glutinating strains of enteroviruses for which this useful property has not yet been demonstrated. Thus far, in addition to the 3 Coxsackie B viruses, we have been able to confirm the specificity of the hemagglutination previously reported(1,8,9,10,11) for only the following types of enteroviruses: ECHO types 3, 6, 7, 11, 12, 13, 19 and Coe virus. We have also been able to demonstrate specific hemagglutination by a strain of ECHO type 20 (but not the prototype), ECHO type 21, Coxsackie A types 20 and 24, and several newly recognized enteroviruses which have not been numbered. There is every reason to believe that this list will be extended.

Although HI responses observed in children infected with Coxsackie B viruses were relatively type-specific, the occurrence of heterologous responses in some children with pre-existing HI antibody suggests that this degree of specificity will not hold true with older individuals. Additional data are obviously desirable on the responses of adults, time of first appearance of HI antibodies, and duration of antibody persistence. In view of the simplicity of the HI procedure as compared with other serologic tests available, it would appear that this technic warrants further investigation as to its usefulness for diagnostic and epidemiologic purposes.

Summary. Agglutination of human erythrocytes by Coxsackie B virus types 1 and 5 is reported. Optimum titers were obtained with a sedimentation temperature of 37°C and the erythrocytes of newborn infants. A hemagglutination-inhibition (HI) procedure employing Coxsackie B virus types 1, 3, and 5 was used to test paired sera from 25 children infected with Coxsackie B virus types 3, 4, and 5. A significant homologous response was detected in every case except one. A few heterologous responses occurred in children with pre-existing HI antibody to one or more of the 3 test antigens.

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Induced Reactivation of Herpes Simplex Virus in Healed Rabbit Corneal Lesions.* (26709)

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The first report of induced reactivation of herpes simplex infection in an experimental animal was that of Good and Campbell(1) who subsequently reported additional observations of induced herpetic encephalitis in rabbits which included virus isolation(2,3). Recently Schmidt and Rasmussen(4) reported the apparent reactivation of herpes

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TABLE I. Reactivation of Herpes Simplex Virus in Rabbit Cornea by Arthus Phenomenon.

Rabbit No.	Date and site of infection	Antibody titer (9/7/60)	Corneal Arthus reaction		Virus release*
			Date	Degree	
148	2/18/60 (L)	1:128	4/27/60	severe	+
			6/ 8/60	"	0
			9/ 7/60	minimal	+
150	4/13/60 (L)	1:8	6/ 8/60	minimal	0
			12/ 7/60	"	0
151	3/21/60 (L, R)	1:512	6/ 8/60	severe	+
			9/ 7/60	"	+
			12/ 7/60	"	+
152	3/21/60 (L, R)	1:512	6/ 8/60	severe	+
			9/ 7/60	minimal	0
			12/ 7/60	moderate	0
153	4/13/60 (L)	1:128	6/ 8/60	minimal	0
			12/ 7/60	"	+
156	7/15/60 (L)	1:128	9/ 7/60	moderate	0
			12/ 7/60	severe	0
157	7/15/60 (L)	1:2048	9/ 7/60	severe	0
			12/ 7/60	"	0
158	7/15/60 (L, R)	1:512	9/ 7/60	severe	0
			12/ 7/60	"	0

L = Left eye; R = Right eye.

* Herpes simplex virus recovery by corneal swabbing during the 14-day post-Arthus period.

simplex encephalitis from the latent state in rabbits by repeated intramuscular injections of epinephrine.

These reported reactivations of herpes simplex from the latent state in animals all have the disadvantage of being at an inaccessible site. The encephalitis induced is frequently fatal and manipulations and detailed observations of these animals are technically difficult. For these reasons, methods for reactivation of healed herpes simplex infection in the skin or mucous membranes were investigated in this laboratory. Repeated attempts at reactivation of healed cutaneous herpes simplex infection in mice, rabbits, and guinea pigs were unsuccessful. The stimuli for attempted reactivation included epidermal sensitization, artificial fever, local and systemic hydrocortisone injections, ultraviolet irradiation and intravenous administration of influenza virus. Attempts at reactivation of healed herpes simplex in the rabbit cornea by ultraviolet irradiation were unsuccessful. The experiments reported here have to do with attempts at reactivation of healed rabbit corneal herpes simplex infection by induction and evocation of local hypersensitivity reactions of the Arthus type.

