JOURNAL OF VIROLOGY, Oct. 1967, p. 988-995
Vol. 1, No. 5

Effect of Polycations on Growth and Dissemination of the Encephalomyocarditis Virus in Mice

JOHN E. CRAIGHEAD

Departments of Pathology, Peter Bent Brigham Hospital and Harvard Medical School, Boston, Massachusetts 02115

Received for publication 27 June 1967

Pathogenicity of the encephalomyocarditis (EMC) virus for adult mice was increased when polycations of diverse type were mixed with virus and inoculated by the subcutaneous or intraperitoneal routes. Diethylaminoethyl (DEAE) dextran, hexadimethrine (polybrene), polymyxin B, polylysine, and calf thymus histone in various concentrations stimulated multiplication of virus in tissues at the injection site and enhanced entry of virus into the blood. The nonpathogenic r^+ variant of EMC which grows locally in tissues but fails to disseminate after subcutaneous and intraperitoneal inoculation was used in most experiments. This virus caused viremia and fatal central nervous system disease only when the polycations were included in the inoculum. DEAE dextran and polybrene stimulated the release of interferon in infected tissues but had no effect in the absence of virus multiplication. Histological studies of tissues from the injection site showed that polycations provoke a mononuclear cell reaction and alter the integrity of connective tissue. However, the mechanism by which the substances enhance virus growth and dissemination was not defined.

Takemoto and Liebhaber described two variants (r^+ and r) of encephalomyocarditis (EMC) virus which differ in the size of plaques formed in L cells under agar (20). During studies in this laboratory to compare the properties of various EMC strains, it was found that the r^+ (small plaque) variant is nonpathogenic for adult mice when introduced by the subcutaneous and intraperitoneal routes but is virulent when inoculated intracranially (J. E. Craighead, Federation Proc. 24:1073, 1963). In contrast, the r (large plaque) variant, like most "wild" strains of EMC, is highly pathogenic when injected by all three routes.

Detailed studies of mice inoculated subcutaneously with the r^+ variant showed that the virus multiplies in tissues near the injection site for periods of as long as 10 days (4). However, viremia fails to occur and virus cannot be recovered from regional lymph nodes, major viscera, or the central nervous system. The r variant also grows in tissues locally after subcutaneous injection, but by 24 hr it can be isolated consistently from the blood. Thus, it would appear that influences external to the cell selectively affect entry of r^+ variant into the blood stream or lymphatics. There are no data to suggest that interferon or antibody produced during the early stages of the infectious process significantly inhibit growth or dissemination of the virus (*in press*).

Liebhaber and Takemoto showed that the r^+ variant is bound electrostatically by the sulfated acid polysaccharides in agar (12). Since polyanions similar to those in agar are found in abundance in the connective tissue (13, 15), we postulated that these naturally occurring substances might bind virus and limit its spread. The polycations diethylaminoethyl (DEAE) dextran and protamine prevent the union of r^+ virus particles with sulfated acid polysaccharides in vitro (11). Accordingly, attempts were made to enhance growth and dissemination of virus in animal tissues by inoculating mixtures of virus and polycations subcutaneously. The results of these studies are recorded here.

MATERIALS AND METHODS

Virus. The r^+ variant of EMC, supplied by K. K. Takemoto, was plaque-purified twice before stock pools were prepared. Reference is made to revertants in the text below. Plaques formed by these viruses either resembled those of r variant or were intermediate in size between r^+ and r. The latter revertant, designated r^i, was found after purification to be relatively insensitive to the inhibitor dextran sulfate (*in preparation*).

Cell culture and plaque technique. Plaque titrations were carried out in L-929 cells with the use of a 1% Noble Agar (Difco) overlay and 35-mm plastic dishes (Falcon Plastics, Los Angeles, Calif.). Counts were made by neutral red staining 48 or 72 hr after inoculation.

Reagents. DEAE dextran hydrochloride (molecular weight $\approx 2 \times 10^6$ and 8×10^4; Pharmacia, Inc., Uppsala, Sweden), hexadimethrine bromide (Polybrene, molecular weight $\approx$ 3,600; Abbott Laboratories, North Chicago, Ill.), poly-L-lysine hydrobromide (molecular weight $\approx 9.2 \times 10^4$; Pilot Chemicals, Inc., Watertown, Mass.), polymyxin B sulfate (Burroughs-Wellcome, Inc., Tuckahoe, N.Y.), calf thymus histone, type II (Sigma Chemical Co., St. Louis, Mo.), and dextran (molecular weight $\approx 2 \times 10^5$ and 2×10^6; Nutritional Biochemicals Corp., Cleveland, Ohio) were prepared in Hanks' balanced salt solution at *p*H 7.4. Toxicity of the substances, as manifested by acute or protracted mortality, was evaluated in subcutaneously inoculated 3-week mice as a preliminary to the selection of dosages for the studies described below.

