# The Effect of Polyinosinic:Polycytidylic Acid on Tumor Metabolism (35006)

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We have reported that the double-stranded synthetic RNA, polyinosinic:polycytidylic acid (poly I:poly C) inhibits the growth of a variety of different types of tumors in mice and rats (1) and also blocks carcinogenesis by dimethylbenzanthracene (2). The mechanism of this action appears to be multifaceted (3). The interferon induced in mice by this compound (4) may inhibit the growth of certain tumors (5). The compound is capable of strongly enhancing cell-mediated rejection of foreign antigens (3, 6, 7). In addition we have reported briefly that there is a decreased incorporation of radioactive precursors into RNA and protein of tumors in *in vivo* tests (3). The present paper presents the documentation for this latter statement.

*Materials and Methods. Tumors.* The MT-1 adenovirus-induced tumor in Balb C mice and the J96132 reticulum cell sarcoma in C57 black Kaplan mice have been described previously (1). The A-RCS reticulum cell sarcoma arose spontaneously in a $CD_2F_1$ male mouse, in Dr. R. H. Adamson's laboratory, and has been carried by subcutaneous trocar transplantation for 100 transplant generations. The two reticulum cell sarcomas were used 12–22 days after implantation, the MT-1 tumor between 8 and 13 days. When used they were about 0.5–1 cm in diameter and were not necrotic. These tumors were kindly supplied by Drs. Alan Rabson, Lloyd Law, and Richard Adamson of the National Cancer Institute.

*Polyinosinic:polycytidylic acid (poly I:poly C).* Poly I and poly C were purchased from P-L laboratories (Milwaukee, Wisconsin). They were dissolved separately at 1 mg/ml in pyrogen free physiological saline, at 44°. The pH was adjusted to 7.6–7.8 with 0.1 *N* NaOH. The separate solutions were filtered through a Millipore filter of 0.2-$\mu$ porosity. The two homopolymers were annealed by mixing at 44° in the ratio of 100 mg of poly I to 93.5 mg of poly C. There was a decrease in optical density at 248 m$\mu$ of about 45% from that expected from the two nonannealed single strands.

Protein was determined by the method of Lowry (8).

The general plan of the experiments was as follows. Three mice, with or without tumors, were inoculated intraperitoneally with 150 or 200 $\mu$g of poly I:poly C solution or with an equal volume of the vehicle. This is the dose that is effective as an antitumor agent. After 20 hr of exposure, 100 $\mu$Ci of $^3H$ uridine (sp act, 5.0 C/mmole), or 10 $\mu$Ci of $^{14}C$ proline (sp act, 220 mCi/mM) were injected, again intraperitoneally. After 1.5 or 2 hr, the animals were sacrificed. The indicated organs were removed, homogenized in cold phosphate buffered saline (PBS), and the homogenate was made to 10% with trichloroacetic or perchloric acid. The precipitate was washed with 1% acid, and dissolved in 0.2 *N* NaOH. Aliquots were used for protein determination. The radioactivity was determined as disintegrations per minute with a Packard scintillation counter filled with an absolute activity analyzer. The data were calculated (dpm/$\mu$g of protein[1]), and are shown as such or were used to calculate the percentage change induced by poly I:poly C treatment. Each experiment was done at least three times.

*Results.* From a large number of experiments, two things became evident. (i) The effect of such treatment on normal organs

[1] Except for the data of Table I where counts per minute were determined.

TABLE I. The Effect of Poly I:Poly C on Incorporation of Radioactive Proline into the Organs of Normal C57 Black Mice.

Specific activity as cpm/$\mu$g of protein.

| Organ | Control | Poly I:poly C treated | Change (%) |
|---|---|---|---|
| Liver | 2.41 | 3.37 | +39 |
| Spleen | 2.25 | 3.39 | +51 |
| Kidney | 2.12 | 4.05 | +91 |
| Thymus | 1.69 | 1.67 | −1 |
| Blood | 0.73 | 1.83 | +150 |
| Heart | 0.57 | 0.51 | −10 |
| Brain | 0.27 | 0.31 | +14 |
| Muscle | 0.21 | .18 | −13 |

was rather variable, depending to some extent on the strain of mouse studied, and (ii) protein and RNA synthesis in all three tumors was strongly inhibited by poly I:poly C treatment.

In general, macromolecule synthesis in normal tissues in Balb C mice were inhibited; in $CDF_1$ mice there frequently was inhibition but not so much as in Balb C mice; and in organs of C57 black Kaplan mice, there sometimes was inhibition, but frequently there was strong enhancement.

Table I shows the specific activities of tissues of nontumor-bearing C57 black mice that had been treated with 200 $\mu$g of poly I:poly C or saline, for 16 hr, then exposed to $^{14}C$ proline for 2 hr.