Materials and methods. The strain of herpes simplex virus used was isolated in chick embryo fibroblast cell culture in this laboratory in 1957 from a labial herpes simplex lesion. A suspension of this virus was prepared in rabbit kidney cell monolayer cultures. This preparation had a titer of $10^{7.5}$ TCD_{50} on rabbit kidney cell culture and was used for initiation of infection and for antibody titrations.

Male albino rabbits of 3-8 lb weight obtained from Blue Spruce Farm, Altamont, N. Y. were used throughout these experiments.

Neutralizing antibody titers are expressed as that dilution of serum neutralizing 100 TCD_{50} of herpes simplex virus on rabbit kidney cell cultures.

Rabbit kidney cell monolayers were employed for isolation and titration of virus and for neutralizing antibody determinations, using appearance of cytopathic effect as an indicator. The maintenance medium for these cultures was 2% calf serum in 199.

Results. (Table I). Five rabbits were infected on the cornea without scarification by dropping approximately 0.1-0.2 ml of undiluted virus suspension ($10^{6.5}$ TCD_{50}) into

the eye and holding the eyelids closed and in firm contact with the globe for about 30 seconds. Three animals were infected unilaterally and 2 bilaterally. An additional group of 3 rabbits was similarly infected, 2 unilaterally and one bilaterally, but the cornea was scarified with a hypodermic needle prior to introduction of the virus.

Within a period of 2-4 days muco-purulent exudate, chemosis and photophobia were noted in the inoculated eyes. Rabbit kidney cell cultures inoculated with the exudate exhibited cytopathic effect similar to that seen with herpes simplex virus. The cornea showed areas of staining when a 2% fluorescein solution was applied indicating damage to corneal epithelium. The acute inflammation subsided and epithelial repair was completed within approximately 14 days, frequently leaving residual corneal scarring and vascularization. Herpes simplex neutralizing antibody was demonstrable in each rabbit so infected. Of 8 rabbits 3 had titers of 1:512 and 3 titers of 1:128; the 2 remaining animals had titers of 1:2048 and 1:8, respectively.

Beginning 6 to 9 weeks after initiation of the corneal infection, the rabbits were prepared for a corneal Arthus reaction by a modification of a technic described by Rich (5). Each animal received 0.5 ml of horse serum subcutaneously and 0.1 ml intradermally at 3-5 day intervals until an area of hemorrhage and edema appeared at site of injection. These lesions sometimes proceeded to eschar formation and ulceration. The desired reaction was obtained after 4-5 injections.

Both eyes of each sensitized rabbit were first swabbed for the presence of virus, then a small bleb was raised with horse serum at the site of corneal scarring in the left eye of each animal. Within 24 hours, varying degrees of reaction were noted, varying from slight corneal opacity to hemorrhage and in some instances ulceration and sloughing of corneal tissue. The acute corneal reaction usually subsided in 7-10 days. Several instances of deep ciliary flush were noted. Degree of reaction closely paralleled amount of corneal vascularization present at time of challenge.

Following the challenge injection of horse serum into the cornea swabbing for virus was done, usually at daily intervals, for a 2 week period. The uninjected right eye of each rabbit was also cultured for virus. (Three rabbits had healed herpes simplex infections of the right eye and 5 had had no infection of the right eye—Table I.) Induction of the Arthus reaction was repeated for a total of 2-3 times at intervals of 2½ to 6 months for each animal. In addition control cultures were taken daily to 3 times weekly for a period of 36 days on 5 rabbits, followed by weekly to twice weekly swabbings and cultures for an additional 6 months interrupted by one to 2 additional attempts to induce reactivation. Positive virus cultures from the challenged left eye during the 14 day period following an Arthus reaction were regarded as evidence of induced reactivation. All other positive cultures from either eye were interpreted as reflecting spontaneous reactivation.