Experimental procedure. A polycation solution was added to an equal volume of balanced salt solution containing an appropriate number of plaque-forming units (PFU) of virus. Within 10 min 0.1-ml samples of the mixtures were inoculated into the lateral abdominal wall of 3- or 12-week male Swiss mice. Observations on mortality were done in groups of 10 or 20 animals over a period of 12 days. Control groups of mice received equivalent amounts of either the substance under test or the virus alone. The virus content of tissues and blood serum in each of four animals was determined at 24-hr intervals after inoculation.

Tissue and serum preparation. Skin, subcutaneous tissue, and muscle from the abdominal wall surrounding the injection site were excised widely *en bloc* and mortar-ground with sand in 3 or 5 ml of diluent. Coarse debris was removed by low-speed centrifugation and the supernatant fluid was recentrifuged at $10,000 \times g$ for 15 min. Animals were bled from the retro-orbital sinus. Diluent was added to the blood to make an approximate serum dilution of 1:10, and the clot was separated after 2 to 4 hr. A diluent made up of 5% heated (56 C for 30 min) chicken serum and Hanks' balanced salt solution was used routinely.

Interferon assays. Tissue homogenates and serum were prepared for interferon assays by dialysis for 48 hr at *p*H 2 and centrifugation for 60 min at $10,000 \times g$. Portions (0.2 ml) of serial twofold dilutions of the test material were incubated with L-cell monolayers for 24 hr. Cultures were washed and then challenged with 50 to 70 PFU of vesicular stomatitis virus. Plaque reduction of 50% or greater at 48 hr was used to determine end points. Selected specimens exhibiting interfering activity were shown to possess the established properties of interferon (1).

Histological studies. The lateral abdominal wall was excised from 3-week animals 24 and 48 hr after the subcutaneous injection of 0.1-ml amounts of a mixture of carbon black and various concentrations of DEAE dextran, polybrene, polylysine, or dextran. After fixation in Formalin, histological sections were prepared by use of the deposits of carbon as a guide in the choice of tissue blocks.

RESULTS

Effect of polycations on infection. Virus growth in tissues at the injection site was enhanced and viremia occurred when DEAE dextran, polybrene, polymyxin B, polylysine, and calf thymus histone were mixed with the r^+ variant and introduced into mice by the subcutaneous route. The effect of the polycation was evaluated by titrations of tissues and of blood serums obtained at intervals after inoculation. Several concentrations of the five substances were tested; the virus dosage employed in different experiments varied somewhat but was consistently smaller than 50 PFU per animal.

The results of studies in 3-week mice during the 48-hr period after inoculation of a mixture of r^+ variant and 50 μg of polybrene are shown in Fig. 1. The data are representative of experiments using this substance at dosages ranging from 50 to 500 μg per animal. More virus (10- to 1,000-fold) was recovered from the tissues of mice receiving the polycation than from appropriate controls. Although the r^+ variant predominated in the tissue homogenates, substantial numbers of r^i and r PFU were present. Virus appeared promptly in the blood. At 24 hr, r^+ PFU usually were found; later, only r^i and r were recovered. Viremia in animals receiving polybrene persisted for 72 to 96 hr, at which time signs of central nervous system involvement became evident. Virus was never isolated from the blood of animals which had not received polybrene, even though it was present in tissues surrounding the injection site for periods of 120 hr.

Similar observations were made with DEAE dextrans of two molecular weights (8×10^4 and 2×10^6) in dosages ranging from 50 to 500 μg. Although repeated comparative studies were not carried out, tissue and serum titers appeared to be greater at 24 hr when the higher concentrations of DEAE dextran were employed. As with polybrene, a heterogeneous population of PFU usually was found in the blood 48 or 72 hr after inoculation. Often r^i or r PFU were recovered from the blood of animals whose tissues yielded a predominant population of the r^+ variant (Fig. 2).