Figure 1 shows an example of inhibition of protein synthesis in tumors in C57 black mice, accompanied by enhancement of such synthesis in normal organs. Figure 2 shows the effect of poly I:poly C on both RNA and protein synthesis, where there is strong inhibition of the tumor metabolism, with a much less pronounded effect on normal organs.

Figure 3 compares two separate experiments with the MT-1 tumor in Balb C mice, in which two different preparations of poly I:poly C were tested for their effects on $^{14}C$ proline incorporation into acid-insoluble components of the cells. Balb C mice have been reported to produce less interferon than C57 black in response to Newcastle disease virus as well as to poly I:poly C (9).

Figures 4 and 5, dealing with the A-reticulum cell sarcoma in $CDF_1$ mice, compare the effect of a 20-hr and a 45-hr exposuure to poly I:poly C on uridine and proline incorporation. As with the other tumor types, this type shows strong inhibition. After 45 hr of exposure to poly I:poly C, it can be seen that there is strong inhibition in some of the normal organs.

Comparable results in experiments with nontumor-bearing Balb C and $CDF_1$ mice showed that the effect of poly I:poly C on normal organs was not dependent on the presence of the tumor.

Augmentation or inhibition of the specific

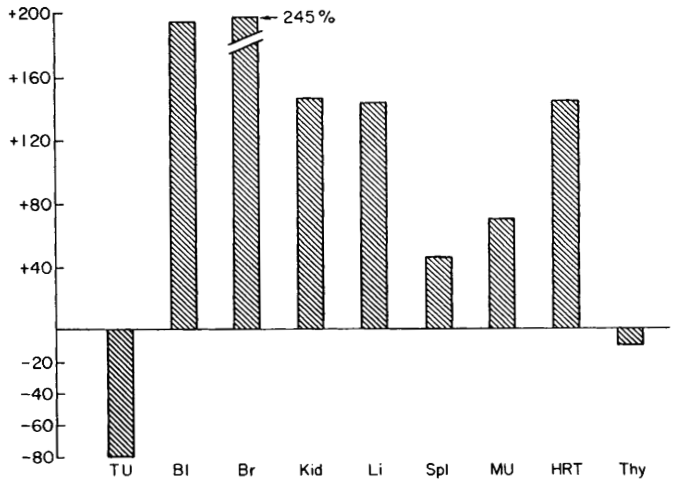


FIG. 1. The effect of poly I:poly C treatment on protein synthesis in organs of C57 black mice bearing the J96132 reticulum cell sarcoma.

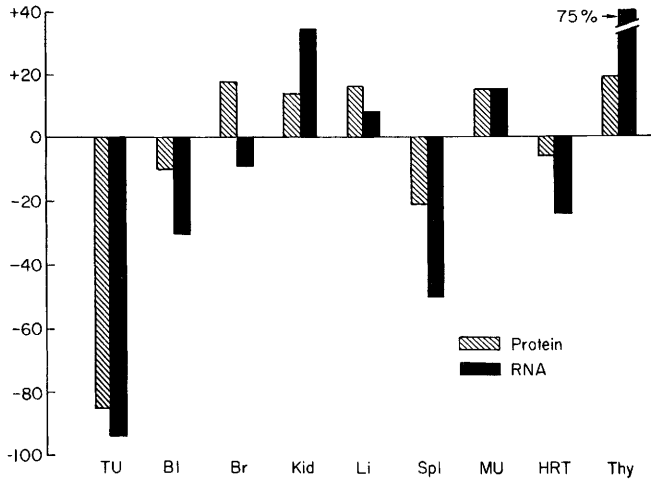


FIG. 2. The effect of poly I:poly C treatment on protein and RNA synthesis in organs of C57 black mice, bearing the J96132 reticulum cell sarcoma.

activity of macromolecules, such as RNA or protein, can represent either increased or decreased rate of synthesis of that macromolecule, or it could reflect changes in the specific activity of the acid-soluble precursors. In some experiments an approximation of the specific activity of the acid-soluble precursors was made. The organs were removd, homogenized rapidly in cold buffered sodium chloride (PBS), and concentrated perchloric acid was added to a final concentration of 10%. After sedimentation of the insoluble portions, the radioactivity in the acid-soluble material was determined. Specific activity was determined by dividing the disintegrations per minute by the optical density at 260 or 280 $m\mu$[2]. The data of Fig. 6 reveal that poly

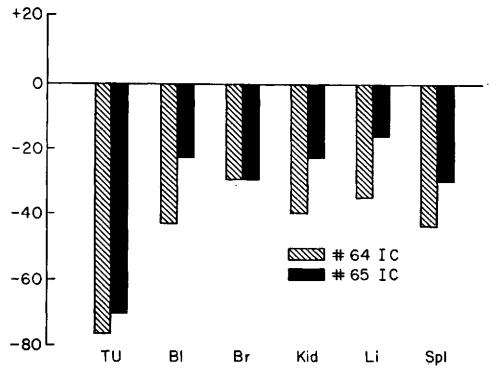


FIG. 3. The effect of two different preparations of poly I:poly C on protein synthesis in organs of Balb C mice bearing the MT-1 tumor.

