changes in the treated tumors suggests that the mode of action of acetic hydrazide on tumor inhibition is not a direct toxic action on the tumor cells. Three mechanisms of action have been considered. The hydrazide portion of the molecule may combine with a carbonyl group and remove an essential compound from the citric acid cycle, or the acetyl moiety can manifest itself through interference with active acetate formation. Busch(5) in experiments on the metabolism of acetate-1-C^{14} in tissues of tumor-bearing rats showed that utilization of the acetate molecule in the tumors was markedly diminished. Acetic hydrazide may further depress this utilization of acetate. In addition acetic hydrazide might, conceivably, interfere with the activity of some amidases.

Summary. Acetic acid hydrazide caused moderate growth inhibition of transplanted mammary carcinoma 755. Possible modes of action are discussed.

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Fluorescent Antibody Studies with Agents of Varicella and Herpes Zoster Propagated *in vitro*.* (21235)

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Investigation of varicella and herpes zoster viruses in the laboratory has been hampered by the lack of a susceptible experimental animal. An alternative approach was indicated by the observation(1) that specific cytopathic changes followed inoculation of roller tube cultures of human tissues with vesicle fluid materials from cases of varicella or herpes zoster. Serial propagation *in vitro* of agents apparently derived from these cases was accomplished, as demonstrated by the regular appearance in subcultures of cytopathic changes that were focal in nature and associated with the presence of intranuclear inclusion bodies. A peculiarity of the agents in the culture system employed was the apparent failure of infectious material to appear in the fluid phase; therefore, tissue suspensions from infected cultures were utilized as inocula for passage *in vitro*. Initially this peculiarity posed technical problems in obtaining immunologic evidence of their identity. The fluorescent antibody technic developed by Coons and coworkers(2,3) was then applied in an effort to clarify the immunologic relationship of the agents under investigation.

This technic was first applied to problems dealing with the growth of viruses *in vitro* by Watson(4) in a study of mumps virus. She employed fluorescein-conjugated antimumps serum, and in general heretofore conjugates have been prepared with each type of antibody under investigation. Recently, one of us (AHC) prepared a fluorescein conjugate with antihuman gamma globulin rabbit serum for the purpose of detecting any specific antibody of human origin after combination with

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its homologous antigen.‡ In the present study this antiglobulin conjugate was employed in an effort to detect virus in infected culture materials. Specifically the reaction between agents considered to be those of varicella or herpes zoster and homologous human antibody was investigated.