Polylysine (Fig. 3), polymyxin B, and calf thymus histone (Fig. 4) enhanced virus growth in tissues when animals were administered either 250 or 500 μg mixed with r^+. Only data obtained with the lower concentration of the substances

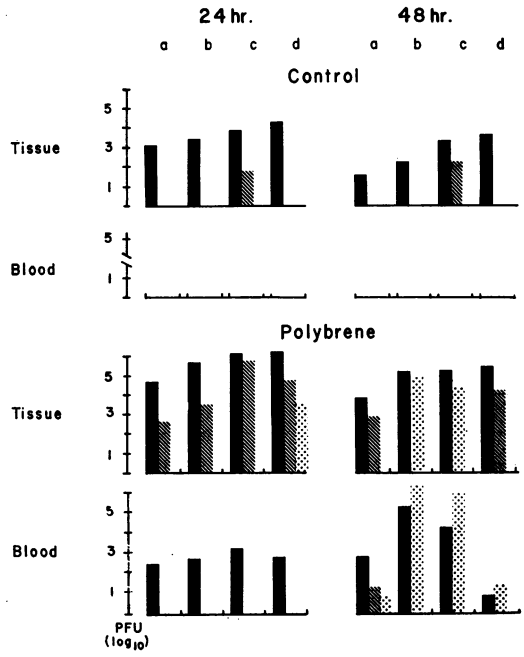

FIG. 1. *Recovery of virus from tissues surrounding the injection site and blood 24 and 48 hr after the subcutaneous inoculation of 3-week mice with 50 PFU of r^+ variant and polybrene (50 μg per animal). In this and subsequent figures, data obtained on four animals (a–d) from each group at the indicated time interval are shown. Tissue and serum specimens were titered in L-cell monolayers under an agar overlay. Solid bar = r^+, lined bar = r^i, dotted bar = r variant.*

are shown in the figures, since results with both dosages were comparable. Virus was recovered from the blood of most animals at some time during the 72-hr period following inoculation. However, the time of onset of viremia was somewhat more variable than with polybrene and DEAE dextran. Similar experiments with two different commercial preparations of protamine were negative.

In the absence of a polycation, approximately 8 PFU of r^+ variant are required to initiate a tissue infection in 3-week animals (*in press*). A calculated 6 and 9 PFU, respectively, were used in experiments with calf thymus histone and DEAE dextran. Growth of virus in tissues was demonstrated only when the virus was inoculated with the polycations (Fig. 4). Studies with small dosages of virus and other polycations were not carried out.

DEAE dextran enhanced r^+ replication in the tissues of 12-week mice. In contrast to results with younger animals, viremia was transient and was infrequently followed by signs of nervous system involvement and death. Studies in 12-week mice with polycations other than DEAE dextran were not attempted.

To determine the specificity of the polycation effect on growth and systemic dissemination of virus, experiments were conducted using the pathogenic r variant and the genetically unrelated, but antigenically similar, M variant of EMC (3). DEAE dextran not only stimulated the early multiplication of r in tissue but shortened

