Pyran and Polyribonucleotides: Differences in Biological Activities

PAGE S. MORAHAN, WILLIAM REGELSON, AND ALBERT E. MUNSON

Departments of Medicine and Microbiology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia 23219

Received for publication 10 March 1972

Maleic anhydride-divinyl ether copolymer (pyran) and the polyribonucleotides are both large polyanions with potent antiviral activity. However, they are biologically quite different. Interferon levels of 100 units or more/ml were associated with antiviral activity of polyribonucleotides. Interferon induction by pyran compounds was not primarily involved in antiviral resistance because preparations that did not induce interferon possessed antiviral activity equal to that of interferon-inducing preparations. Both polyriboinosinic-cytidylic acid [poly (rI.rC)] and pyran increased the immune response to sheep erythrocytes in the Jerne hemolytic plaque-forming cell (PFC) assay, but their modes of immunoadjuvant action differed. On peak day, poly (rI.rC)-treated mice demonstrated 5.1 × 10^4 PFC/spleen (557 PFC/10^6 nucleated cells) and pyran-treated mice exhibited 4.5 × 10^4 PFC/spleen (299 PFC/10^6 nucleated cells), as compared with 2.7 × 10^4 PFC/spleen (261 PFC/10^6 nucleated cells) in controls. The compounds also differed in phagocytic alteration; polyribonucleotides did not affect phagocytosis whereas pyran produced a biphasic response. Both polyribonucleotides exhibited toxic inhibition of liver microsomal enzyme metabolism of type I and type II drugs. However, whereas pyran sensitized mice 50-fold to the lethal effects of endotoxin, the polyribonucleotides did not significantly sensitize mice to endotoxin.

Pyran copolymer (maleic anhydride-divinyl ether copolymer) and the polyribonucleotides are large polyanionic compounds with potent antiviral activity (6, 14, 22). The two groups of compounds also share other characteristics. They both induce interferon (6, 20), possess antitumor activity (8, 12, 20, 23), and are immunologocal adjuvants (2, 3, 18, 24). However, in experiments attempting to determine the mode of antiviral action of these compounds, we have found fundamental biological differences between the two groups of polyanions.

The results reported here indicate that interferon production is well correlated with the antiviral activity of the polyribonucleotides against encephalomyocarditis (EMC) virus infection of mice. Immunological adjuvant activity and alteration of reticuloendothelial system (RES) phagocytic activity, as measured with sheep erythrocytes, appear relatively unimportant. Our results demonstrate that the protection elicited by pyran is most likely not due to the interferon system, as has been suggested by several sources (5, 13, 19). Our results suggest that enhancement of the immune response or intracellular virucidal activity may be more important mechanisms of antiviral activity for the pyran compounds.

MATERIALS AND METHODS

Polyribonucleotides and pyran preparations. Synthetic double-stranded complexes of polyriboinosinic-cytidylic acid [poly (rI.rC)] and polyriboadenylic-uridylic acid [poly (rA.rU)] were dissolved or diluted in 0.03 M NaCl at 4°C and were used within 72 hr of solution. Complexes were kindly supplied by L. D. Hamilton, Brookhaven National Laboratory, and the National Institutes of Health [standard poly (rLrC), lot no. K4, October, 1970]. Pyran preparations synthesized by Hercules, Inc., between 1964 and 1969 were kindly supplied by D. Breslow. Anhydrous pyran preparations were put into 0.15 M NaCl, and solution was completed by raising the pH to neutrality.

Mice. Adult male and female mice (20 to 25 g) from the inbred strain of NYLAR-A Swiss mice were used for all experiments.

Interferon titers. Serum pools from two to five mice were collected by cardiac puncture 2 hr after intravenous (iv) injection of polyribonucleotides or 24 hr after iv injection of pyran, when interferon titers are maximal (6, 15). Sera were frozen at −20°C until interferon assays were performed by plaque reduction of vesicular stomatitis virus Indiana strain in L929...
cells. Interferon titer is defined as the reciprocal of the greatest dilution of serum which reduced control plaque formation by 50%. Calculations of titers were made by the method of least squares. All values were corrected to a simultaneously assayed standard murine interferon which titered 945 ± 89 (standard error) over 25 assays. This plaque assay was approximately four times less sensitive than an assay of interferon performed by measuring yield reduction of GD-VII virus hemagglutinin in L929 cells. This second assay, which measured 90% of the activity contained in the National Institutes of Health standard murine interferon preparation, was used in some experiments.