The results of these studies are graphically illustrated in Fig. 1. Rabbits 156, 157 and 158 are omitted from consideration here because virus isolation attempts were entirely negative in these animals. It is of interest that these 3 rabbits had been infected using a corneal scarifying technic while the remaining 5 rabbits were infected without scarification.

One rabbit (151) had induced reactivations on 3 occasions, one (148) on 2 occasions, and 2 (152 and 153) each had one induced reactivation. In summary, of 19 attempts to induce reactivation, 7 were followed by virus release within a period of 1-12 days. No correlation was noted between degree of hypersensitivity reaction and incidence of virus excretion provided at least a minimal reaction was obtained.

When virus release occurred after attempted induction, it was demonstrable for 3-7 days except for one instance in rabbit 148 where it was detected on only one day. Virus recovery during control study periods was usually for a period of one day. It is possible that these periods would have been

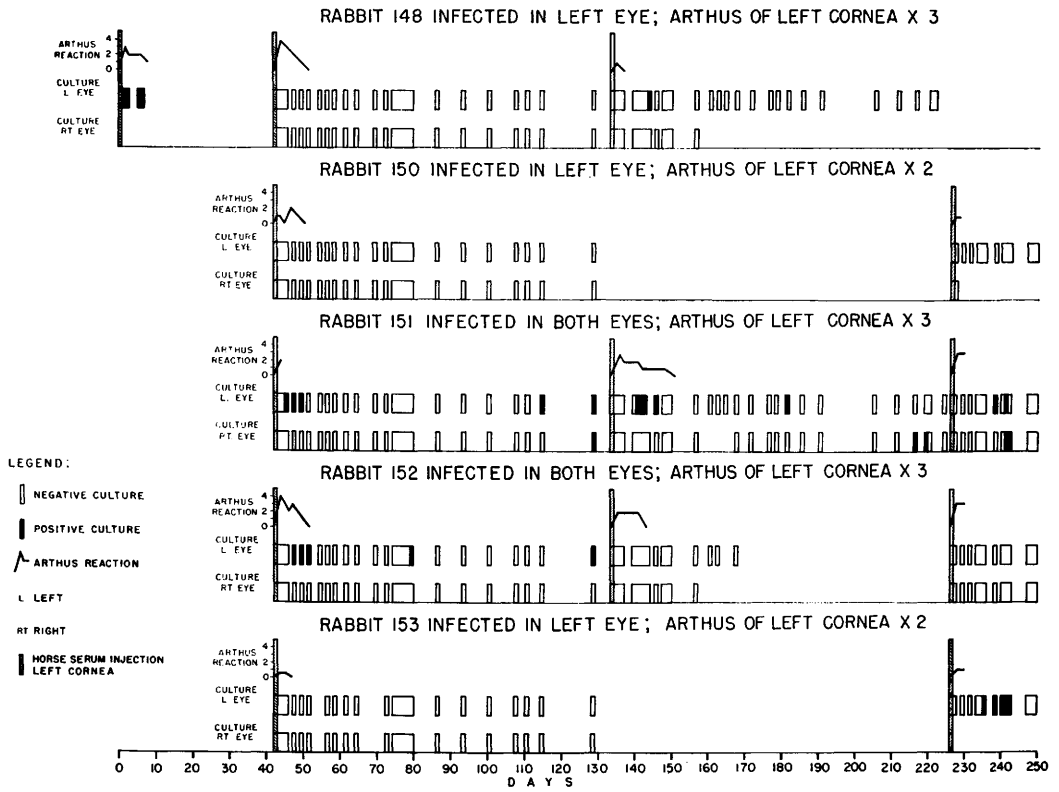

FIG. 1. Reactivation of herpes simplex virus in rabbit cornea by Arthus reaction.

longer had daily specimens been taken. There were no instances of recovery of virus from a previously uninfected eye.

It was noted that in rabbits 148, 151 and 152 where multiple induced reactivations occurred, the responses were noted at longer intervals after each challenge.