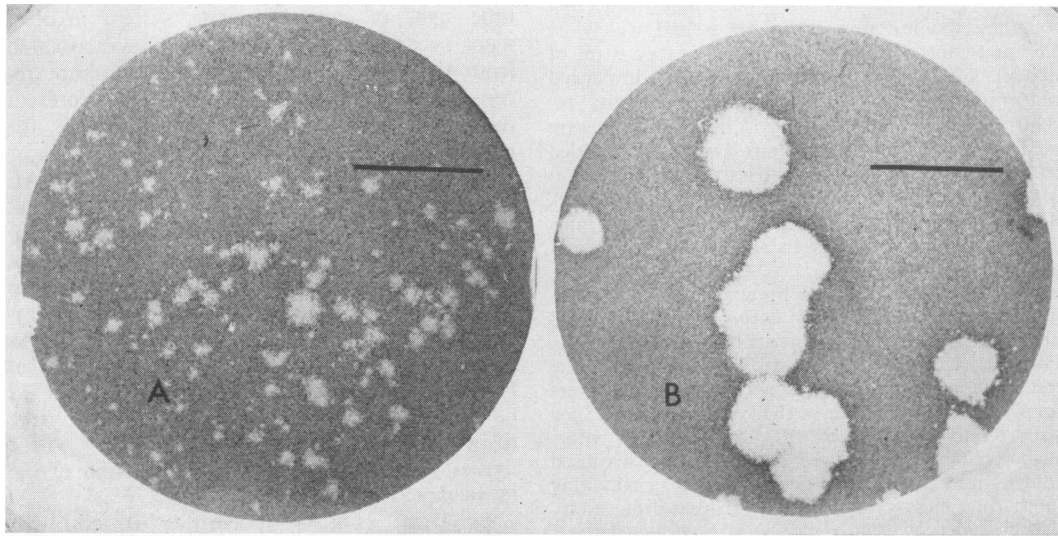

FIG. 2. *Agar overlaid, monolayer plate cultures of L cells inoculated with a tissue homogenate (A) and serum (B) from a 3-week mouse killed 48 hr after subcutaneous inoculation of 50 PFU of r^+ mixed with DEAE dextran (500 μg per animal). Plates were stained with neutral red and photographed 3 days after inoculation. By day 5, plaques in plate B increase to a diameter of 8 to 12 mm. Size of plaques in plate A increased only slightly or not at all. Scale = 1 cm.*

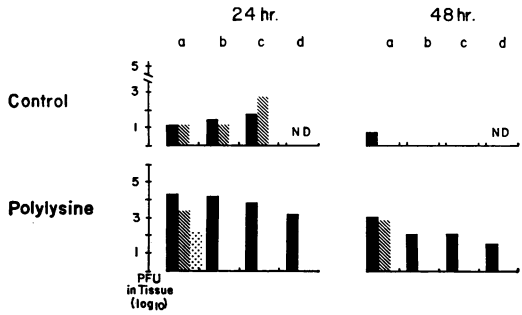

FIG. 3. *Recovery of virus from tissues surrounding the injection site 24 and 48 hr after subcutaneous inoculation of 3-week mice with 30 PFU of r+ variant and polylysine (250 μg per animal). ND = not done.*

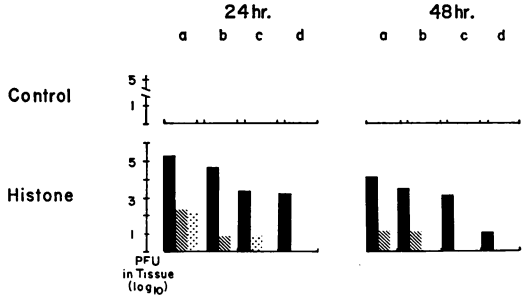

FIG. 4. *Recovery of virus from tissues surrounding the injection site 24 and 48 hr after subcutaneous inoculation of 3-week mice with 6 PFU of r+ variant and calf thymus histone (250 μg per animal).*

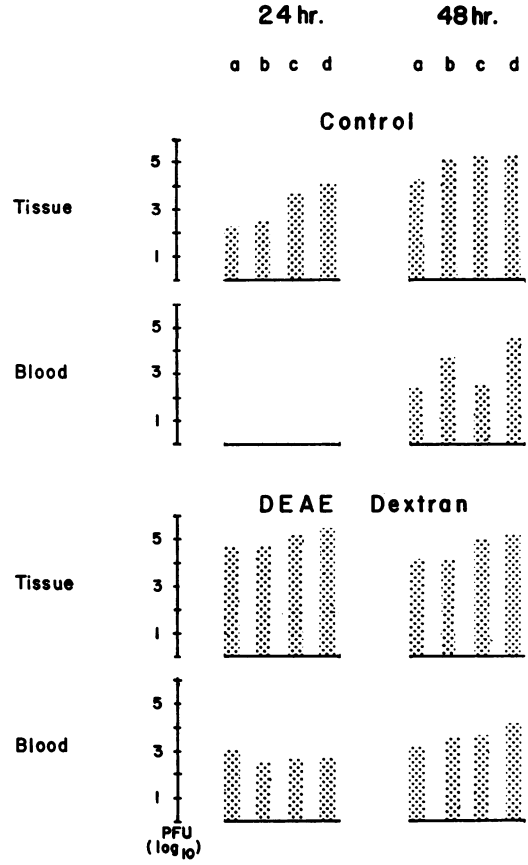

FIG. 5. *Recovery of virus from tissues surrounding the injection site and serum 24 and 48 hr after subcutaneous inoculation of 3-week mice with 8 PFU of r variant and DEAE dextran (molecular weight 2 × 10⁶, 50 μg per animal).*

the interval between inoculation and the appearance of viremia. The data shown in Fig. 5 were obtained with 3-week animals. An identical effect was observed with 12-week mice. Results with the M variant of EMC were similar.

Dextran (molecular weight 2×10^6) in various concentrations had no effect on growth of r^+ variant at the injection site or on the occurrence of viremia.