Antiviral activity. Mice were inoculated iv with the polyanions and were challenged iv 24 hr or 7 days later with 50 LD₅₀ of encephalomyocarditis (EMC) virus. The EMC ATCC strain VR129 was prepared as a 10% brain homogenate from infected weanling mice.

Immunoadjuvant activity. Mice were inoculated with the polyanions and later with approximately 10⁹ sheep erythrocytes (sRBC). For hemolysis antibody measurement, mice were inoculated with sRBC 3 hr after polyanions, and titers were determined in individual sera obtained by cardiac puncture 7 days later (five mice per group). Mean hemolysis titers ± standard error were determined and compared with control titers. For splenic hemolysin plaque-forming cell (PFC) measurement, mice were inoculated with sRBC 24 hr after polyanions, individual spleens were removed 3, 4, 5, and 6 days after antigen administration, and PFC were determined by the method of Jerne and Nordin (10). Both total PFC per spleen and PFC/1₀⁶ nucleated spleen cells were determined (five mice/group).

Phagocytic activity. Mice were inoculated iv with the polyanions, and at intervals thereafter iv with chromium-5¹-labeled sRBC. At 60 min after sheep cell injection, the per cent uptake of labeled cells into the liver, spleen, lungs, and thymus was determined. The mean per cent uptake from eight mice per group was calculated and compared with the mean per cent uptake in control mice (16, 18).

Sensitization of mice to endotoxin. Mice were inoculated iv with the polyanions and were challenged 24 hr later iv with various doses of Salmonella typhosa 0901 lipopolysaccharide (Difco). Ten mice per endotoxin dose were used. Deaths occurring within 72 hr were recorded. The LD₅₀ of endotoxin in treated and control mice was determined by the Cornfield-Mantel modification of Karber's method (4).

Inhibition of drug metabolism in vivo. Mice were inoculated with the polyanions, and at intervals thereafter iv with hexobarbital (80 mg/kg). Sleeping time was determined for individual mice (10 mice per group) and compared with the controls (25).

Inhibition of microsomal mixed functional oxidase enzymes in vitro. Mice were inoculated with polyanions and livers were removed 24 hr later. The 9,000 × g liver supernatant fractions from five individual livers were incubated for 30 min with either a type I substrate (aminopyrine) or a type II substrate (aniline) together with all of the necessary cofactors (7, 11). The metabolism of aminopyrine to formaldehyde or of aniline to p-aminophenol was measured.

Statistical evaluation. Means with standard deviations and standard errors were calculated for all data, and Student's t test was used to determine significant differences from control values.

RESULTS

Interferon production and antiviral activity. Pyran, poly (rL.rC), and poly (rA.rU) differed markedly in ability to induce serum interferon and to protect mice against subsequent challenge with EMC virus. Poly (rL.rC) complexes (2 mg/kg, iv) induced high levels of serum interferon 2 hr after inoculation, and protected mice challenged 24 hr later with EMC virus (Table 1). Antiviral protection was short-lived; mice challenged 7 days after poly (rL.rC) administration were not significantly protected (Table 1). The poly (rA.rU) complex induced very low levels of serum interferon and only slightly protected mice from subsequent challenge with EMC either 1 or 7 days later (Table 1). Thus, for the polynucleotides, antiviral action generally appeared to parallel serum interferon production (22).

All of the pyran preparations (25 mg/kg, iv)

| Table 1. Interferon production and antiviral protection against encephalomyocarditis virus (EMC) |
|-------------------------------|-------------------------------|-----------------------------|
| Protective agent | Interferon productiona (units/ml of serum) | Per cent protection against EMCb |
| | 24 hr | 7 days |
| | | |
| None | <20 | 0 | 0 |
| Poly (rL.rC) P-L | 1,850 | 65 | 20 |
| Poly (rL.rC) CK19 | 4,130, 3,436, 2,720, 5,500 | 75 | 23 |
| Poly (rL.rC) CK22 | 540, 630, 29, 602 | 80 | NDc |
| Poly (rA.rU) CK5 | <20 | 25 | 10 |
| Poly (rA.rU) CK6 | 36, 53, 43 | 40 | ND |
| Pyran NSC 46015 | 85, 48 | 75 | 80 |
| Pyran XA124-177 | 90, 32 | 66 | 79 |
| Pyran NSC 46015-D | <20, <20 | 75 | 80 |