Materials and methods. Tissue culture materials infected with agents isolated from cases of varicella or herpes zoster were exposed to acute or convalescent phase sera collected from cases of varicella or herpes zoster, or to sera from cases of recurrent herpes simplex. To control the specificity of the reaction uninoculated tissues were included in each experiment, and, on occasion, tissues infected *in vitro* with herpes simplex virus were also studied. Cultures were prepared and maintained as previously outlined(1) in medium containing no human serum. Tissue fragments were planted in a plasma layer on long coverslips (No. 1; 11 x 50 mm) that were introduced into roller tubes. After cellular growth was well established the virus inoculum was added: the period of cultivation prior to inoculation varied from 6 to 15 days for cultures of human embryonic skin-muscle tissue and from 11 to 27 days for human foreskin tissue. The inoculum, in the case of the varicella and zoster agents, consisted of a tissue suspension prepared by grinding the cells removed from infected tissue cultures in nutrient medium: 0.1 to 0.3 ml was introduced per culture. That containing herpes simplex virus consisted of a 1:20 dilution in isotonic phosphate buffer (pH 7.1) of infected tissue culture fluid, and 0.1 ml was introduced per roller tube. All inoculated coverslip cultures were maintained until definite focal lesions appeared. In the varicella and zoster experiments the cultures were terminated as follows when cytopathic lesions had become apparent: one group on the 7th day after inoculation, 6 groups on the 8th day, 3 on the 10th, 2 on the 11th and one set each on the 13th, 15th and 19th days. Focal lesions appeared within 48 hours in the preparations inoculated with herpes simplex virus and the cultures were harvested at that time. The coverslip preparations were removed and washed by dipping 3-4 times in isotonic phosphate buffer, and air dried in an incubator at 37°C for one to two hours. They were then covered with acetone in a Petri dish for 10 minutes and again dried. The relatively dense central area of each implanted tissue fragment was dissected free with the point of a scalpel and discarded, leaving the thinner outgrowth on the slip. The preparations were placed in Petri dishes at −15°C until exposed to serum and examined. The storage period usually was 1 to 5 days, although fluorescence was observed with preparations kept at −15°C for 8 days. Unsatisfactory results were obtained in one experiment with coverlip cultures stored for 50 to 60 days after drying. In each experiment a number of coverslips from uninoculated cultures were similarly handled. The procedure of examination for antigen in the coverslip cultures was as follows. Paired preparations infected with one strain of virus were overlayered for 30 minutes with a 1:10 dilution of human serum in phosphate buffer; one coverslip receiving acute phase and the other convalescent phase serum from the same patient. (In the early experiments, the human sera were twice absorbed with mouse liver powder as previously described(2), but it was ascertained that this step could be eliminated without resultant "nonspecific staining.") The coverslips were washed by gentle agitation in buffered saline (0.15 M NaCl containing 0.01 M phosphate, pH 7) for 10 minutes and then overlayered for 30 minutes with anti-human gamma globulin prepared in rabbits and labeled with fluorescein; this had been twice absorbed with dried mouse liver powder. The preparations were again washed in saline for 10 minutes and mounted by placing the inverted preparation on a drop of reagent glycerol containing 1 part in 10 of buffered saline on a slide. The stained cultures were examined under the fluorescence microscope and photographs taken as described(2,3,5).

Virus strains and sera studied. A. *Agents from cases of varicella.* Four strains were studied; 3 have previously been mentioned

‡ Concurrently and independently a similar technic has been developed by Dr. David Gitlin at the Children's Medical Center, Boston, employing conjugated antigamma globulin sera.

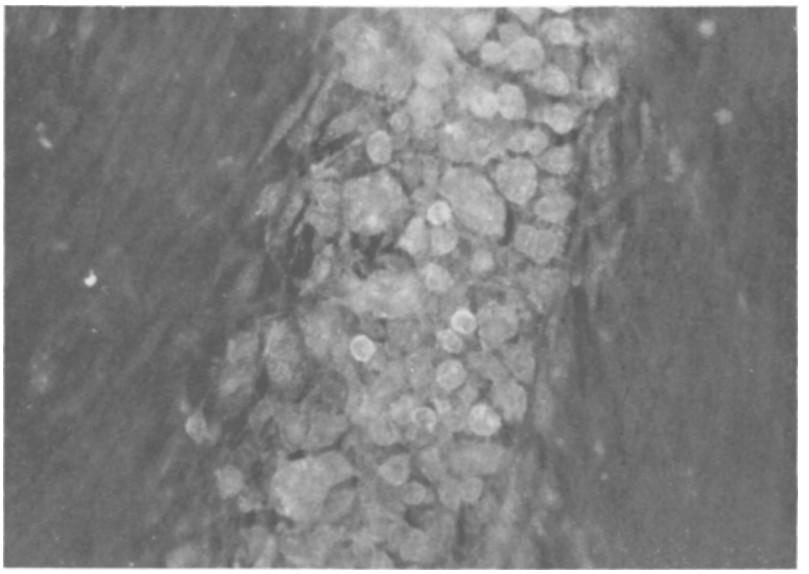

FIG. 1. Focal varicella lesion stained by fluorescent technic in human embryonic skin-muscle tissue culture; 10 days after inoculation with 14th passage Wel. strain material. Exposed to convalescent varicella serum #1555B. 250×.