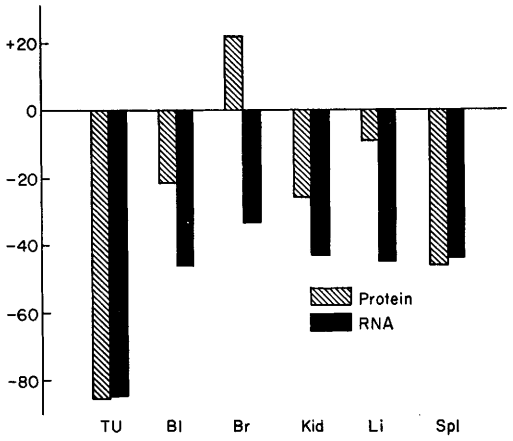


FIG. 4. The effect of a 20-hr exposure to poly I:poly C on RNA and protein synthesis in organs of $CDF_1$ mice bearing the A reticulum cell sarcoma.

[2] This procedure does not of course, give the true specific activity of the precursor pools of proline and uridine. The procedure measures relative specific activity in the control and poly I:poly C-treated samples, assuming that poly I:poly C does not alter the amount or composition of the acid soluble fraction of the cells. This assumption may not be valid. Without much more detailed analysis than is warranted for this experiment, these aspects of the effect of poly I:poly C on acid-soluble precursor pools cannot be analyzed. However, the question originally asked for these experiments, namely, is the altered rates of incorporation of $^{14}C$ proline and $^{3}H$ uridine into protein and RNA just a reflection of changes in acid-soluble pools can be answered.

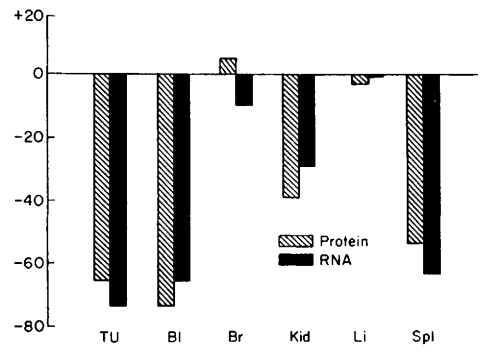


FIG. 5. Same as Fig. 4, except that exposure to poly I:poly C was 45 hours.

I:poly C treatment is associated with many changes in the radioactivity of the acid-soluble fractions but there is no consistent correlation between the direction of that change, and the direction of the change in radioactivity in the corresponding acid-insoluble material. Therefore the changes in acid-soluble precursors is not the immediate cause of the changes in acid-insoluble fractions.

*Discussion.* The data presented here bear on two aspects of the action of poly I:poly C—its ability to inhibit tumors, and its toxicity in mice. There is, in general, a relatively greater inhibition of macromolecule synthesis in the tumors than in other organs of the mice. This would be consistent with the fact that there is almost always observed a decrease in growth of the tumors in animals treated with poly I:poly C or an actual diminution in tumor size when treatment is started after tumors have already developed. The inhibition of macromolecule synthesis by poly I:poly C may be a direct cause of the inhibition of tumor growth, or it conceivably could arise because the tumor growth was being inhibited by some other mechanism. One possible mechanism, for example might be the demonstrated enhancement by poly I:poly C of lymphocyte action against foreign antigens (3, 5, 6). This latter possibility would be consistent with the fact that tumor metabolism is usually much more inhibited than is the metabolism of normal tissues. However, it is to be noted that there are strong effects of the poly I:poly C on macromolecule metabolism in many of the normal organs. It is hard to imagine that this effect is related to enhanced immunological activity. It would be more likely to be attributable to a direct effect of the chemical. The latter interpretation, at least with regard to the effect on normal organs, is consistent with the findings, in mouse tissue culture, of altered