* Mice were inoculated iv with polynucleotides (2 mg/kg) or pyran (25 mg/kg), and serum collected either 2 hr (polynucleotides) or 24 hr (pyran) later. Each value represents a pool of serum from two to five mice.

b Mice inoculated as above were challenged iv with approximately 20 to 50 LD₅₀ of EMC. Per cent protection = the difference between control and experimental per cent mortality 14 days after infection.

c Not done.
induced strong antiviral protection against EMC inoculated either 1 or 7 days later (Table 1). However, none of the preparations induced more than 100 units of serum interferon 24 hr after inoculation, at a time when pyran-induced interferon is maximal (14, 15). When the more sensitive interferon assay for yield reduction of GD-VII virus hemagglutinin was used, interferon titers still were less than 100 units. Levels of interferon undetectable by either assay occurred after administration of pyran 46015-D and certain other experimental pyran preparations which possessed good antiviral activity. Both the interferon-inducing and noninducing pyran preparations showed equal antiviral activity on a dose response basis (unpublished data). There was no correlation between detectable serum interferon levels and antiviral potency of the pyran compounds, which is in contrast to the polyribonucleotides.

**Effects on phagocytosis.** The polyribonucleotides and pyran also differed in effect on the phagocytic activity of the mouse. Neither poly (rLrC) nor poly (rA,rU) complexes profoundly altered phagocytosis of sheep erythrocytes by the liver, spleen, lungs, or thymus (Table 2). Splenic phagocytosis was slightly, although significantly, depressed 24 hr after polyribonucleotide inoculation. At the 1 to 2 mg/kg dose, both poly (rLrC) and poly (rA,rU) caused the slight changes in splenic phagocytosis, yet only poly (rLrC) was antivirally effective. Thus, alterations in RES phagocytic activity did not appear relevant to the observed antiviral protection of poly (rLrC).

Pyran produced a marked biphasic phagocytic response: first, a depression of several days' duration, followed by phagocytic enhancement (Table 2). At 24 hr after pyran inoculation, there was a pronounced depression of sheep cell uptake into the liver (52.2 to 5.2%). By 7 days after inoculation, phagocytosis by spleen, liver, and lungs were all markedly enhanced (Table 2). During both the depressed and the stimulated phagocytic states, pyran induced antiviral protection. Phagocytic activity of the liver, and perhaps of the spleen and lungs, did not appear important for the antiviral action of pyran.

**Immunoadjuvant activity.** Pyran and the polyribonucleotides also differed in mechanism of immunoenhancing activity. All three polyonions caused marked immunoenhancement of serum hemolysin antibody titers. Control hemolysin titers (log₂) were 7.9 ± 0.32; titers in various poly (rLrC)-treated mice were 11.1 ± 0.18, 9.7 ± 0.22, and 10.5 ± 0.17; in poly (rA,rU) CW7-treated mice, they were 10.7 ± 0.46; and in pyran 46015-treated mice, they were 11.9 ± 0.22. All of the polyonions significantly enhanced serum antibody.

When splenic antibody-forming cells (PFC) were measured 3, 4, 5, and 6 days after antigen administration, both poly (rLrC) and pyran caused immunoenhancement of total splenic PFC (Fig. 1). On day 5, the PFC were significantly increased from 27,500 ± 8,100 PFC/spleen in controls to 51,100 ± 16,800 PFC in poly (rLrC)-treated mice and to 45,300 ± 19,000 PFC in pyran-treated mice. However, only poly (rLrC) significantly enhanced the specific activity of splenic cells in antibody production (Fig. 1).
On day 5, there were 261 ± 69 PFC/10^6 cells in controls, a similar 299 ± 158 PFC in pyran-treated mice, but an increased 557 ± 217 PFC in poly (rI.rC)-treated mice. Pyran evidently enhanced immunity by increasing the total number of nucleated spleen cells, and thereby the total antibody response, rather than by increasing the specific activity of antibody-producing cells (Fig. 1).

