% WSR.
FIG. 3c. Nonreaginic serum (UN); buffer contaminated with 0.02% WSR.
FIG. 3d. Nonreaginic serum (UN); buffer contaminated with 0.04% WSR.
FIG. 3e. Nonreaginic serum (UN); buffer contaminated with 0.06% WSR.

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.