(1). Inocula derived from the 10th to 12th tissue culture passage of the McE. strain were employed in four experiments; from the 11th to 14th *in vitro* pasage of the Wel. strain in 2 experiments; and from the 3rd to 7th passage of the Pat. strain in 3 experiments. An additional strain, designated Cic., was isolated postmortem from the lung of a case of generalized varicella(6); in a single experiment with this strain, second culture passage material provided the inoculum. B. *Agents from cases of herpes zoster.* Three agents were employed. One strain, Sto., previously mentioned(1), was used as 5th culture passage material. Strain Blu., isolated *in vitro* from the vesicular eruption of a 37-year-old male with characteristic unilateral lumbar lesions, provided the inocula for 3 experiments in the form of 3rd to 9th culture passage material. Strain Bag., isolated from a 61-year-old woman with a typical thoracic lesion, was employed in a single experiment as 4th culture passage material. C. *Herpes simplex virus.* The Rod. strain, originally isolated by Dr. F. C. Robbins from the lung of a patient with fatal generalized herpes, was used as pooled infective fluids derived from the 4th tissue culture passage. D. *Sera studied.* Serum specimens collected from 10 patients with varicella, from 5 with herpes zoster, and from 5 with recurrent herpes simplex were examined; they had been stored in the frozen state. Those from cases of herpes simplex were obtained through the courtesy of Dr. M. M. Stark and had been shown by him to possess high levels of herpetic complement fixing antibody(7). Dr. J. J. Finn, Jr. kindly provided the herpes zoster material.

Experimental. Infected coverslip preparations in the absence of serum as seen under the fluorescence microscope showed a faint blue-gray background luminescence that permitted visualization of the normal tissue and of the focal lesions, but showed no fluorescence. A total of 33 uninfected culture preparations examined in the presence of convalescent phase sera from cases of varicella or herpes zoster were similarly negative. Preparations infected with the agent of varicella when examined in the presence of sera collected before the 5th day of illness revealed minimal or no fluorescence, whereas in the presence of sera collected during early convalescence the focal collections of rounded cells were strongly fluorescent. As a further check on the specificity of the reaction, a preparation showing un-

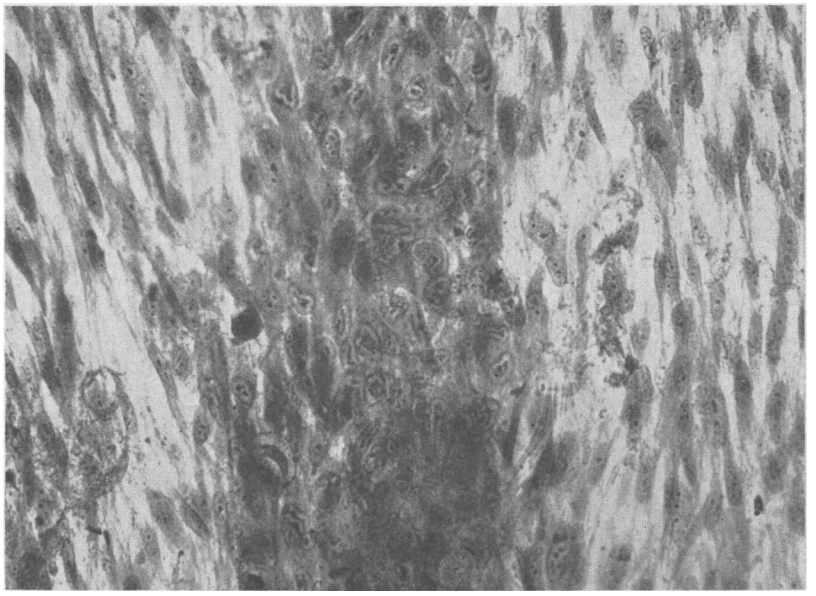

FIG. 2. Focal varicella lesion stained with H and E in culture of human embryonic skin-muscle tissue; 7 days after inoculation with 10th passage Wel. strain material. Fixed: Bouin's. 250×.