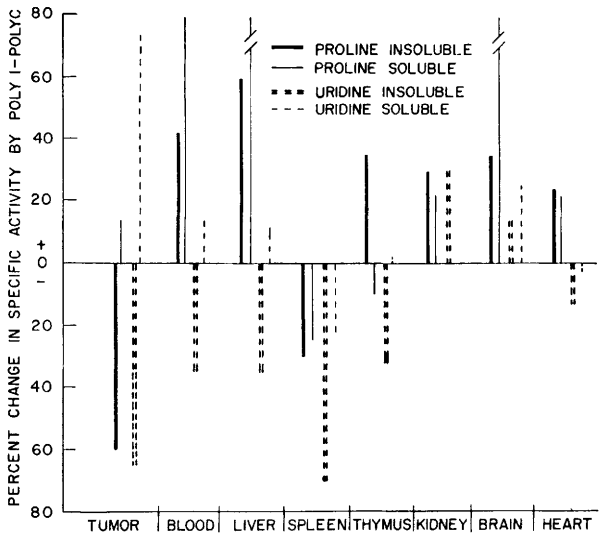


FIG. 6. Comparison of the effects of poly I:poly C on the specific radioactivities of $^{3}H$ uridine and $^{14}C$ proline in acid-soluble and acid-insoluble fractions of organs from $CDF_1$ mice bearing the A reticulum cell sarcoma.

RNA (10), and protein metabolism (3), and altered protein metabolism in slices of organs of rats exposed to poly I:poly C (11).

The fact that poly I:poly C leads to augmentation of the specific activity of precursor pools in some organs, and an inhibition in others rules out the possibility that alterations in macromolecule radioactivity is only a reflection of a generalized alteration in precursor transport.

The differences among the different strains of animals, while not absolute, is relatively reproducible. It should be pointed out that in our hands, Balb C mice, in which poly I:poly C causes the strongest inhibition of macromolecule synthesis, also is the most sensitive to the clinical toxicity and lethality brought about by higher doses of the compound. It has been reported that C57 black mice produce 2–3 times as much interferon in response to poly I:poly C as do Balb C mice (9).

The general disturbance of macromolecule synthesis seen in the cells of the mice treated with poly I:poly C could well result in an alteration of the ability of these organs to carry out the functions that these cells ordinarily are required to perform. It also is possible that the ability of these cells to support virus growth might be modified by mechanisms which have no relationship to the interferon mechanism.

These findings have implications relative to the possible use of poly I:poly C in therapy in man, both in viral disease and in malignancies. The data presented here were obtained with doses of poly I:poly C that were not toxic, in the sense that these doses do not cause death or marked illness during the time of observations. However, biochemical alterations of the magnitude found could be considered evidence of a more subtle type of toxicity. What physiological or pathological consequences might follow such biochemical changes can only be conjectured, but certainly their possibility is cause for concern when applied to man. However, the desirable actions of the compound conceivably might be separable from the undesirable ones, by suitable alteration in the molecular architecture.

*Summary.* The synthetic double-stranded RNA, polyinosinic: polycytidylic acid (poly I:poly C), strongly inhibits *in vivo* RNA and protein synthesis in three different tumors in mice. Macromolecule synthesis in normal organs of these mice may be inhibited or enhanced, depending to some extent on the strain of mouse. Administration of poly I:poly C to the mice also leads to alterations in the specific radioactivity of acid-soluble precursor pools, sometimes increasing and sometimes decreasing such specific activity. There was no discernible relationship between the nature of the alteration in precursor specific activity and the direction of effect on macromolecule synthesis.

1. Levy, H. B., Law, L. W., and Rabson, A. S., Proc. Amer. Ass. Cancer Res. **10**, 49 (1968); and Proc. Nat. Acad. Sci. U.S. **62**, 357 (1969).

2. Gelboin, H., and Levy, H. B., Science **167**, 205 (1970).

3. Levy, H. B., Asofsky, R., Riley, F., Garapin, A., Cantor, H., and Adamson, R., Proc. 2nd Conf. Antiviral Substances, N.Y. Acad. Sci. (1969), in press.

4. Field, A. D., Tytell, A. A., Lampson, G. P., and Hilleman, M. R., Proc. Nat. Acad. Sci. U.S. **58**, 1004 (1967).

5. Gresser, I., Bourali, C., Levy, J. P., Fontaine-Brouty-Boye, D., and Thomas, M. T., Proc. Nat. Acad. Sci. U.S. **63**, 51 (1969).

6. Cantor, H., Asofsky, R., and Levy, H. B., J. Immunol. **104**, 1035 (1970).

7. Braun, W., Proc. Int. Cong. Chemother., 6th (1969) in press.

8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

9. DeMaeyer, E., and DeMaeyer, J., Proc. 2nd Conf. Antiviral Substances, N.Y. Acad. Sci. (1969), in press.

10. Margolis, S., and Levy, H. B., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 816 (1969).

11. Bhatnagar, R., and Levy, H. B., unpublished observations.

Received May 7, 1970. P.S.E.B.M., 1970, Vol. 135.