used for phosphorylated hesperidin. In the data obtained with the corticotrophin in gelatin plus phosphorylated hesperidin (Table IB) a summation effect is evident. There is the possibility that here the anti-proteolytic effect of the phosphorylated hesperidin (Table II) may also be a factor in that the gelatin is not acted upon by tissue proteinases and thus the gelatin retains its depot effect for a longer period of time. Thus the overall action of the gelatin-phosphorylated hesperidin combination may be the result of an increased depot effect of the gelatin due to the anti-proteolytic action of the phosphorylated hesperidin as well as the anti-hyaluronidase effect of this compound. The findings discussed above point to the use of a depot agent—anti-hyaluronidase vehicle for extending the duration of effect of other parenterally administered substances.

Preliminary clinical studies confirm the animal data in that the effectiveness of ACTH administered in phosphorylated hesperidin is as effective if not superior to gelatin preparations.

Summary. 1. Subcutaneous administration of purified corticotrophin in gelatin; in phosphorylated hesperidin; and in gelatin plus phosphorylated hesperidin results in an enhanced effect of the hormone upon the adrenal cortex as measured by adrenal ascorbic acid. The combination of gelatin plus phosphorylated hesperidin has been shown to be most effective in extending the duration of effect of the corticotrophin. 2. The mechanism of action is attributed to the antihyaluronidase as well as the anti-proteolytic properties of the phosphorylated hesperidin.

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Age Susceptibility Pattern of the Rat to Epidemic Keratoconjunctivitis Virus. (20236)

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Recent investigations(1,2) indicate that the viruses of epidemic keratoconjunctivitis (EK) and St. Louis encephalitis (SLE) are closely related. The two viruses would appear to be so closely related that it is not possible to differentiate them by serological methods. By studying the host range of the two viruses it was noted, however, that the viruses are not identical. It was observed(3) that while both viruses induce a fatal encephalitis in mice only the EK virus was capable of initiating a fatal encephalitis in guinea pigs and rabbits. It was also observed that EK virus, like SLE, does not produce any clinical evidence of infection in young adult rats following intracerebral inoculation of the virus(3). Since it has already been demonstrated that 7- or 8-day-old rats are highly susceptible to SLE virus,

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TABLE I. Susceptibility of Rats of Different Ages to Epidemic Keratoconjunctivitis Virus Injected Intracerebrally.

Age	No. tested	Wt		Results		
		Avg, g	Range, g	Mortality Total	%	Day of death*
7 days	6	13.5	11– 16	6	100	5, 7, 7, 7, 8, 8
7	5	12.7	12– 14	5	100	6, 6, 6, 6, 7
12	2	14.5	14– 15	0	0	S, S
12	9	20.2	16– 22	7	78	7, 7, 7, 7, 8, 8, 9, S, S
3 to 4 wk	16	43.0	40– 50	0	0	S (16)†
7 to 8 wk	16	141.3	126–162	0	0	S (16)†

* Numeral indicates day of death; S = survival.
† S (16) = 16 survived.

whereas rats 21 days of age or older are resistant, as far as clinical infection is concerned (4,5), studies were conducted to determine whether rats manifest a similar age susceptibility pattern toward EK virus and thus to obtain further information regarding the relationship between the 2 viruses.

Materials and methods. Virus and animals. The strain of EK virus used in the studies was obtained from Dr. Isaac Ruchman. A single virus suspension was used and was prepared from virus-infected brains of 3- to 4-week-old Swiss mice. The infected brains were homogenized in a Waring blendor together with sufficient inactivated, undiluted rabbit serum to make a 10% suspension of the infected brains. The homogenate was centrifuged at about 2000 RPM for 5 minutes to sediment gross particles. The supernatant fluid was removed and immediately distributed in 2 ml amounts in screw-capped vials. The virus suspension in the vials was "quick frozen" by rolling the vials in a mixture of dry-ice and alcohol. The frozen virus was then stored at about −70°C in a dry-ice cabinet. When needed it was thawed by placing a vial of the frozen virus in a 37°C water bath. The mouse intracerebral LD/50 titer(6) of the virus suspension, after storage at about −70°C for 4 days, was 1 x $10^{-8.3}$. The rats used in the studies were obtained from Sprague-Dawley, Madison, Wisc. All rats which were younger than 21 days of age when tested were reared in this laboratory and an accurate record of their ages was available. Older rats were shipped when 21 days of age. All rats inoculated when they were younger than 21 days of age were allowed to remain with the mother until they were at least 21 days of age. The rats were weighed on the day they were inoculated and the average weight and weight range of each group determined. All rats were observed twice daily for 21 days after inoculation of the virus.