Both poly (rI.rC) and poly (rA.rU) were immunoadjuvants, yet only poly (rI.rC) possessed antiviral activity against EMC. It seems unlikely, therefore, that immunoenhancement is a significant mode of antiviral action of poly (rI.rC) in this instance.

**Effects on endotoxin detoxification.** Pyran markedly sensitized mice to the lethal effects of endotoxin when endotoxin was administered 24 hr after pyran (17), decreasing the LD₅₀ of S. typhosa lipopolysaccharide 150-fold. Polyribonucleotides at high doses have also been reported to possess endotoxin sensitizing action (9), and poly (rI.rC) inoculated intraperitoneally simulta-

neously with endotoxin does sensitize mice (W. C. Rose and S. G. Bradley, unpublished data). However, with the 1 to 2 mg/kg iv doses used in these studies, neither poly (rI.rC) nor poly (rA.rU) markedly increased lethality due to endotoxin (Table 3). This is in direct contrast to the ability of pyran to sensitize mice profoundly to endotoxin.

**Effects on drug metabolism.** With respect to other biological parameters, pyran and the polyribonucleotides both inhibited the ability of liver microsomal mixed functional oxidase enzymes to metabolize certain drugs. When mice were inoculated with the polyanions, and tested in vivo for ability to metabolize hexobarbital (type I substrate drug), both polyanions inhibited drug metabolism. Significant inhibition, and therefore prolonged sleeping time, occurred 24 and 48 hr after polyanion inoculation (Table 4). Sleeping time prolongations ranged from 41 to 200% increases at 24 hr and from 105 to 170% increases at 48 hr. Drug metabolism, and therefore sleeping times, were normal by 6 days after pyran or poly-nucleotide administration (Table 4).

The inhibition of drug metabolism seen in vivo was also observed in vitro with 9,000 × g liver supernatant fractions obtained from treated mice. Metabolism of both a type I substrate (aminopyrine) and a type II substrate (aniline) were markedly inhibited by inoculation of mice with polyribonucleotides or pyran. Inhibition of aminopyrine metabolism ranged from 28 to 60%; inhibition of aniline metabolism ranged from 51 to 55% (Table 5). Thus, both groups of polyanions altered the ability of the hepatic microsomal enzymes to metabolize drugs. This biological activity has also been observed with other antiviral agents, including the chlorite-oxidized oxyamylose polyanion and endotoxin.

**Table 3. Sensitization of mice to endotoxin by polyanions**

| Drug          | Endotoxin LD₅₀ (mg/kg iv) | 95% Confidence limits |
|---------------|---------------------------|-----------------------|
| None          | 33.6                      | 30.0-37.4             |
| Poly (rI.rC) P-L | 37.5                    | 33.6-41.8             |
| Poly (rA.rU) CK6 | 43.9                   | 39.7-48.4             |
| Pyran XA124-177 | 0.66                    | 0.57-0.78             |

* Mice were inoculated iv with polyribonucleotides (2 mg/kg) or pyran (25 mg/kg) and were challenged iv 24 hr later with various doses of Salmonella typhosa lipopolysaccharide. Deaths occurring within 72 hr were recorded and the LD₅₀ was determined.
TABLE 4. Prolongation of hexobarbital sleeping time in mice pretreated with polyantionts

| Drug          | Hexobarbital sleeping timea |
|---------------|-----------------------------|
|               | 24 hr | 48 hr | 7 days |
| **Experiment 1** |       |       |       |
| None          | 36.6 ± 2.0 | 45.1 ± 1.6 | 36.9 ± 2.8 |
| Poly (rI.rC) TY15 | 51.6 ± 3.2 (S) | 98.6 ± 7.8 (S) | 48.0 ± 6.4 |
| Poly (rA.rU) CW7 | 64.0 ± 4.6 (S) | ND | 40.0 ± 3.7 |
| **Experiment 2** |       |       |       |
| None          | 54.9 ± 4.5 | 30.0 ± 2.6 | ND |
| Poly (rI.rC) P-L Ref. | 102.6 ± 9.4 (S) | 61.6 ± 8.4 (S) | ND |
| Poly (rA.rU) CK6 | 80.4 ± 4.3 (S) | 81.1 ± 4.5 (S) | ND |
| **Experiment 3** |       |       |       |
| None          | 34.4 ± 2.4 | 40.1 ± 3.2 | 38.6 ± 2.4 |
| Pyran XA124-177 | 103.4 ± 12.2 (S) | 84.8 ± 6.6 (S) | 75.3 ± 6.2 |

