Antiviral Activity of Chlorite-Oxidized Oxyamylose, a Polyacetal Carboxylic Acid

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Intraperitoneal injection of chlorite-oxidized oxyamylose (COAM) protected mice against mingo, vaccinia, Semliki Forest, and influenza APR8 viruses. Topical administration in the eye of rabbits partially inhibited the development of experimental herpetic keratoconjunctivitis. COAM resembled polyacrylic acid in many aspects, but it was markedly less toxic. For systemic administration, the therapeutic index was on the order of magnitude of 1:300 to 1:500. Although the *in vivo* antiviral effect of COAM were off faster than that of polyacrylic acid, protection lasted for several weeks. Against mengovirus, such prolonged protection was achieved only when polymer and virus were injected intraperitoneally. Protection against intravenous vaccinia virus was not dependent on the injection route of COAM. Experiments on the mode of action of COAM pointed to macrophages as possible mediators of the antiviral effect. The fact that small amounts of interferon appeared in the serum after administration of high doses of COAM suggests that interferon may play a role in the induction of antiviral resistance by COAM.

In a previous communication (2), we reported on a novel type of antiviral substances. The chlorite-oxidized oxypolysaccharides (CO-oxypolysaccharides) are polycarboxylic acids. In contrast to polyacrylic acid and pyran copolymer, for which antiviral activity was described earlier (3, 4, 6, 7, 9-11, 13; P. De Somer, Proc. Int. Symp. on Interferon, Lyon, France, 1969, *in press*; W. Regelson and T. C. Merigan, Proc. Amer. Ass. Cancer Res., 1967, Abstr. 219), the CO-oxypolysaccharides have a backbone which, in addition to (C-C)-bonds, contains acetal linkages. This makes these compounds more likely to be biodegradable than polyacrylic acid and may also give them the advantage of being less toxic.

In the present work, one member of the CO-oxypolysaccharides group, CO-oxyamylose (COAM), was extensively studied. Its antiviral effect in different systems is described herein, and available information on its mode of action and pharmacology are presented.

MATERIALS AND METHODS

The preparation of polyacrylic acid and COAM was described in previous papers (2, 6). Single-stranded polyanionic acid (poly-I_n) and polycytidylic acid (poly-C_n) were obtained from P-L Biochemicals, Milwaukee, Wis. Stock solutions containing 1

mg/ml were kept frozen at -20°C. Double-stranded poly-I_n:C_n was prepared by mixing equal volumes of stock solutions of poly-I_n and poly-C_n and adding MgCl₂ to a final concentration of 5×10^{-3} M.

Cell cultures were grown in a modified Eagle's medium containing lactalbumin hydrolysate and 10% calf serum. Mouse macrophages were obtained by peritoneal washing of adult NMRI mice, 4 days after intraperitoneal injection of 0.5 ml of proteose-peptone (Difco). The cells were plated, washed three times, and refed after 45 min to remove small lymphocytes.

Semliki Forest virus was grown in mouse embryo fibroblast monolayers. Influenza APR8 and Newcastle disease virus (NDV) were propagated in 11-day-old embryonated chicken eggs. Herpesvirus (oral strain) was grown on first-passage rabbit kidney cells. Sindbis virus was grown in primary chick embryo monolayers. Vaccinia virus used for the mouse tail lesion test (1) was obtained as a calf lymph preparation from the State Vaccine Office, Brussels, Belgium.

RESULTS

Vaccinia tail lesion and mengovirus protection tests. A first system in which COAM was studied was the vaccinia tail lesion protection test in mice (1). Groups of 12 mice (12 to 14 g) were injected intraperitoneally with decreasing doses of COAM as indicated in Fig. 1. One day later, they were given an intravenous injection of vaccinia virus. Pox lesions on the tail were counted 7 days later. Figure 1 shows a significant

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FIG. 1. Influence of COAM on the number of vaccinia virus-induced pox on the tails of mice. Each point represents the averaged pox count of 12 mice (female NMRI mice, 12 to 14 g). COAM was given intraperitoneally 24 hr before intravenous injection of vaccinia virus.

and progressive decrease in pox number as the dose of COAM was increased. Under the conditions of our laboratory, a 50% reduction in pox number was statistically significant at the $P < 0.05$ level. For this reason, 50% reduction was chosen as an end point for determinations of lowest active doses. The particular batch of COAM for which the test is shown in Fig. 1 had a "50% inhibitory dose" of 55 µg/mouse or 4.2 mg/kg.