Experimental. Behavior of rats of different ages following intracerebral inoculation of the virus. Groups of rats varying from 7 to 52 days of age were injected intracerebrally with a 10% suspension of virus. All animals received 0.03 ml of virus except the 7- to 8-week-old rats which received 0.1 ml, 0.05 ml being injected into each cerebral hemisphere. The results appear in Table I. All of the 7-day-old animals developed a fatal encephalitis. In these young animals the first indications of infection, abdominal distention together with weakness of the posterior extremities and a tendency to fall to one side when walking, were seen between the 4th and 5th days following inoculation. They also showed humped (hyperflexed) backs and became hyperexcitable, frequently biting the handler. Terminal signs were emaciation, convulsions, prostration, and in one instance paralysis of the posterior extremities. The 2 litters tested when 12 days old showed different degrees of susceptibility. A fatal encephalitis developed in 7 rats of a litter of 9, whereas both rats of a litter of 2 survived. It should be noted that the smallest animal in the litter of 9 weighed more than either animal in the smaller litter. The four 12-day-old rats which survived apparently did not completely escape infection. Although never showing characteristic signs of infection with EK virus, their fur was ruffled and they failed to grow at a normal rate during the 4- to 5-day period during which the other 12-day-old rats either showed characteristic signs or died. The course of the dis-

TABLE II. Susceptibility of Young Rats to Epidemic Keratoconjunctivitis Virus Instilled into the Nose.

Age, days	No. in each litter tested	Wt		Results	
		Avg, g	Range, g	% mortality	Day of death
7	6	9.2	8 –11	100	5, 6, 7, 8, 8, 8
7	2	19	18.5–19.5	100	7, 8
7	1	16	—	100	9
12	12	19.1	18 –21	100	8 on 8th day, 4 on 9th day

ease in the 12-day-old rats, which developed a fatal encephalitis, was essentially the same as that noted for the 7-day-old animals. All of the 3- to 4-week-old rats as well as all of the 7- to 8-week-old animals appeared to be resistant in that none of these animals developed a clinically apparent infection.

Behavior of 7- and 12-day-old rats following nasal instillation of the virus. Since 7-day-old rats were uniformly susceptible to EK virus injected directly into the brain and because some 12-day-old rats were found to be susceptible when the virus was so injected, an experiment was done to determine the behavior of young rats following intranasal inoculation of the virus. The results appear in Table II and show that both age groups were uniformly susceptible. The data presented in Tables I and II might suggest that 12-day-old rats are more susceptible to intranasally inoculated virus than to virus injected directly into the brain. Another possible explanation may be that at or about 12 days the susceptibility pattern changes so that in any large group of rats of this age some would be found to be resistant and others susceptible regardless of whether the virus were inoculated intranasally or intracerebrally. A similar observation has been reported for the behavior of Japanese B encephalitis virus in 12-day-old rats(7). The signs observed in the 7- and 12-day-old rats, which had the virus instilled into the nose, were the same as those already noted for 7-day-old rats which had been inoculated intracerebrally.

