Pathogenesis of Fatal Encephalomyocarditis (EMC) Virus Infections in Albino Rats. (22041)

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This paper presents a study of encephalomyocarditis virus infections, carried out by means of virus titrations and histopathologic studies of various organs of albino rats moribund from disease. EMC virus can produce paralysis and fatal encephalitis in albino rats although other species of rats and mongooses, when inoculated with this agent, appear to die principally from myocarditis(1). The subject has additional interest in that previous investigators(2-6) have reported only inapparent infections in albino rats.

Materials and methods. Experimental animals. Albino rats were random bred. Some were originally obtained from Makerere College Medical School and others from Veterinary Research Laboratory, Kabate, Kenya. Hooded rats, which originated at Lister Institute, Elstree, England, were employed for a few experiments. Mice were of the Swiss albino strain. *Virus.* Of 3 strains of EMC virus employed, 2 had been isolated from animals in monkey runs of the Virus Research Institute. The principal one we used was originally obtained from an infected baboon and had been carried through 6 intracerebral passages in mice; the second strain was isolated from a rhesus monkey and had been passaged 16 times in mice; the third strain was one originally ioslated from a chimpanzee in Dania, Florida(7), and had been passed 78 times in mice. This last strain was obtained from Dr. Karl Habel, National Institutes of Health, Bethesda, Md. The rhesus and Florida strains were studied for comparative purposes. *Virus isolations from inoculated animals.* Ten percent tissue suspensions in meat infusion broth containing penicillin and streptomycin were centrifuged at 4000 rpm for ½ hour prior to intracerebral inoculation of 0.03 ml into mice 5 weeks of age. For titrations, groups of 4 mice were used for each 10-fold dilution. Blood obtained by cardiac puncture was heparinized to prevent clotting. Urine, aspirated from the bladder at necropsy, was obtained from only a few animals. Samples of gut were removed routinely from the lower ileum and laid open and flushed free of intestinal contents in 3 changes of broth. Formed feces were obtained by incision of the rectum. Storage for all specimens was at −20°C. Under these conditions, little or no diminution of virus titer in frozen specimens appeared to take place within 4 months. A majority of suspensions, nonetheless, were titrated in mice shortly after being harvested. Inoculated mice were then observed for 8 days for paralysis and death. Specificity of illness induced was judged by a characteristic incubation period of 3-5 days and the clinical picture, of which paralysis of the hind legs was a cardinal feature. Fifty percent end-points were calculated by the method of Reed and Muench(8). *Neutralization tests.* Spot identifications of viruses isolated were made by means of neutralization tests performed with normal and EMC-immune rabbit sera. The antiserum was prepared by immunization of rabbits with brain suspensions of mice injected with the Florida strain of EMC virus. All sera were inactivated at 56°C for ½ hour prior to mixing with an equal volume of virus suspension that would give final dilutions containing 100 or more LD_{50} of virus. Serum-virus mixtures were inoculated into mice intraperitoneally in volumes of 0.1 ml after ½ hour of incubation at room temperature.

Results. EMC virus (baboon strain) was carried through 15 passages in albino rats 3 to 5½ weeks of age by intraperitoneal inoculation of 10% suspensions of brains of ill rats. Repeated passage did not result in any ob-

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TABLE I. Virus Titrations of Various Organs in Albino Rats Infected with EMC Virus. Rats were sick 24 hours or less when sacrificed.

Rat	Strain of virus	Age inoc. in days	Blood	Brain	Fem. mus.	Heart	Spleen	Ileum	Feces	Urine	Incubation period, days
113W	Baboon	5	4.6*	7.7	4.5	2.7	2.3	—	—	—	2
114W	"	11	3.7	7.5	5.3	3.5	3.3	4.5	0	0	3
47W	"	25	<1	4	1	1	1	0	0	—	3
117W	"	32	<1	5.2	0	1	1.5	0	0	—	4
133W	"	72	0†	4	0	<1	<1	0	—	—	9
127W	Florida	30	0	4.5	1.4	0	1.5	0	0	—	4
128W	Rhesus	29	0	5.5	0	2.5	0	0	0	—	5

* Titers expressed as inverse log of dilution killing 50% of mice inoculated.
† 0 = No virus isolated from 1:10 suspension.