Effect of polycations on interferon release. Data on the recovery of interferon from mice inoculated subcutaneously with the r^+ variant are presented in Table 1. Interferon was not demonstrated in animals receiving the virus alone, but was readily detected in both the tissues and blood of mice which had been given DEAE dextran or polybrene mixed with the virus. The presence of interferon was associated with virus titers of greater than 10^3 PFU. Similar results were obtained with the r variant. Two different dosages of DEAE dextran were employed in the experiments shown in Table 2. Although the data are limited, it would appear that increased amounts of interferon were released by the tissue of animals receiving the larger dosages of DEAE dextran. DEAE dextran and polybrene in the concentrations employed in these studies failed to stimulate the elaboration of interfering substances in the tissues and blood of uninfected mice. Since polycations in low concentrations increase plaquing efficiency of vesicular stomatitis virus (*in preparation*), tissues from animals receiving one or the other of the two polycations were used routinely as controls in interferon assays.

Effect of polycations on mortality due to infection. Figure 6 shows the cumulative mortality at daily intervals for mice which had been inoculated with mixtures of the r^+ variant and various concentrations of polybrene. Death of the animals usually occurred between the 4th and 7th day, and was preceded by the paralysis and encephalitis characteristic of EMC virus.

TABLE 1. *Virus and interferon in tissues and serum at intervals after the subcutaneous inoculation of 43 PFU of r+ variant mixed with DEAE dextran or polybrene*

Polycation	24 hr				48 hr				72 hr			
	Tissue		Serum		Tissue		Serum		Tissue		Serum	
	V[a]	I[b]	V	I	V	I	V	I	V	I	V	I
Balanced salt solution	1×10^2	0	0	0	3×10^2	0	0	0	6×10^2	0	0	0
DEAE dextran, 50 μg per animal	6×10^4	10	1×10^1	0	1×10^4	15	3×10^4	150	1×10^4	12.5	7×10^3	33
Polybrene, 50 μg per animal	7×10^5	10	0.3×10^1	0	2×10^4	12.5	4×10^3	0	2×10^4	30	1×10^4	150

[a] V = virus recovered from tissues (PFU per animal) or serum (PFU per milliliter).
[b] I = Interferon recovered from tissues (units per animal) or serum (units per milliliter).

TABLE 2. *Virus and interferon in tissues and serum at intervals after the subcutaneous inoculation of 8 PFU of r variant mixed with DEAE dextran*

Polycation	12 hr				24 hr				36 hr			
	Tissue		Serum		Tissue		Serum		Tissue		Serum	
	V[a]	I[b]	V	I	V	I	V	I	V	I	V	I
Balanced salt solution	1×10^2	0	0	0	3×10^3	0	0	0	1×10^5	0	7×10^3	0
DEAE dextran, 50 μg per animal	3×10^3	0	0.3×10^1	0	3×10^5	0	5×10^2	30	7×10^4	40	3×10^4	30
DEAE dextran, 500 μg per animal	1×10^3	0	0.5×10^1	0	7×10^5	80	3×10^4	130	5×10^5	160	2×10^4	260

[a] V = Virus recovered from tissues (PFU per animal) or serum (PFU per milliliter).
[b] I = Interferon recovered from tissues (units per animal) or serum (units per milliliter).

Results identical to those in Fig. 6 were obtained with DEAE dextran over a wide range of dosages.

Similar but more limited studies were carried out with histone, polylysine, and polymyxin B (Fig. 7). A substantial proportion of the animals receiving r+ variant mixed with both 250 and 500 μg succumbed as a result of a generalized EMC infection. However, deaths were distributed more irregularly over the 10-day period after inoculation than with either polybrene or DEAE dextran, and the percent mortality was lower. This observation may reflect the variable occurrence of viremia noted after the administration of histone, polylysine, and polymyxin.

Animal mortality was increased significantly when mixtures of r+ variant and DEAE dextran were introduced by the intraperitoneal and intramuscular routes. The effective range of polycation dosage by these routes was not determined. In the experiments described above, mice were injected within the 10-min period immediately after the addition of polycation to virus. Results were not affected when a mixture of DEAE dextran and r+ was held at 4 C for 24 hr before inoculation.