To determine whether COAM, like polyacrylic acid (3), can protect mice over a period of several weeks, groups of 12 mice (12 to 14 g) were injected intraperitoneally with different doses of COAM (3, 0.3, and 0.03 mg per mouse). One series of groups was given vaccinia virus intravenously after 7 days; the other one, after 14 days. Table 1 shows the results expressed as per cent reduction as compared with the control group. Protection was observed in mice challenged with vaccinia virus 7 days after a sufficient dose of COAM; no protection was present after 14 days. In this respect, COAM clearly differed from polyacrylic acid, which, under identical conditions, protected the mice for over 2 months against vaccinia virus (3). Such prolonged protection was explained by the persistence of the compound in the tissues. The fact that protection induced by COAM wore off may have been due to slow degradation of COAM in the organism. Alternatively, it may be explained by development of refractoriness to COAM, analogous to the hyporeactive state occurring after administration of other interferon inducers (5, 8, 11, 14). The following experiment was carried out to distinguish between these two possibilities. Each of the mice in four groups of 36 mice was injected with 0, 0.1, 0.3, or 1 mg of

TABLE 1. Reduction in pox development by COAM administration at different times before intravenous vaccinia inoculation^a

Dose of COAM (intraperitoneally) mg/mouse	Time interval between COAM and vaccinia virus	
	7 days	14 days
3	-49 ($P < 0.05$)	+12 (NS)
0.3	-43 ($P < 0.0025$)	-20 (NS)
0.03	+11 (NS)	-26 (NS)

^a Anti-vaccinia virus effect is given by the change (per cent) in average pox count in groups of 12 mice (female NMRI, 12 to 14 g). In parentheses, P values for comparison with the control groups are given; NS = not significant ($P > 0.05$).

COAM. After 2 weeks each dosage group was split in three subgroups, and the mice in each subgroup received 0, 0.3, or 1 mg of COAM. One day after this injection, vaccinia virus was given intravenously to all mice. Table 2 shows the average number of pox lesions recorded for each subgroup. It can be seen that residual activity of the first injection was minimal (statistically not significant). The second dose had the expected effect, regardless of whether or not the animals had been pretreated with COAM. These results rule out the possibility that the mice became refractory to the antiviral effect of COAM, and favor the interpretation that the drug is broken down or eliminated.

Suggestive reductions in pox count were noted when COAM was administered 1 day after vaccinia virus. No influence was observed when the compound was given beyond that time. It can be concluded that COAM inhibits an early step in the pathogenesis of vaccinia virus infection. This would be consistent with the hypothesis that it acts by stimulating the interferon mechanism. Indeed, a certain time would be required before interferon is produced and starts to act. By that time, the virus infection would be well advanced. Moreover, it is known that interferon does not influence the development of pox lesions when given after the inoculation of vaccinia virus (3).

The duration of the antiviral activity induced by COAM was also tested in the mengovirus protection test. Groups of 12 mice (20 g) were given COAM intraperitoneally in different doses and at different time intervals before and after intraperitoneal mengovirus inoculation. Each group was observed for 2 weeks after virus inoculation, and surviving mice were counted each day. A mortality-time curve was drawn, and the time (days) of 50% mortality was graphically

TABLE 2. *Anti-vaccinia virus effect of COAM after repeated intraperitoneal doses^a*

Second dose (given 14 days after the first one)	First dose			
	0 mg	0.1 mg	0.3 mg	1 mg
mg/mouse				
0	21.9 (3.02)	14.4 (2.38)	16.4 (3.70)	16.4 (3.33)
0.3	3.6 (0.58)	4.1 (0.94)	3.4 (0.91)	5.4 (0.94)
1.0	3.8 (0.89)	8.8 (1.27)	5.3 (1.31)	2.6 (0.39)