a Sleeping time in minutes (mean ± standard error). Mice were inoculated iv with pyran (25 mg/kg) or polyribonucleotides (2 mg/kg in experiment 1 and 1 mg/kg in experiment 2), and with hexobarbital (80 mg/kg iv) at the designated intervals thereafter. (S) = P < 0.05. ND = not done.

TABLE 5. Inhibition of aminopyrine and aniline metabolism by liver microsomal enzymes

| Drug          | Dose (mg/kg) | Formaldehyde (µg per mg of protein per 30 min) | p-Aminophenol (µg per mg of protein per 30 min) |
|---------------|--------------|-----------------------------------------------|-----------------------------------------------|
|               |              |                                               |                                               |
| None          |              | 0.744 ± 0.047a                                | 2.45 ± 0.13a                                  |
| Poly (rI.rC) |              | 0.540 ± 0.051                                | 1.19 ± 0.05                                  |
| P-L Ref...    |              |                                               |                                               |
| Pyran         |              | 0.300 ± 0.046                                | 1.10 ± 0.11                                  |
| XA124-177     |              |                                               |                                               |

a Mice were inoculated iv two times, 24 hr apart. Livers from five mice/group were removed 24 hr after the last injection. Production of formalde- hyde from aminopyrine and p-aminophenol from aniline were measured in individual liver 9,000 × g supernatant fractions.

DISCUSSION

Pyran and the polyribonucleotides appear to elicit quite distinct biological responses. Interferon production by polyribonucleotides appears to be well correlated with antiviral protection against EMC virus. Complexes of poly (rA.rU), which induced very small amounts of interferon, were not antivirally effective. Although certain pyran preparations induced interferon, interferon is apparently not primarily involved in the anti- viral protection produced by pyran polyonanys. To invoke the action of interferon, one would have to postulate (i) that very small levels of undetectable interferon are sufficient, (ii) that interferon induced by pyran possesses increased or different antiviral activity than interferon induced by viruses, poly (rI.rC) or poly (rA.rU), or (iii) that both are true. The argument that undetectable levels of interferon are sufficient seems unlikely because amounts of interferon less than 100 units/ml induced by either poly (rA.rU) or decreased doses of poly (rI.rC) were not effective against EMC, suggesting there is a minimal and detectable level of interferon necessary for antiviral activity (P. S. Morahan et al., Proc. Nat. Acad. Sci. U.S.A., in press). In addition, equal doses of pyran preparations that induced or did not induce measurable levels of interferon conferred equal antiviral protection, indicating that interferon production was not necessary for antiviral resistance. It is possible that pyran-induced interferon does not get into the circulation, but stays in tissues. However, Merigan found six times more interferon in serum than in spleens of pyran-treated mice (12), and Pindak et al. (19) found very little (2 to 27 units) in either serum or spleens. There might be more evidence for interferon involvement if pyran were more effective against interferon-sensitive than interferon-insensitive viruses. However, pyran is effective against the EMC-MM-Mengo viruses, vaccinia virus, Friend leukemia virus, and vesicular stomatitis virus, but is less effective against vesicular stomatitis virus, which is very sensitive to interferon (5, 13, 14). The argument that pyran interferon is functionally different from other interferons appears unlikely, because interferons induced by various interferon inducers possess generally similar physiochemical characteristics and biological properties whenever they have been examined. Thus, the antiviral action of pyran is probably mediated by mechanisms different from the interferon system.

Activation of the RES, at least as measured by phagocytosis of such particles as sRBC, colloidal carbon, and lipid emulsion, also appears unlikely
as a primary mode of antiviral action for either group of polyanion. Polyrribonucleotides affected the RES very slightly at 1 mg/kg iv, and both the inactive poly (rA.rU) and active poly (rL.rC) acted similarly. At higher doses (8 mg/kg, iv) poly (rL.rC) does significantly depress phagocytosis (21). These effects may be related to toxic effects of the drug. Pyran protected mice from EMC during both the RES depressed and stimulated phases, suggesting that phagocytosis per se is not a mode of antiviral action. However, it is possible that protection of pyran may be related to viral phagocytosis.