served change in virulence. Although rats of all ages might become paralyzed and die, susceptibility, which was 100% in suckling animals, became increasingly unpredictable in rats over 3-4 weeks of age. Severe crippling of one and frequently of all 4 extremities gave rats a helpless appearance and occasional rats had a hemorrhagic, watery exudate along the eyelids. Death usually supervened in one to 3 days. The incubation period was 2-3 days in rats under 2 weeks of age, and 4-6 days, rarely up to 11 days, in older rats. Table I presents comparative titrations of various tissues from albino rats of increasing ages which were killed when moribund. All, except for 47W, were given the same inoculum of 12th passage rat brain intraperitoneally. Suckling rats (113W and 114W) had considerable amounts of virus in all organs tested with highest titers in brain tissue, whereas the older rats (47W, 117W, and 133W) had little recoverable virus from any organs except brain. Seventeen other rats, from 22 to 39 days of age and representing 4th to 14th passages of EMC virus, were also sacrificed when moribund and studied by methods similar to those just described. Although titrations in many cases were limited to blood, brain, and femoral muscle, titers obtained were essentially the same as those obtained for rats 47W, 117W, and 133W (Table I). None of these older rats exhibited the widespread distribution of virus encountered in suckling animals. Two rats, 3 to 4 weeks of age, inoculated intraperitoneally with 13th-passage brain suspensions, developed paralysis of the hind legs within 4 days. Brains, when harvested and pooled, had a titer of $10^{-4.5}$. Lumbar spinal cord from one rat had a titer of $10^{-2.5}$ and from the other of $10^{-2.8}$.

Principal histologic changes encountered in limited examinations of sick rats were observed in the brain. In brains of 3 rats examined, lesions consisted of scattered foci of degeneration and cellular infiltration with lymphocytes and macrophages. No study was made of the localization of these lesions. Lesions were also found in spinal cords from 3 rats examined. These included focal hemorrhages, perivascular infiltration with polymorphonuclear leucocytes, lymphocytes, macrophages, and degeneration and loss of nerve cells. Definite myositis was found in both of two rats examined. These rats, inoculated intraperitoneally at 3-4 weeks of age, had developed crippling of the extremities 5 days later. As illustrated in one rat (Fig. 1), lesions included degeneration of muscle fiber, disappearance of fiber, and a mild infiltration of round cells and polymorphonuclear leucocytes.

Viremia. Albino rats, other than suckling animals, exhibited minimal or no viremia. To determine whether viremia of greater degree might take place during the incubation period, 9 rats 26 days of age, were inoculated intraperitoneally. At 7 hours after inoculation and then daily for 6 days, blood was obtained from alternating pairs and pooled for testing. No virus was recovered from 1:10 suspensions of blood obtained from well rats during these periods, although 7 of the 9 eventually became paralyzed. Blood obtained from a pair with paralysis 3 days after inoculation had a virus titer of $10^{-2.7}$. Two other pairs of rats, however, which became ill 5 and 6 days after in-