^a Vaccinia virus was given 1 day after the second dose of COAM. Figures represent the average pox count on the tail 7 days after vaccinia virus inoculation. The standard error is given in parentheses ($N = 12$).

determined. A shift of 1 day in 50% mortality time is significant at the $P < 0.05$ level. As shown in Table 3, a dose of about 0.1 mg of COAM per mouse (i.e., 5 mg/kg) was sufficient to give significant protection over a 24-hr period. Higher doses were needed to obtain protection for longer periods: a single dose of 3.2 mg/mouse (i.e., 160 mg/kg) was sufficient to keep the mice resistant for 30 days.

Obviously, protection against mengovirus was of longer duration than that against vaccinia virus. The following experiment suggests that this may be related to the fact that, in the regular mengovirus protection tests, both COAM and mengovirus were injected intraperitoneally.

A mengovirus protection test was carried out in which COAM was given subcutaneously, intraperitoneally, or intravenously at dosages indicated in Table 4. A vaccinia tail lesion protection test was carried out in parallel. Protection against mengovirus was markedly less pronounced when COAM was given subcutaneously than when it was given intraperitoneally. In the vaccinia tail lesion test, subcutaneous and intraperitoneal administration were equivalent. Intravenous administration, in which the dose was restricted by acute toxicity to 0.3 mg/mouse, was ineffective in the mengovirus test but was as effective as the other administration routes in the vaccinia tail lesion test. These results suggest that interaction of mengovirus and COAM takes place at the level of peritoneal cells, whereas a similar interaction between COAM and vaccinia virus is more diffusely distributed over the organism. Probably, after intraperitoneal injection, active concentrations of COAM are maintained in the peritoneum for a longer time than in the other tissues. This would explain a more prolonged effect against mengovirus than against vaccinia virus.

Antiviral activity in other in vivo test systems. Antiviral activity of COAM was also tested in mice injected intraperitoneally with Semliki Forest virus. Groups of 10 weaned female NMRI

TABLE 3. *Protection against mengovirus in mice given COAM intraperitoneally at different time intervals before or after the virus*

Dose of COAM (mg/mouse)	Time of COAM administration, days before (–) or after (+) intraperitoneal mengovirus injection			
	–30 days	–15 days	–7 days	–1 day
3.20	1.7 ^a	>7	>7	3.3
1.00				1.8
0.32	1.0	0.6	4.3	1.4
0.10				1.6
0.03	–0.4	0	0.1	0.7

^a Anti-mengovirus effect given as days of delay in 50% mortality time in groups of 12 mice (female NMRI, 20 g). A delay of 1 day is statistically significant at the $P < 0.05$ level. With the 3.2-mg dose, 50% mortality was delayed 0.2 days when the drug was given 1 or 2 days after virus injection.

mice were injected intraperitoneally with COAM (2 mg/mouse) or polyacrylic acid (0.5 mg). One day later, the animals were given an intended LD₅₀ of Semliki Forest virus intraperitoneally. A group of nontreated mice was included. Figure 2 shows an increased survival time in the treated mice. A similar test was carried out by means of an intranasal challenge with an intended 10 LD₅₀ of influenza APR8 virus. An increased survival time of 1 day was observed in mice treated with 2 mg of COAM per mouse, and an increase of 2 days occurred in those given 10 mg/mouse (Fig. 3).

Finally, COAM was tested in herpetic keratitis of rabbits. Groups of two rabbits were used. Herpes infection of the eye was established by superficial scarification of the cornea and subsequent inoculation of herpesvirus (oral strain, 0.01 ml of undiluted tissue culture fluid containing 10⁷ plaque-forming units/ml). The treatment consisted of ocular instillation of 0.01 ml of saline (control group), COAM at 50, 5, and 0.5 mg/ml, or the double-stranded ribonucleic acid polyribonucleosinic-ribocytidylic acid