Graded concentrations of dextran (molecular weights of 2×10^5 and 2×10^6), sucrose, protamine sulfate, and two commercial preparations of testicular hyaluronidase failed to affect r+ pathogenicity when the substances were inoculated subcutaneously with the virus. Mortality attributable to disseminated EMC infection was not increased by the administration of cortisone acetate (1.25 mg per day) to 3-week mice for 10 days preceding r+ inoculation and at daily intervals thereafter. This dosage of steroid substantially increased mortality in mice infected

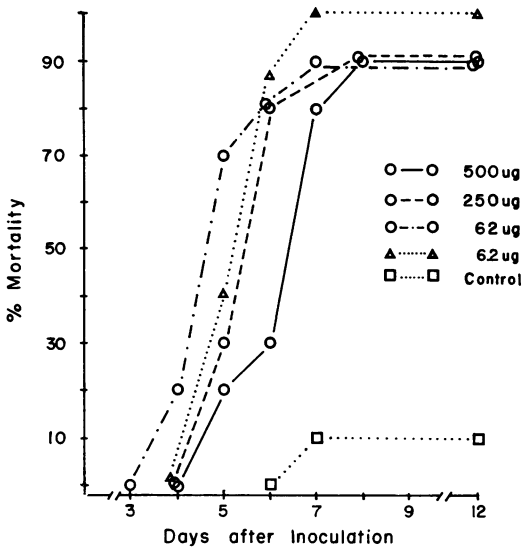

FIG. 6. *Cumulative mortality in groups of 3-week mice inoculated subcutaneously with 25 PFU of r+ variant and polybrene in various concentrations.*

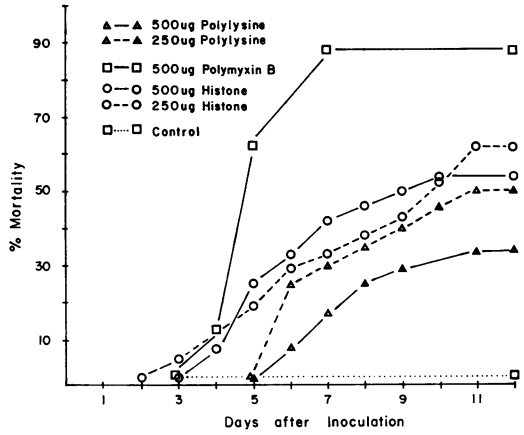

FIG. 7. *Cumulative mortality in groups of 3-week mice inoculated subcutaneously with r+ variant and polycations. The dosages of virus were: polymyxin B, 25 PFU; polylysine, 20 PFU; calf thymus histone, 6 PFU.*

with the relatively nonpathogenic M variant of EMC.

Tissue studies. Histological sections of tissue surrounding the subcutaneous injection site were prepared from animals which had been administered mixtures of carbon black and DEAE dextran, polybrene, polylysine, dextran, or 2 N sucrose. Prominent accumulations of mononuclear cells, edema, and vascular congestion (Fig. 8) were found immediately beneath the dermis 24 hr after the administration of DEAE dextran over a wide range of dosages (12.5 to 500 μg). Carbon particles were distributed extensively in connective tissue and were located frequently within macrophages. These cells also contained varying amounts of an eosinophilic, periodic acid Schiff-staining material presumed to be DEAE dextran. Changes in the tissues of animals which had been given polybrene and polylysine were less prominent. Indeed, in many specimens the number of accumulated mononuclear cells was no greater than in mice which had received dextran or hypertonic sucrose. The extent of the alterations, however, varied from animal to animal. Attempts to define histochemically changes in the polysaccharide composition of connective tissue after polycation injection by use of toluidine blue in an acid *p*H range yielded inconclusive results.

DISCUSSION

Liebhaber and Takemoto showed that the r+ variant of EMC virus is electrostatically bound by the naturally occurring sulfated acid polysaccharides in agar (12). Since this variant is nonpathogenic for adult mice when inoculated by the subcutaneous or intraperitoneal routes, we postulated that sulfated acid polysaccharides in the peritoneal cavity (14) and ground substance