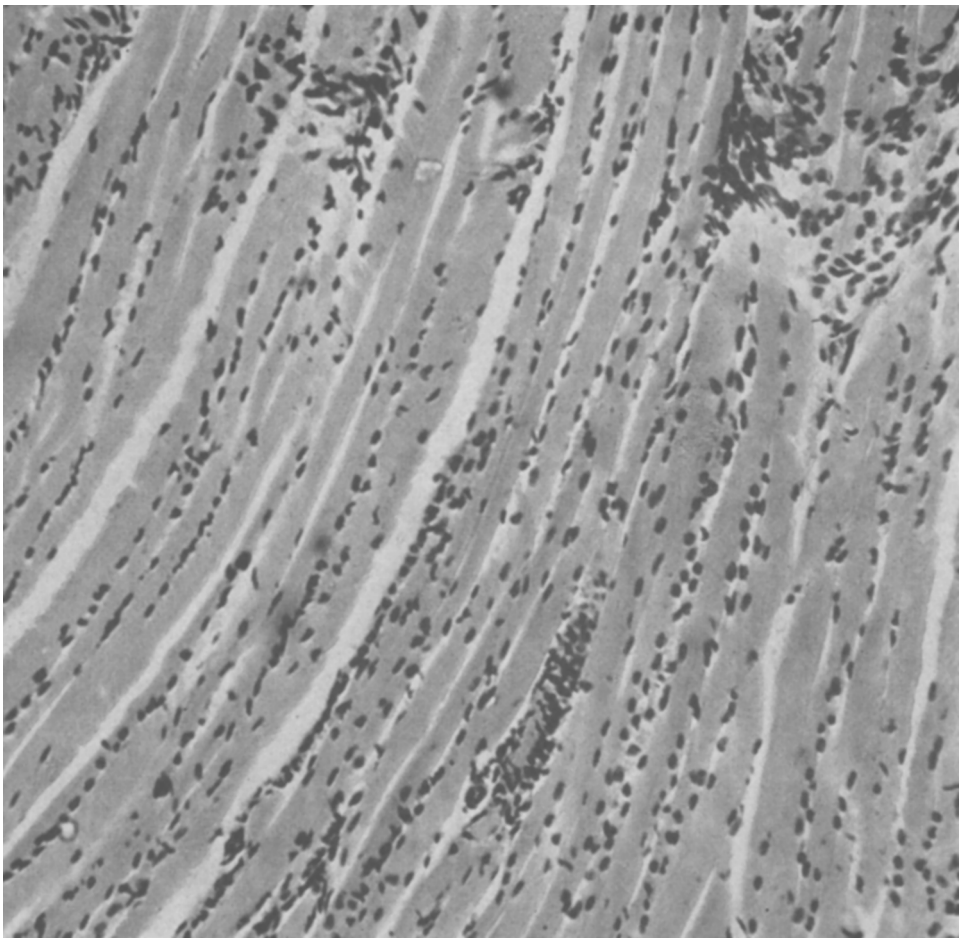

FIG. 1. Myositis in rat skeletal muscle (H & E × 170).

oculation, had no demonstrable viremia.

Inoculation of pregnant rats. Experiments on pregnant rats were performed to determine 1) if disease might be transmitted to offspring and 2) whether this virus, by growth in uterine muscle, might interrupt pregnancy. Seven rats, presumed to be pregnant, were inoculated intraperitoneally. Four of these gave birth to healthy litters 4 to 6 days after inoculation. Mothers and offspring remained well. Two rats, which proved not to be pregnant, developed paralysis of the hind legs, one in 7 days (weight 132 g) and one in 11 days (weight 236 g). Brains of both contained virus in high titer. The 7th rat gave birth in 3 days and at 7 days after inoculation she began dragging her hind-quarters but was still nursing. Of mice inoculated with a 1:10 brain suspension from this rat, only one of 3 died. Such a minimal amount of virus in an inoculated animal was regarded as being of questionable significance. No virus was recovered from a young one sacrificed a day after the mother became ill.

Ingestion and inhalation of virus. Albino and hooded rats were susceptible to infection both by ingestion and by inhalation. They are considered together, since no strain difference in susceptibility was ever apparent throughout present experiments. Of 18 rats 25 to 29 days of age inoculated intranasally with infected rat brain suspensions containing 10^{-3} to 10^{-6} LD_{50} of virus, 9 became paralyzed and died within 4 to 11 days. Two of the ill rats were tested for virus which, though readily recovered from other organs, was not

found in lung suspensions. Rats became ill less readily as a result of ingestion. Thus, 23 rats 3 to 4 weeks of age were fed infected carcasses of rats and mice. Of these, 2 were subsequently found dead and 2 developed paralysis, one in 6 days and another 2 weeks after feeding. EMC virus was recovered from brains of the ill rats. Seven of the survivors, tested for neutralizing antibodies 2½ to 3 weeks after an infective meal, were negative.

Comparative effect of other strains of EMC virus. A final question arose as to whether findings obtained in albino rats resulted from use of a particular strain of EMC virus, the one originally isolated from a sick baboon. For comparative purposes, the 2 other strains were inoculated into albino rats. These strains killed all rats under 2 weeks of age. Of 4 rats, 29-30 days of age, inoculation of the Florida strain intraperitoneally led to death of 2 and paralysis of another within 4 days. Inoculation of 3 rats of similar age with the rhesus strain led to paralysis of all in 5 days. Two rats were alive but crippled after 14 days. One acutely ill rat from both series was sacrificed. Patterns of virus distribution (rats 127W and 128W, Table I) did not differ essentially from those observed in albino rats inoculated with the baboon strain. Myocardium of rat 128W, however, had a titer of $10^{-2.5}$. Possibly this relatively high titer was related to the fact that the rhesus strain of EMC virus, as used in this experiment, had been previously passed through 7 intraperitoneal passages in multimammate rats(1).