TABLE 4. Influence of administration route of COAM on its antiviral activity

Dose (mg/mouse)	Treatment		Vaccinia test (% reduction in pox number) ^b	Meningovirus test (no. of days of delay in 50% mortality time) ^c
	Injection route ^a	Time of injection (days before virus)		
3	ip	1	88 ($P < 0.001$)	>7.7
0.3	ip	1	81 ($P < 0.001$)	>7.7
3	sc	1	79 ($P < 0.001$)	4.3
0.3	sc	1	46 ($P < 0.005$)	1.7
0.3	iv	1	69 ($P < 0.001$)	-0.9
3	ip	7	74 ($P < 0.001$)	>7.4
0.3	ip	7	6 (NS)	>7.4
3	sc	7	85 ($P < 0.001$)	1.1
0.3	sc	7	32 (NS)	0.4
0.3	iv	7	40 ($P < 0.05$)	0

^a Intraperitoneal (ip), subcutaneous (sc), or intravenous (iv).

^b See footnote to Table 1.

^c See footnote to Table 3.

FIG. 2. Effect of intraperitoneal injection of COAM and polyacrylic acid (PAA) on experimental Semliki Forest virus infection in mice.

(poly- $I_n:C_n$) at a concentration of 1 mg/ml. The treatment was started 4 hr before herpesvirus infection and was given three times daily until the fourth day after infection. From then on, only one daily dose was given. The corneal lesions were maximal on day 5, at which time conjunctivitis was also present. On day 11, the keratitis was stabilized (scar formation) and conjunctivitis had disappeared. Table 5 shows the individual and averaged keratitis scores on days 5 and 11. It can be seen that COAM inhibited the development of herpetic keratitis but was at least 50 times less effective than poly- $I_n:C_n$.

Toxicity studies. Evidence that COAM is

FIG. 3. Influence of intraperitoneal administration of COAM on experimental infection of mice with influenza APR8 virus.

degraded or eliminated from the organism was obtained from comparative data on toxicity after a single dose and after repeated doses. For determination of acute toxicity, groups of 5 or 10 mice were injected intraperitoneally with increasing doses of COAM (0, 160, 500, and 1,600 mg/kg). The animals were observed for 1 month. Mortality occurred only in the group receiving 1,600 mg/kg; the animals died between days 2 and 5 after injection. The calculated LD_{50} was 1,250 mg/kg. The surviving animals gained weight normally during the whole observation period. At autopsy, a moderate splenomegaly was observed in COAM-treated groups. In a separate test, it was found that, after a single dose of COAM (3 mg/mouse, intraperitoneally), splenomegaly was maximal (220%) on day 4 and was restored to almost normal (134%) on day 14. The leukocyte count remained within the variation spread of the controls.

Toxicity after repeated administration was determined as follows. Groups of 10 mice were injected intraperitoneally with 0, 160, and 500 mg of COAM per kg. The injections were given on days 0, 7, 14, 20, 33, 40, 48, 54, and 61, i.e., one injection weekly for 10 weeks with a 1-week interruption. Five mice from each group were sacrificed on day 29, i.e., after four injections. The remaining five were killed at day 71, i.e., 10 days after the ninth (last) injection. The group receiving 500 mg/kg weekly showed a 4% decrease in weight gain; the group receiving 160 mg/kg gained weight normally. The spleen weight was slightly increased after four (171%) as well as after nine injections (144%). The fact that the animals tolerated nine injections of 500 mg/kg (i.e., a total of 4,500 mg/kg, or 3.6 times the acute LD_{50}) suggests that COAM is not

TABLE 5. Influence of topical administration of COAM on experimental herpetic keratitis in rabbits in comparison with polyribonucleosinic-ribocytidylic acid (poly-I_n:C_n)

Rabbit no.	Treatment ^a	Keratitis score ^b					
		Day 5			Day 11		
		Left	Right	Avg	Left	Right	Avg
1	Phosphate-buffered saline	2.5	2.5	2.5	3.0	2.0	2.2
2		2.5	2.5		2.0	2.0	
3	COAM, 0.5 mg/ml	2	3	2.6	2.0	2.5	2.2
4		3	2.5		2.5	2.0	
5	COAM, 5 mg/ml	0	2	1.4	1.0	1.0	1.4
6		2	1.5		2.5	1.0	
7	COAM, 50 mg/ml	0	1	1.5	0	0.5	0.8
8		3	2		1	1.5	
9	Poly-I _n :C _n , 1 mg/ml	1	1	0.2	0	0.5	0.2
10		0	0		0.5	0	

^a Instillation in the eye, 0.01 ml three times daily during 4 days and once daily thereafter. Treatment started 4 hr before herpesvirus inoculation.