Spontaneous virus release (unrelated to injection of horse serum) occurred in only 2 of 8 rabbits (151 and 152). These 2 and 2 others (148 and 153) also released virus after Arthus reactions were induced. Neither spontaneous nor Arthus-related virus release was noted at any time in the 4 remaining rabbits.

Three rabbits (151, 152 and 158) had healed bilateral infections. Neither induced nor spontaneous virus release was detected in rabbit 158. Virus was recovered in each of 3 attempts at Arthus-induced release from the left eye of rabbit 151. Spontaneous release of virus was noted on 6 occasions in 119 control cultures from rabbit 151. These 6 positive cultures were equally divided between the 2 eyes. Rabbit 152 released virus once from the challenged left eye after Arthus reaction. Two other attempts at reactivation were unsuccessful. Spontaneous virus release was noted twice in 85 control cultures from this rabbit. This spontaneous shedding involved the left eye, in which Arthus-induced virus release had occurred.

Discussion. Within an arbitrarily defined period of 14 days during which time the effects of the Arthus reaction were presumably active, there were 7 instances of virus release following 19 attempts at induction. It is probably significant that during this same period virus release was not detected in the unchallenged corneas of rabbits in which bilateral infection had been induced.

The occurrence of spontaneous reactivations makes it desirable to extend these studies before one can state definitely that local anaphylaxis induces herpes simplex virus reactivation in healed rabbit corneal lesions. It seems probable that the isolation of herpes simplex virus from 2 of 28 excised

post-infection rabbit corneas reported by Sery(6) may have coincided with similar spontaneous reactivation of infection—perhaps immediately prior to excision.

The repeated isolation of herpes simplex virus with and without attempts at induction and interspersed by long periods of absence of virus release is of considerable interest. Corneal herpes simplex in the rabbit thus closely parallels the syndrome of recurrent human cutaneous, ocular and mucous membrane herpes simplex infection and affords an accessible model for experimental study.

Summary. Herpes simplex virus was inoculated into corneas of 8 rabbits with the induction of herpetic keratitis which proceeded to healing. The animals were then sensitized to horse serum and an Arthus reaction was induced in the healed cornea. Herpes simplex virus was repeatedly recovered in rabbit kidney cell culture from swabbings taken from these corneas. This phenomenon was observed 7 times in 19 attempts at induction. While there were several instances of spontaneous virus release, none occurred during the 14 day post-Arthus period in the unchallenged eyes of animals in which bilateral infection had been induced previously.

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Beta-2A (=Beta-3-II=Gamma-1A) Mouse Leukemia with "Flame Cells" in Leukemic Infiltrations and Degenerative Lesions in Muscles.* (26710)

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Gamma globulin-like paraproteins have been described in various mouse transplantation lines characterized by plasmacellular leukemic(1-6) or myelomatous (7-10) lesions. More recently a transplantation line associated with macroglobulinemia and with microscopic lesions comparable to those seen in Waldenström's macroglobulinemia in man was described(11). This report concerns the new observation that the paraprotein of a transplantation line of leukemia appeared immunoelectrophoretically to be of a type homologous to the recently described human beta-2A (=gamma-1A) myeloma proteins (12-13). In addition, some of the cells ("flame cells") present in the leukemic infiltrations resembled those very recently considered to be pathognomonic of human beta-2A myelomas(14). Degenerative lesions in cardiac and skeletal muscles similar to those described in thiamine-deficient mice(15) were also observed.

Material and methods. Methods of paper- and immunoelectrophoresis etc. have been described(5,16,17). For estimation of hexose content of paraproteins, total protein-bound hexose (anthrone method) was related to scannings of PAS-stained paper electrophoreses of whole sera(18). Ultracentrifugation was performed at 60,000 rpm in an oil-driven ultracentrifuge (Svedberg type) on serum diluted 4 times in phosphate buffer, pH 6.66, containing 0.2 M sodium chloride. The *transplantation line* originated in a 28-months old, untreated (CBA × DBA/2) F_1 mouse(19) with enlarged lymph nodes and

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