An immunoadjuvant role in antiviral protection engendered by poly (rL.rC) also appears unlikely, because both the antivirally active poly (rL.rC) and inactive poly (rA.rU) were immunological enhancers. Immunological enhancing action by pyran may play a role in its antiviral action, and the immunoadjuvant action of pyran merits additional study with viruses as antigenic stimuli. Recently, Campbell and Richmond (Bacteriol. Proc., p. 190, 1972) noted that pyran increased the protective effect of foot and mouth disease virus vaccine. The different modes of immunoadjuvant action between pyran and poly (rL.rC) are intriguing. Enhancement of antibody production by pyran is apparently proportional to splenic enlargement induced by pyran, whereas antibody enhancement by poly (rL.rC) is not associated with increases in the number of spleen cells. This has been suggested by separate investigations with pyran (18) and polyribonucleotides (2). However, these differences in immunoadjuvant activity are more readily apparent in this simultaneous study of pyran and poly (rL.rC). The mechanisms involved are still largely unknown.

Pyran and the polyrribonucleotides also differed in their effects on the ability of the mouse to detoxify endotoxin. Polyrribonucleotides have been reported to sensitize mice to the lethal effects of endotoxin (9). However, in this study, with relatively small doses (1 to 2 mg/kg, iv) of poly (rL.rC) that possessed equivalent antiviral activity to pyran (25 mg/kg, iv), profound differences in endotoxin sensitization were noted. Pyran treatment reduced the endotoxin LD50 50-fold, whereas polyrribonucleotide treatment did not significantly alter the LD50 from control values. Thus poly (rL.rC) differs from many resistance-inducing compounds that sensitize mice to endotoxin, including chlorite-oxidized oxamyllose polyanion, zymosan, and Bacillus Calmette Guérin.

Pyran and the polyrribonucleotides, although both polyanionic compounds and potent antiviral agents, thus appear to differ fundamentally. They affected the RES differently, were different types of immunoadjuvants, and differed in ability to sensitize mice to endotoxin. Their only activities in common were inhibition of liver microsomal enzymes and production of antiviral resistance. However, antiviral action of polyrribonucleotides is generally correlated with ability to induce interferon, whereas the action of pyran does not appear to be mediated primarily through the interferon system. Recently, Billiau, Muyembe, and de Somer (1) suggested that peritoneal macrophages are important in polycarboxylate-mediated antiviral resistance, because protection occurred only when the drug and virus were inoculated intraperitoneally. However, we have observed that pyran inoculated either iv or ip conferred protection against EMC given by either route, indicating the protection was systemic rather than local. Increased intracellular virucidal activity or immune adjuvant activity may be involved in the antiviral protection conferred by pyran. The mechanism of antiviral activity of pyran is apparently distinct from that of the polynucleotides.

ACKNOWLEDGMENTS

We thank Ann Munson for expert technical assistance.

This investigation was supported by Public Health Service grant CA10537 from the National Cancer Institute and by grants from the Council for Tobacco Research, the Irwin Sistrubger Memorial Medical Foundation, and the Samia Grotto Foundation.