^b (1) Barely visible lesion; (2) clearly visible opacification covering less than one-fourth of the cornea; (3) opacification covering one-fourth to one-third of the cornea; (4) opacification covering more than one-third of the cornea.

retained by the organism. Probably it is degraded or excreted, or degraded and then excreted. Studies in which radioactively labeled COAM is being used are now under way, and are expected to give quantitative information in this regard.

Mode of action of COAM. Several types of experiments were carried out to elucidate the mechanism of antiviral activity of COAM in vivo. As has been described in a previous paper (2), certain CO-oxypolysaccharides, including COAM, inhibit the development of cytopathic effect in tissue cultures challenged at low input multiplicity and in the presence of the compounds. For polyacrylic and polymethacrylic acid, a similar effect was noted, and a closer study led us to assume that it was due to inhibition of adsorption of the virus (6).

At 18 hr after intraperitoneal injection of COAM, interferon was detectable in the serum (2). Attempts to demonstrate the induction of interferon-like resistance of COAM in vitro in rabbit kidney cells, human skin fibroblasts, or mouse macrophages were unsuccessful; cells incubated for 24 hr with subtoxic levels of COAM, then washed free from COAM and subsequently challenged with vesicular stomatitis virus (VSV) at high input multiplicity, showed undiminished multiplication of the virus. However, when parallel macrophage cultures were established from mice treated with COAM and from control mice, a marked difference in response to virus challenge was observed. The experiment was carried out as follows. A group of mice was injected intraperitoneally with proteose-peptone

and COAM (2 mg/mouse); control mice received only proteose-peptone. Five days later, the macrophages of both groups were harvested and plated. After 45 min, the plates were washed 3 times with tissue culture medium and refed. Microscopically, there was no difference between the two sets of cultures. Part of the cultures of each set were challenged immediately with VSV or mengovirus at an approximate input multiplicity of 10 plaque-forming units/cell. Excess virus was removed after 1 hr by three careful washes. The cultures were refed and incubated for 24 hr. The second part of the cultures was challenged the next day in an identical way. The results of a representative experiment are shown in Table 6. It can be seen that both VSV and mengovirus yields were significantly lower in macrophages harvested from COAM-treated mice. These results show that the macrophage population of mice treated with COAM is partially resistant to virus infection. Such resistance could be responsible for the observed antiviral effect of COAM in the whole animal.

Another way in which COAM might increase resistance to viral infection could be by modifying the host response, e.g., by accelerating or increasing interferon production or antibody formation. In an investigation of these possibilities, groups of mice (20 to 25 g) were injected intraperitoneally with COAM (2 mg/mouse). Interferon responsiveness was measured 7 days later by intravenous injection of Sindbis virus (10^9 plaque-forming units/mouse), NDV (10^{10} EID₅₀/mouse), or poly-I_n:C_n (25 μ g/mouse).

TABLE 6. *Growth of VSV and of mengovirus in control mouse peritoneal macrophages and in macrophages from mice pretreated with COAM*

Origin of macrophages ^a	Time of virus challenge (hr after explantation)	Virus yield (log ₁₀ plaque-forming units/ml)	
		VSV	Mengo-virus
Control mice.....	1	5.4	6.8
Mice injected with COAM.....	1	4.8	5.1
Control mice.....	24	5.8	6.8
Mice injected with COAM.....	24	4.9	5.4

^a Mice were injected intraperitoneally with 0.5 ml of proteose-peptone with or without COAM (2 mg/mouse) added. Macrophages were harvested 4 days later.