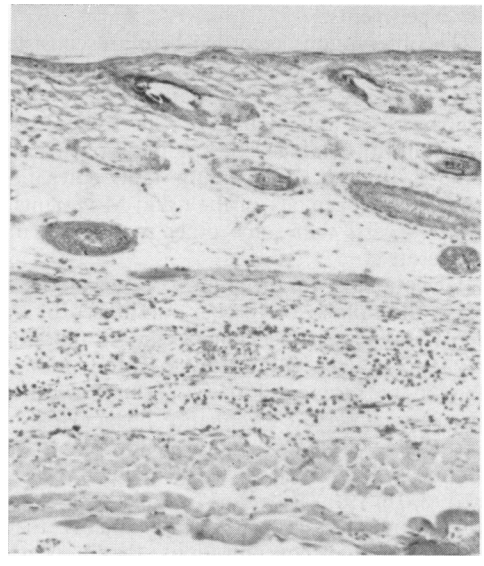

FIG. 8. *Skin, subcutaneous tissue, and muscel from lateral abdominal wall of 3-week mouse sacrificed 24 hr after subcutaneous inoculation of 250 μg of DEAE dextran (molecular weight 2 × 10⁶) mixed with carbon black. Note the mononuclear cell infiltrate, edema of connective tissue, and scattered accumulations of carbon beneath the dermis. Hematoxylin and eosin stains, 5-μ section.*

of connective tissue (13, 15) might tie up virus in a similar fashion and inhibit its spread in animal tissues. The work reported here was undertaken initially in a search for methods to test this hypothesis.

Plaques of the r^+ variant increase in size when DEAE dextran and protamine are added to an agar overlay (11). Presumably, the polycations interfere with binding of the virus particle by the agar inhibitor and permit spread of virus to neighboring, susceptible cells in the monolayer. We reasoned that it might be possible to enhance dissemination of virus in the tissues of the intact animal and permit access to vascular channels by introducing virus mixed with polycations.

The macromolecules employed in this study were selected because of their highly basic charge and diverse chemical make-up. The five active substances bridge a wide range of molecular weights. All are polydisperse. Polybrene has been employed clinically to counteract the anticoagulant properties of heparin (9); inhibition of r^+ variant growth by this polyanion is reversed by DEAE dextran (11). The toxicity of polymyxin for mice can be prevented by the systemic administration of heparin (7). It is of interest that two commercial preparations of protamine, another heparin antagonist, were inactive in our experiments.

The observations recorded here are consistent with the notion that r^+ virus particles interact with naturally occurring sulfated acid polysaccharides in animal tissues. Our results, however, fail to establish the validity of this hypothesis. Indeed, the finding that polycations enhance pathogenicity of the r and M variants of EMC suggests that some other mechanism may be operative.

Polycations in appropriate concentrations increase the uptake of foreign substances by cultured cells. Ryser and Hancock found that basic polyamino acids stimulate the imbibition of albumin by sarcoma 180 cells (18). Polycations have been observed to enhance phagocytosis by leukocytes (5) and the adsorption of foreign ribonucleic acid and virus by animal cells in vitro (8, 16, 19). The means by which polycations act under these circumstances remains to be defined. At high concentrations, they appear to exert a direct "toxic" effect on the cell membrane and thus increase permeability (17). Although it is possible that the substances used in our experiments affected susceptibility of individual cells of the intact animal, evidence accumulated in this laboratory has shown that DEAE dextran, polybrene, and polylysine over a wide range of concentrations not only inhibit uptake of r^+ particles by L cells but fail to stimulate growth of the virus in already infected cells (*in preparation*). Likewise, Colter et al (2) noted that protamine blocks the receptor sites on L cells for the small plaque variant of mengovirus.

Histological studies on tissues from the subcutaneous site of inoculation indicated that mononuclear cells accumulate in connective tissue promptly after the introduction of polycations. It is possible that these cells supported virus growth or transported phagocytized virus to susceptible cells distant from the inoculation site. The reproducibility of our results and the variable monuclear response to different polycations and among individual animals makes such an explanation seem unlikely. The effect of polycations appears to be specific. Neutral or negatively charged macromolecules and the spreading factor, hyaluronidase, failed to enhance virus growth or dissemination. The fate and distribution of virus in connective tissue as well as the histological and histochemical changes after polycation injection are presently under investigation.

DEAE dextran and polybrene stimulated the release of interferon in the tissues of infected animals. This effect was associated with increased virus multiplication at the injection site. Since polycations alone were ineffective, they would appear to enhance interferon production by a mechanism which differs from that of the anionic polysaccharide, statolon (10). Mononuclear phagocytes may have played a role in the elaboration of interferon, in view of their abundance in tissues after polycation injection and their demonstrated capacity to stimulate release of the substance (6). It seems unlikely that interferon significantly influenced the course of the local infection or dissemination of virus.

Aside from their theoretical interest, the observations recorded here may have practical implications. It would be of interest to determine whether or not polycations increase the susceptibility of animals used in virus isolation attempts. It remains to be determined whether or not the polycation effect is limited to the EMC group of viruses. Thus far, our efforts to enhance replication or affect the virulence of coxsackie group B and sindbis viruses in adult mice have failed.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grants AI-6481, AI-5494, and HE-6370 from the National Institutes of Health.