LITERATURE CITED

1. Billiau, A., J. J. Muyembe, and P. de Somer. 1971. Mechanism of antiviral activity in vivo of polycarboxylates which induce interferon production. Nature N. Biol. 232:183-186.
2. Braun, W., and M. Nakano. 1967. Antibody formation: stimulation by polyanalycin and polycytidylic acids. Science 157: 819-821.
3. Braun, W., W. Regelson, Y. Yajima, and M. Ishizuka. 1970. Stimulation of antibody formation by pyran copolymer. Proc. Soc. Exp. Biol. Med. 131:171-175.
4. Cornfield, J., and N. Mantel. 1950. Some new aspects of the application of maximum likelihood to calculations of the dose response curve. J. Amer. Statist. Ass. 45:181-210.
5. de Clerco, E., and T. C. Merigan. 1969. Local and systemic protection by synthetic polynionic interferon inducers in mice against intranasal vesicular stomatitis virus. J. Gen. Virol. 5:359-363.
6. Field, A. K., A. A. Tytell, G. P. Lampson, and M. R. Hilleman. 1967. Inducers of interferon and host resistance. II. Multistranded synthetic polynucleotide complexes. Proc. Nat. Acad. Sci. U.S.A. 58:1004-1010.
7. Gram, T. E., J. T. Wilson, and J. R. Fouts. 1968. Some characteristics of hepatic microsomal systems which metabolize aminopterin in the rat and rabbit. J. Pharm. Exp. Ther. 159:172-181.
8. Hamilton, L. D. 1971. Immunogenic polynucleotides, p. 107-128. In R. F. Beers and W. Braun (ed.), Biological effects of polynucleotides. Springer-Verlag, New York.
9. Huang, K., and M. E. Landay. 1969. Enhancement of the lethal effects of endotoxins by interferon inducers. J. Bacteriol. 98:1110-1111.
10. Jene, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. Science 140:605.
11. Kato, R., and J. R. Gillette. 1965. Effect of starvation on NADP-dependent enzymes in liver microsomes of male and female rats. J. Pharm. Exp. Ther. 150:279–284.
12. Levy, H. B., L. W. Law, and A. S. Rabson. 1969. Inhibition of tumor growth by polyninosinic-polycytidylic acid. Proc. Nat. Acad. Sci. U.S.A. 62:357–360.
13. Merigan, T. C. 1967. Induction of circulating interferon by synthetic anionic polymers of known composition. Nature (London) 214:416–417.
14. Merigan, T. C., and M. S. Finkelstein. 1968. Interferon-stimulating and in vivo antiviral effects of various synthetic anionic polymers. Virology 35:363–374.
15. Merigan, T. C., and W. Regelson. 1967. Interferon induction in man by a synthetic polyanion of defined composition. N. Engl. J. Med. 277:1283–1287.
16. Morrow, J. H., and N. R. DiLuzio. 1965. The fate of foreign red cells in mice with altered reticuloendothelial function. Proc. Soc. Exp. Biol. Med. 119:647–652.
17. Munson, A. E., and W. Regelson. 1971. Sensitization to endotoxin by pyran copolymer. Proc. Soc. Exp. Biol. Med. 137:553–557.
18. Munson, A. E., W. Regelson, W. Lawrence, and W. R. Wooles. 1970. Biphasic response of the reticuloendothelial system (RES) induced by pyran copolymer. J. Reticuloendothel. Soc. 7:375–385.
19. Pindak, F. F., J. P. Schmidt, D. J. Giron, and P. T. Allen. 1971. Interferon levels and resistance to viral infection associated with selected interferon inducers. Proc. Soc. Exp. Biol. Med. 138:317–321.
20. Regelson, W. 1967. Prevention and treatment of Friend leukemia virus (FLV) infection by interferon-inducing synthetic polyanions. Advan. Exp. Med. Biol. 1:315–332.
21. Regelson, W., A. E. Munson, W. R. Wooles, W. Lawrence, and H. Levy. 1969. The reticuloendothelial (RES) action of interferon inducers: the biphasic action of pyran copolymer and polynucleotides on phagocytic and immunologic response. Colloques de l’Institut National de la Sante et de la Recherche National, No. 6, L’Interferon, p. 381–390.
22. Richmond, J. Y., and L. D. Hamilton. 1969. Foot-and-mouth disease virus inhibition in mice by synthetic double-stranded RNA (polyninosinic and polycytidylic acids). Proc. Nat. Acad. Sci. U.S.A. 64:81–85.
23. Sandberg, J., and A. Goldin. 1971. Use of first generation transplants of a slow growing solid tumor for the evaluation of new cancer chemotherapeutic agents. Cancer Chemother. Rep. 55:233–237.
24. Turner, W., S. P. Chan, and M. A. Chirigos. 1970. Stimulation of humoral and cellular antibody formation in mice by poly I-C. Proc. Soc. Exp. Biol. Med. 135:334–338.
25. Wooles, W. R., and A. E. Munson. 1970. The effect of stimulants and depressants of reticuloendothelial activity on drug metabolism. J. Reticuloendothel. Soc. 9:465–477.