The animals were bled 3 hr after poly-I_n:C_n injection or 8 hr after injection of viruses. Serum interferon levels in COAM-pretreated animals amounted to 1,000, 2,000, and 3,200 units/ml after, respectively, poly-I_n:C_n, Sindbis virus, and NDV injections, in comparison with 1,000, 6,300, and 3,200 units/ml in the corresponding control groups. These data show that 7 days after injection of COAM, at a time when the mice are efficiently protected against either vaccinia or mengovirus, neither hyper- nor hyporeactivity existed with respect to induction of interferon.

Similarly, the antibody levels against Sindbis virus were determined in control mice and in mice treated with COAM. The antigen was given either subcutaneously together with COAM (2 mg/mouse), or intravenously 3 days after intraperitoneal injection of COAM (2 mg/mouse). In neither case could a significant alteration in antibody response be noted.

Finally, the possibility that COAM might act by altering the clearance of circulating virus was ruled out by the observation that the disappearance of intravenously injected mengovirus was not affected by pretreatment of the mice with COAM (3 mg/mouse, 3 days before administration of mengovirus).

DISCUSSION

COAM, a polycarboxylic acid with a backbone consisting of (—C—C—O—C—O—) sequences, is a potent antiviral substance. At a dose of 4.2 mg/kg intraperitoneally, it protected mice against vaccinia virus infection. At a dose of 25 mg/kg, this effect extended over a period

of 7 days. COAM also protected mice against lethal mengovirus infection. A dose of 5 mg/kg was sufficient for 24-hr protection, but by increasing the dose to 160 mg/kg anti-mengovirus activity was extended over a period of 1 month. Modest antiviral activity was also observed in three other systems. Mice injected intraperitoneally with COAM were partially protected against lethal Semliki Forest and influenza APR8 virus challenges. Moreover, herpetic keratitis in rabbits was partially inhibited by topical administration of COAM, but the compound was at least 50 times less active than poly-I_n:C_n.

The mechanism of antiviral activity is not clear. Structurally and from a point of view of biological activity, COAM has some similarities to polyacrylic acid or pyran copolymer (3, 4, 6, 7, 9-11, 13; De Somer, *in press*; Regelson and Merigan, Proc. Amer. Ass. Cancer Res., 1967, Abstr. 219). On the basis of its polyanionic nature, antiviral activity could be predicted. Like polyacrylic and polymethacrylic acids, COAM inhibited cytopathic effect in tissue cultures provided the cultures were challenged at a low input multiplicity and in the presence of the compounds. For polymethacrylic acid, this effect was correlated with inhibition of virus adsorption or penetration, or both (6). Presumably a similar mechanism is operating for COAM. This type of antiviral activity is certainly not due to interferon, as cultures pretreated with COAM did not produce interferon and were not resistant to a high input viral challenge.

When different polyanions are compared, there is no correlation between *in vitro* inhibition of cytopathic effect (i.e., interaction with viral adsorption and penetration) and *in vivo* antiviral effect (De Somer, *in press*). Polymethacrylic acid inhibited cytopathic effect but did not reduce vaccinia lesions in mice (3). Certain CO-oxypolysaccharides did not inhibit cytopathic effect at concentrations which were not toxic for the cells, but yet gave good protection against mengovirus and vaccinia virus in mice (2). Hence, it is doubtful whether interaction with viral adsorption is responsible for the observed *in vivo* effects. Experiments on the clearance of intravenously injected viruses pointed to the same conclusion: polymethacrylic acid inhibited the disappearance of intravenously injected Sindbis virus (*our unpublished results*), whereas COAM did not affect the clearance of mengovirus.

Another antiviral parameter which COAM has in common with polyacrylic acid and pyran copolymer is the induction of interferon *in vivo*.