Discussion. It is apparent from the data presented that very young rats are susceptible to clinical infection with epidemic keratoconjunctivitis virus following either intracerebral or intranasal inoculation of the virus. As rats grow and develop, however, they, become increasingly resistant so that when they are 3 to 4 weeks of age they are no longer susceptible even though the virus is injected directly into the brain. Duffy and Sabin have reported a similar age-susceptibility pattern for St. Louis encephalitis virus(4,5). Seven- and 8-day-old rats were found to be susceptible to St. Louis encepalitis virus when the virus was inoculated by either the intracerebral or intranasal route. On the other hand 21-day-old rats were found to be refractory to the virus in that they did not develop a clinically apparent infection when St. Louis encephalitis virus was injected directly into the brain.

It is evident, therefore, that the age-susceptibility pattern of the albino rat to both epidemic keratoconjunctivitis virus and St. Louis encephalitis virus is similar because 7-day-old rats are uniformly susceptible to both viruses and a high degree of susceptibility is seen in 12-day-old rats. However, rats 3 to 4 weeks of age appear to be uniformly resistant to clinical infection with either virus in that rats of this age fail to develop a clinically apparent infection even though the viruses are injected by the intracerebral route.

Summary. 1. A uniformly fatal encephalitis develops in 7-day-old rats following either intracerebral injection or intranasal instillation of epidemic keratoconjunctivitis virus. 2. Most of the 12-day-old rats tested were found to be susceptible to infection with epidemic keratoconjunctivitis virus but were not uniformly so since some animals in this age group survived. 3. Rats 3 to 4 weeks of age as well as rats 7 to 8 weeks of age apparently are refractory to epidemic keratoconjunctivitis virus since they did not develop a clinically apparent infection following intracerebral injection of the virus.

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Production of Generalized Shwartzman Reaction with Group A Streptococcal Factors.* (20237)

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Recent studies by Thomas, Denny and Floyd(1-3) suggest that the mechanisms which bring about the generalized Shwartzman reaction may play a role in the pathogenesis of rheumatic fever and other disorders. Their studies involve the preparation of rabbits for the generalized Shwartzman reaction by infection with group A streptococci and provocation of the reaction by the injection of toxins from Gram-negative organisms. They observed that heat-killed group A streptococci and culture filtrates did not possess the property of preparing rabbits for this reaction and it was necessary to utilize products of Gram-negative organisms for the provoking injection. This paper is concerned with observations which indicate that when group A streptococci multiply in an *in vivo* environment a soluble material is produced which possesses the property of preparing rabbits for the generalized Shwartzman reaction. This reaction may be provoked in rabbits, prepared with this factor, not only by toxins from Gram-negative bacteria, but, in addition, by reduced filtrates of group A streptococcal cultures containing active streptolysin O.

Materials and methods. Streptococcal skin lesion extract. The preparation and extraction of this lesion extract was described by Watson and Cromartie(4). This technic consists of intracutaneous injection of 30 ml of an 18-hour Todd-Hewitt broth culture of type 28 group A streptococci in about 30 sites over the shaved abdomen and thorax of 2 kg New Zealand white rabbits. The moribund animals were killed 24 hours after injection of the streptococci, the lesion ground in a meat grinder and extracted overnight at 2°C by stirring with 1 to 2 ml of saline per g of tissue. This was filtered through gauze and centrifuged in the Spinco 30 head at 25,000 rpm for ½ hour. This supernatant was filtered through a Selas bacterial filter and kept frozen in sealed ampules at −70°C. Four lots of this material were used in the work reported in this paper. They are referred to as streptococcal lesion extract 108, 109, 112, or 116. The total nitrogen of extracts 108 and 109 was 1.7 mg N per ml. Extract 116 contained 3.24 mg N per ml. As a preparing dose 0.5 ml of extract 108 or 109 was used. A preparing dose of extract 116 was 0.25 ml. Lesion extract 112 was prepared as above and was then concentrated 10 fold by lyophilization and dialyzed against physiological saline for 24 hours.

Bacterial toxins. Typhoid toxin was prepared from N-28-1 strain of *Salmonella typhosa*. The organism was cultured 9 days in nutrient broth under semi-anerobic conditions. It was then filtered through a Selas bacterial filter and stored in the refrigerator under aseptic conditions Six lots of the toxin were produced. The potency of each lot was

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