stained foci in the presence of acute phase sera was unmounted, washed, exposed to convalescent phase serum and conjugate, and re-examined; the foci then stained brilliantly. The pattern of fluorescence observed in cultures infected with the varicella and zoster strains was identical, being sharply limited to the focal collections of rounded cells. Fig. 1 pictures such an area stained by the fluorescence method after exposure to convalescent serum; for comparison, a comparable lesion stained with hematoxylin and eosin is shown in Fig. 2. Staining of cells infected with the agent of varicella as observed under higher magnification is pictured in Fig. 3 and 4. Interpretation on a cytologic basis of localization of the staining was difficult. In certain of the marginal fibroblasts adjacent to a cytopathic focus the nuclei were outlined with stained granules and there was a lesser degree of cytoplasmic staining. The periphery of those cells that had rounded up was usually brightly fluorescent, and such cells frequently contained irregular granular masses of fluorescent material; it was not clear whether these were cytoplasmic or nuclear in location, and while certain of the masses resembled stained intranuclear inclusions a definite interpretation was not possible.

The fluorescent foci observed in preparations infected with herpes simplex virus in the presence of sera from cases of recurrent herpes were similar. However, the stained areas lacked the sharp demarcation observed with the other agents, and scattered stained cells were general throughout the preparation. There appeared to be less rounding of infected cells, and in those wherein morphology was little altered, definite cytoplasmic staining was observed with a more intense nuclear staining.

The degree of fluorescence observed with the varicella, zoster and simplex agents in the presence of acute and convalescent phase homologous and heterologous human sera was compared. The reactions were graded from 0 to 4+, with ± indicating minimal and 4+ indicating intense fluorescence, as summarized in Table I. Results similar to those listed, but not recorded, were obtained with varicella strain Cic. when tested with paired varicella and paired zoster sera and with an additional zoster strain, Bag., when tested with paired zoster sera. The several varicella and zoster strains reacted in an identical manner with

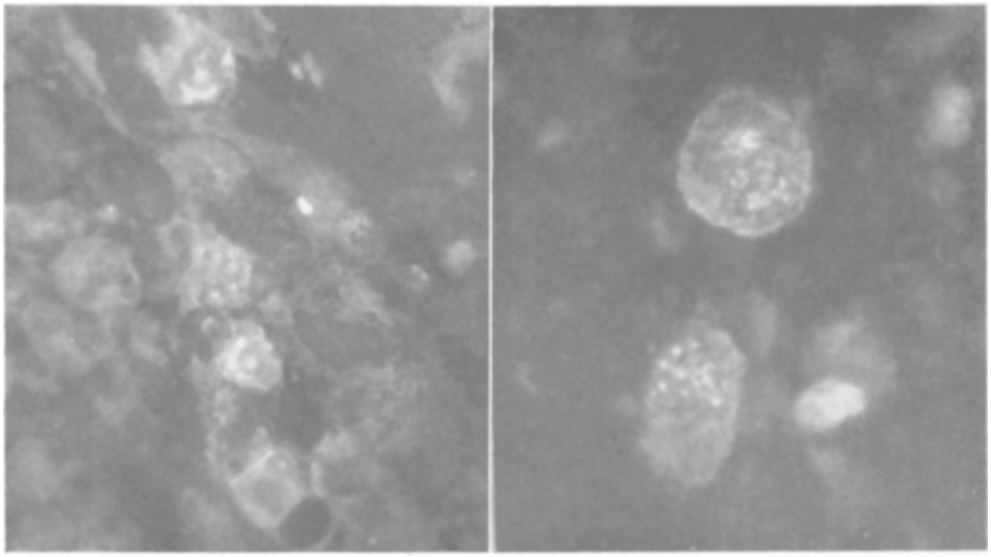

Fig. 3 Fig. 4

FIG. 3. Edge of focal varicella lesion stained by fluorescent method in culture of human foreskin tissue; 14th day after inoculation with McE. strain. Exposed to convalescent varicella serum #1006C. About 320×.