The assistance of Mary Francis McLane and Catherine Layne gratefully is acknowledged.

LITERATURE CITED

1. BARON, S., AND C. E. BUCKLER. 1963. Circulating interferon in mice after intervenous injection of virus. Science **141**:1061–1063.
2. COLTER, J. S., M. A. DAVIES, AND J. B. CAMPBELL. 1964. Studies of three variants of mengo encephalomyelitis virus. II. Inhibition of interaction with L cells by an agar extract and by protamine. Virology **24**:578–584.
3. CRAIGHEAD, J. E. 1966. Pathogenicity of the M and E variants of the encephalomyocarditis (EMC) virus. I. Myocardiotropic and neurotropic properties. Am. J. Pathol. **48**:333–342.
4. CRAIGHEAD, J. E. 1967. Polycation effect on virulent nonpathogenic r^+ variant of encephalomyocarditis (EMC) virus. Nature **214**:716–717.
5. DEVRIES, A., J. SALGO, Y. MATOTH, A. NEVO, AND E. KATCHALSKI. 1955. Effect of basic polyamino acids on phagocytosis *in vitro*. Arch. Intern. Pharmacodyn. **104**:1–9.
6. GLASGOW, L. A., AND K. HABEL. 1963. Interferon production by mouse leukocytes *in vitro* and *in vivo*. J. Exptl. Med. **117**:149–160.
7. HIGGINBOTHAM, R. D. 1959. The mast cell. Trans. Conf. on Polysaccharides in Biology, 4th Madison, N.J., p. 159–210.
8. KAPLAN, M. M., T. J. WIKTOR, R. F. MAES, J. B. CAMPBELL, AND H. KOPROWSKI. 1967. Effect of polyions on the infectivity of rabies virus in tissue culture: construction of a single-cycle growth curve. J. Virol. **1**:145–151.
9. KIMURA, E. T., P. R. YOUNG, AND G. H. BARLOW. 1962. A study with low and high molecular weights of hexadimethrinebromide—an anti-heparin agent. Proc. Soc. Exptl. Biol. Med. **111**:37–41.
10. KLEINSCHMIDT, W. J., AND E. B. MURPHY. 1965. Investigations on interferon induced by statolon. Virology **27**:484–489.
11. LIEBHABER, H., AND K. K. TAKEMOTO. 1961. Alteration of plaque morphology of EMC virus with polycations. Virology **14**:502–504.
12. LIEBHABER, H., AND K. K. TAKEMOTO. 1963. The basis for the size differences in plaques produced by variants of encephalomyocarditis virus. Virology **20**:559–566.
13. LOEWI, G., AND K. MEYER. 1958. The acid mucopolysaccharides of embryonic skin. Biochim. Biophys. Acta **27**:453–456.
14. MERGENTHALER, D. D., AND G. H. PAFF. 1956. Peritoneal mast cells as a possible source of circulatory heparin in the rat. Anat. Rec. **126**:165–175.
15. MUIR, H. 1964. Chemistry and metabolism of connective tissue glycosaminoglycans (mucopolysaccharides), p. 101–154. *In* D. Hall [ed.] Intern. Rev. Conn. Tissue Res., vol. 2. Academic Press, Inc., New York.
16. PAGANO, J. S., AND A. VAHERI. 1965. Enhancement of infectivity of polio virus RNA with diethylaminoethyl-dextran (DEAE-D). Arch. Ges. Virusforsch. **17**:456–464.
17. RYSER, H. J. P. 1967. Studies on protein uptake by isolated tumor cells. III. Apparent stimulations due to pH, hypertonicity, polycations or dehydration and their relation to the enhanced penetration of infectious nucleic acids. J. Cell Biol. **32**:737–750.
18. RYSER, H. J. P., AND R. HANCOCK. 1965. Histones and basic polyamino acids stimulate the uptake of albumin by tumor cells in culture. Science **150**:501–503.
19. SMULL, C. E., AND E. H. LUDWIG. 1962. Enhancement of the plaque-forming capacity of poliovirus ribonucleic acid with basic proteins. J. Bacteriol. **84**:1035–1040.
20. TAKEMOTO, K. K., AND H. LIEBHABER. 1961. Virus polysaccharide interaction. I. An agar polysaccharide determining plaque morphology of EMC virus. Virology **14**:456–462.