Discussion. Induction of paralysis and death in albino rats is in contrast to reports of others(2-6) who described only inapparent infections. Although it is difficult, if not impossible, to explain differences in results, several factors should be considered. One is that only suckling rats are fully susceptible. A lesser number of older rats develop frank disease. As a rough estimate, based on overall experiments, 30% of rats inoculated intraperitoneally at 30 days of age and 10% of rats inoculated when 60 days or older might be expected to develop paralysis. These percentages would doubtless be increased by intracerebral inoculations. A second factor might be that some stocks of albino rats are resistant. Our results, however, were obtained with 3 different strains obtained in Africa and, in an additional experiment, non-inbred albino rats (Osborne-Mendel strain) from the National Institutes of Health, Bethesda, Md., proved susceptible to EMC virus. Strain of virus did not appear to be a factor of importance, since EMC virus originating from Dania, Florida, also led to death and paralysis. The widespread infection with viremia encountered in suckling rats and the limited distribution of virus in adults were findings somewhat parallel to those described by McLaren and Sanders (9). These investigators found that EMC virus grew well in muscle and brain of 5-day-old mice, but not as well in muscle af 30-day-old mice, although brains of the latter remained fully susceptible.

A question of importance is to what extent are titers the result (a) of intrinsic growth of virus within an organ and (b) of residual blood contained in the organ. If titers were secondary effects of a viremia, one would expect to observe 2 types of relationship. First, blood-containing organs such as the spleen should have relatively high titers and, second, any excised specimen of muscle or washed piece of ileum might be expected to have a titer at least 2 logs lower than that of the blood, if one may make estimates from blood volumes calculated by Kaliss and Pressman (10) for mouse organs in which blood vessels were tied off. As shown in Table I for suckling rats, virus presumably multiplied in all organs tested.

Summary. 1. EMC virus can produce a crippling and usually fatal illness in albino rats of all ages. 2. Suckling rats are more susceptible than older rats and experience a greater degree of systemic infection. 3. In older rats, the disease is chiefly localized in the central nervous system, although myositis was likewise observed.

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Glucagon Content of Pancreatic Tissue Devoid of Alpha Cells.* (22042)

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In spite of the large amount of work done in the past 30 years to determine the site of origin of glucagon, there is as yet no general agreement as to whether or not glucagon is the alpha cell hormone(1-5). Some have considered that the alpha cell is the source of glucagon because of differences in insulin requirement of alloxan(6) and metahypophyseal diabetes(7) as compared to that of surgical diabetes(8). This opinion is supported by the presence of a hyperglycemic factor in extracts from normal, alloxanized, and alloxanized duct ligated pancreas(3). An alternative explanation for the differences in insulin requirement was that in surgical diabetes intestinal absorption of nutriments is limited(9). This latter view was controverted by other experiments with duct ligated alloxan diabetic dogs(10). A hyperglycemic factor, apparently identical with glucagon, is present in the stomach of the dog. This was considered to confirm the origin of this substance from the alpha cell of the pancreas because of supposed similarity of argentaffin cell of the stomach with the alpha cell(1). Furthermore in one report, gastro-intestinal argentaffin cells are implicated in disturbances in carbohydrate metabolism(11). However, other workers have not found any correlation between tumours of these cells and diabetes(12). Similarly, glucagon could not be extracted from the stomach of the hog despite the presence of argentaffin cells(1). The lack of identity between the alpha cell and the intestinal argentaffin cell, originally demonstrated by Masson in 1924, has recently been confirmed(13, 14). It would therefore be erroneous to consider that the presence of a hyperglycemic factor in tissues containing argentaffin cells supports the conclusion that the alpha cells serve as the source of glucagon. The discovery of chemical agents apparently capable of damaging alpha cells, rather than clarifying this problem, gave further contradictory results(3-5). Some workers reported alleviation of diabetes by cobalt(15,16), and diethyldithio carbamate(16). Others reported no effect of either of these agents or of synthalin A on the severity of alloxan diabetes (13,17,18). In some studies extracts from the pancreas of cobalt treated animals have been reported to contain lesser quantities of glucagon than normal(4,19). On the other hand, one of us(5,20,21), using qualitative methods of glucagon assay could detect no significant differences between normal and cobalt treated pancreas. Others have reported that cobalt treatment increases the glucagon content of the rabbit pancreas, while synthalin A causes its complete disappearance(22). In this connection, it is of interest that the lesions reported by various investigators after cobalt, are not always identical; it seems to vary with the animal species, the dose and the route of

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