Passively administered interferon can protect mice against several viral infections, including that with vaccinia virus (3). However, this protection is of short duration. Hence, if one wants to hold interferon responsible for protection induced by COAM or any of the polyanionic polymers, one has to assume that after a single dose of these substances interferon is released for several weeks. Evidence that this occurs is not available. After administration of polyacrylic acid, pyran copolymer, or COAM, circulating interferon is detectable only during the first 24 to 48 hr. Similarly, one could object that polymers which do not induce circulating interferon, such as the CO-oxydextrans, give excellent protection against vaccinia virus and mengovirus (2). However, by measuring circulating interferon, one only detects those amounts which flow over from the producing tissues. Furthermore, the presence of a few units per milliliter would be sufficient to inhibit viral growth effectively, and such small quantities would not be demonstrable in serum preparations. One objection to this thesis is that continued release of interferon is not in accord with the hyporeactivity which has been reported after induction with different stimuli, including polyanions (5, 8, 11, 14). However, mice pretreated with COAM, and subsequently stimulated with viruses or poly- $I_n:C_n$, produced the same amounts of circulating interferon as control animals. Thus, in the case of COAM, refractoriness with respect to interferon induction seems to be minimal. Similarly, COAM injection protected against virus infection *in vivo*, regardless of whether or not the animals had received previous treatment with COAM.

When macrophages, harvested from mice pretreated with COAM, were challenged *in vitro* with VSV or mengovirus, at high input multiplicity, less new virus was produced than in control macrophage cultures. Whether this type of intracellular resistance was correlated with interferon cannot at present be ascertained. COAM did not induce interferon or interferon-like resistance *in vitro* in a number of different cell cultures, including mouse peritoneal macrophages.

Another type of experiment also pointed to peritoneal cells as possible mediators of the antiviral effect of COAM. It was found that, at the same dosage level, COAM was significantly more effective against mengovirus when given by intraperitoneal injection than when given by intravenous or subcutaneous injection. Similarly, a prolonged protection against mengovirus was achieved only when polymer and virus were given in the peritoneal cavity. In the vac-

cinia virus tail lesion test, protection was independent of the injection route of the polymer. This strongly suggests that, with mengovirus, an early intraperitoneal event, necessary for multiplication or spread of the virus in the organism, or for both, is blocked by COAM. With vaccinia virus, which is inoculated intravenously, this block would occur at another site(s) in the organism which is as effectively reached by intraperitoneal injection as by subcutaneous or intravenous injection.

An important point of discussion with respect to the possible clinical application of antiviral polyanions is their biodegradability. Pyran copolymer and polyacrylic acid are poorly excreted (J. F. Niblack, Proc. Int. Symp. on Interferon, Lyon, France, 1969, *in press*) and are probably not enzymatically attacked by the cells (10). This may explain their prolonged activity but also part of their high toxicity. The LD_{50} of COAM for mice was 300 to 500 times higher (1,250 mg/kg) than the smallest active antiviral dose (2.5 to 4.2 mg/kg). For polyacrylic acid this ratio is less favorable, the active dose in mice being on the order of magnitude of 4 to 40 mg/kg depending on the batch tested, and the LD_{50} being about 150 mg/kg. That COAM is essentially a biodegradable or excretable compound was indicated by the observation that the antiviral effect wore off faster than for polyacrylic acid, and that mice tolerated, without weight loss or gross pathological aberrations, as much as 3.6 times the acute LD_{50} of COAM over a 1-month period.

As a unifying working hypothesis, we propose that COAM is taken up by macrophages, either intraperitoneal or other. These cells would break down the drug slowly and would produce interferon in small quantities. Initially, enough interferon would be released to become detectable in the circulation. Later, the production rate would decrease so that circulating interferon cannot be measured any more. The macrophages which produce interferon would become resistant to virus infection themselves, and would also confer a certain degree of virus resistance on other cells. Certain viruses, probably those which need macrophages for multiplication or spread through the organism, or for both, would thus be effectively blocked by COAM. Corroborating evidence that the macrophages are essential intermediates in mengovirus infection in mice is found in the observation that specific antimacrophage antibodies can protect mice against lethal mengovirus infection (12).

So far, the studies on the antiviral effects of polyanions indicate that long-term protection and lack of toxicity are mutually exclusive. The

fact that COAM and the other chlorite-oxidized polysaccharides seem to combine biodegradability and long-term protective effects is a step forward. Although the development of an ideal antiviral compound remains the prime objective of research on interferon inducers, our study suggests that investigations on the mechanism of *in vivo* antiviral action of the polyanions will also allow a reconsideration of the pathogenesis of a number of experimental viral infections.

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