FIG. 4. Preparation as in Fig. 3. High power view of cells in varicella lesion. 600×.

paired sera from either disease. Antibody combining with both agents was absent or present in low titer in acute phase sera from varicella or zoster cases and then increased during the course of the illness. On the other hand, no comparable rise in antibody was observed when acute and convalescent phase sera from cases of varicella or zoster were compared in the presence of simplex virus. In the paired sera from one zoster case, a high level of antibody to simplex virus was observed in both specimens. The sera from recurrent cases of herpes simplex that were examined all reacted strongly in the presence of the homologous virus.

Discussion. Previously, on the basis of morphologic evidence, serial propagation *in vitro* of agents apparently derived from cases of varicella and of herpes zoster was described. Now, through the use of the fluorescent antibody technic, immunologic evidence has been educed that provides additional support for the thesis that the agents are those responsible for varicella and herpes zoster. Also, the close relationship, if not identity, of agents isolated from the two clinical entities is further suggested by their similar behavior when employed as antigen in the fluorescent antibody method. Antibody capable of reacting with the varicella or zoster antigen appeared in the serum of patients with varicella around the 3rd to 5th day after the appearance of vesicles. It is of interest that Amies(8) observed the development of agglutinins for varicella elementary bodies after a similar interval. Recently corroborative immunologic evidence has been obtained in support of the findings here described. From tissue culture materials infected with the agent of varicella a satisfactory complement fixing antigen has been prepared(9); with this antigen, the development of complement fixing antibody has been demonstrated in paired serum specimens obtained from cases of varicella and from cases of herpes zoster.

The observations here reported indicate the potential usefulness of an alternative technic for the demonstration of virus propagated *in vitro*. In preliminary experiments we have observed that cultured human myometrial cells infected with Type I poliomyelitis virus fluoresce following exposure to convalescent phase sera and then to the antiglobulin conjugate(10). The method may prove par-

TABLE I. Results of Fluorescent Antibody Staining of Tissue Cultures Infected with Varicella, Herpes Zoster and Herpes Simplex.

Day ill-ness*	Agents used to infect cultures: Varicella strains McE.	Wel.	Pat.	Zoster strains Blu.	Sto.	Simplex strains Rod.
	Sera from varicella cases:					
1	0	0	0	0		
23	4+	3+	+	3+		
3	0		0			
39	+		+			
1	0	0	0	0	0	
9	3+	4+	3+	3+	4+	
3			±	+		
13			4+	4+		
3	0	0	±	+	0	±
9	4+	4+	4+	4+	4+	+
2	0	0				
650	0	0				
5	+	±	+	+	±	
52	4+	3+	3+	4+	4+	
1	±	0	0	0	0	+
7	4+	4+	4+	4+	3+	+
2			0			
9			3+			
35			2+			
5			2+			
9			3+			
31			3+			
	Sera from zoster cases:					
2			0	+		
41			3+	3+		
2	0			0	0	±
51	3+			2+	+	±
4	±			0	0	4+
27	3+			4+	4+	4+
8	4+			4+	3+	
32	4+			4+	4+	
6				+		
28				3+		
	Sera from simplex cases (recurrent):					
			±			4+
			±			4+
	0			0	0	2+
	±			0	0	3+
				0		3+
				0		3+
				+		3+
				+		3+
				±		4+
				±		4+

* Dated from appearance of vesicles.

ticularly useful for the detection of viruses that do not manifest overt cytopathogenicity *in vitro*.

Summary. Tissue culture preparations infected with agents originally derived from the eruptive lesions of cases of varicella and herpes zoster, as well as control preparations infected with the virus of herpes simplex, were studied by a modification of the fluorescent antibody technic. Employing the infected preparations as antigen, fixation of antibody from human sera derived from cases of varicella, herpes zoster or herpes simplex was detected by the use of a fluorescent antihuman gamma globulin conjugate. Antibody reacting with the varicella and herpes zoster antigens to an almost identical degree appeared during convalescence in serum specimens derived either from cases of varicella or from cases of herpes zoster. Antibody reacting with herpes simplex virus was demonstrated uniformly only in a group of sera derived from cases of recurrent herpes simplex. Immunologic evidence was thus obtained to support the thesis that the etiologic agents of varicella and herpes zoster have been isolated and propagated *in vitro*.

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