# INSTABILITY OF POLYRIBOSOMES DERIVED FROM RATS PRETREATED WITH THE HEPATOCARCINOGEN DIMETHYLNITROSAMINE

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Administration of DMNA* to rats produces within 2 hours a decreased rate of amino acid incorporation into ribosomal protein, an increased response of polypeptide synthetic activity to poly U and a reduced number and size of polyribosomes, accompanied by a corresponding increase in 80-S particles (Mizrahi and Emmelot, 1964). The question thus arose whether and to what extent the inhibition of amino acid incorporation, exhibited by the DMNA-treated ribosomes after incubation, was related to the breakdown of polyribosomes observed in freshly-prepared ribosomal preparations. In the present investigation it is shown that during incubation a further breakdown of DMNA-polyribosomes as compared with control preparations takes place, suggesting a cytoplasmic effect of DMNA, and not an interference of DMNA with the synthesis of messenger-RNA.

METHODS - After being fasted overnight, male hybrid rats (U X R-Amsterdam), weighing 200-250 g., were killed 5 h. after an intravenous injection of 100 mg. DMNA/kg.

* Abbreviations: DMNA = dimethylnitrosamine; poly U = polyuridylic acid; RNP = ribonucleoprotein.

The microsomal fraction was separated by centrifugation of the postmitochondrial supernatant (prepared as previously described, Mizrahi and Emmelot,1964) at 105.000 x g for 50 min. and then resuspended in the homogenizing medium at a protein concentration of 10 mg./ml. The microsomes were further subfractionated into membrane-free and membrane-bound RNP particles by layering 15 ml. of the microsomal suspension in 45 ml. centrifuge tubes on a discontinuous gradient consisting of equal volumes of 2.0 and 1.5 M sucrose of the same ionic composition as the homogenizing medium (Bloemendal et al.,1964). Centrifugation was carried out for 12 h. at 70.000 x g in an "elephant" rotor of a P30K Phywé centrifuge. Membrane-free RNP particles were recovered from the bottom of the centrifuge tube. The fraction floating between the 1.5-2.0 M sucrose layers, consisting of fragments of rough endoplasmic reticulum (Benedetti et al.,1964), was freed from contaminating free particles and smooth membranes by using a 5-20 % linear sucrose gradient which was itself layered on a 50 % sucrose solution, as described by Henshaw et al.(1963). Membrane-bound ribosomes were reisolated from a peak located in the 20-50 % intersurface. Amino acid incorporation assays and sucrose-gradient analyses were, unless otherwise indicated, carried out as described previously (Mizrahi,1965).

RESULTS AND DISCUSSION - As in the case of ribosomes isolated with the use of detergents (Mizrahi and Emmelot,1964), the ability of membrane-free RNP particles, isolated as described under Methods, to support amino acid incorporation was reduced and exhibited an increased capacity to be stimulated by poly U when the rats were pretreated with

DMNA (unpublished experiments). Associated with this, a dissociation of polyribosomes to 80-S ribosomes was also observed (Fig.1).

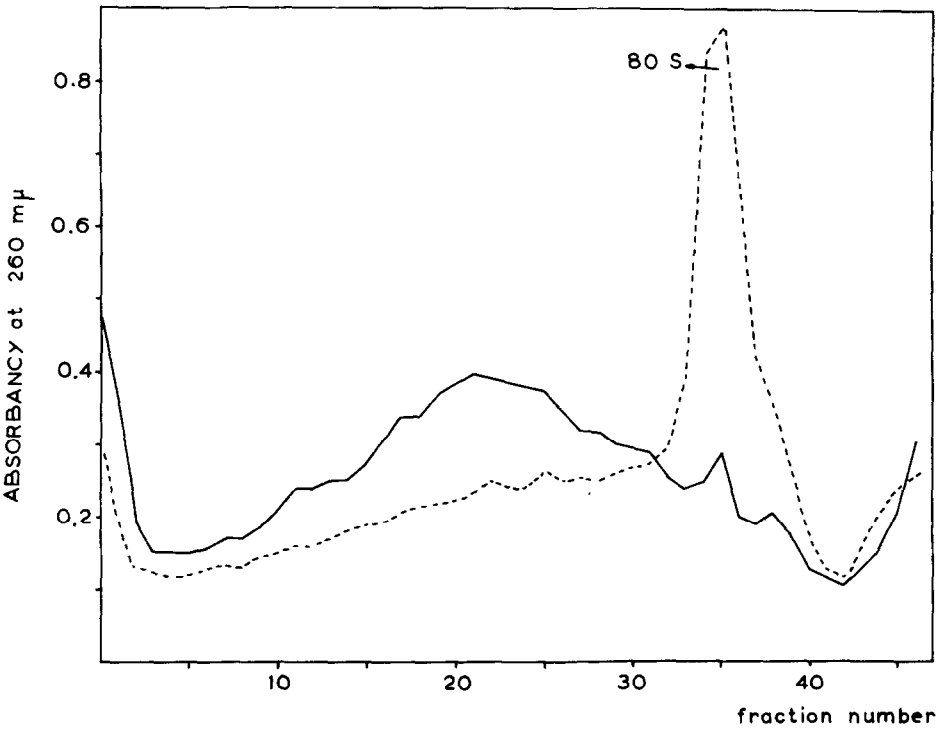


Fig.1. Sucrose-gradient centrifugation profiles of membrane-free RNP particles isolated from normal (solid lines) and DMNA-treated (broken lines) rat liver.

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The heavy ribosomal material of both normal and DMNA-treated ribosomes (fractions 0-10; Fig.1) were collected, concentrated and assayed for amino acid incorporation in the absence and presence of poly U at two time intervals. As illustrated in Table I, the polyribosomes isolated from DMNA-liver were less effective in incorporating phenylalanine than were the corresponding control preparations.

TABLE I - Incorporation of ($^{14}$C)phenylalanine into protein by polyribosomes separated from mebrane-free RNP particles

| Exp. No. | Time of incubation | Counts/min./mg. protein − poly U Control | − poly U DMNA | % inhibition | + poly U Control | + poly U DMNA |
|---|---|---|---|---|---|---|
| 1 | 5 min. | 445 | 336 | 25 | 667(1.5) | 735(2.2) |
| | 60 " | 2344 | 1316 | 44 | 3498(1.4) | 4507(3.4) |
| 2 | 5 " | 346 | 277 | 20 | 482(1.4) | 473(1.7) |
| | 60 " | 2406 | 966 | 60 | 3215(1.3) | 3702(3.8) |

Equal amounts of normal and DMNA-treated preparations corresponding to 0.1 mg. protein and 0.9 mg. 105.000 x g normal supernatant protein were incubated in a standard incorporation mixture containing 0.07 µc (U-$^{14}$C)phenylalanine (66.2 mc/mM) and 50 µg poly U where indicated. Reactions were carried out at 37° in a final volume of 0.2 ml. After the specified incubation times, proteins were plated on millipore filters. Listed in parentheses: factor stimulation of amino acid incorporation by poly U

The decrease was relatively small (20-25 % after 5 min. incubation and became more pronounced (44-60 %) when the reaction was prolonged to 1 h. Addition of poly U to the treated particles raised their ability to incorporate ($^{14}$C)phenylalanine to the levels observed for the normal particles, if not more. The relatively increased extent of stimulation observed with the DMNA-polyribosomes in the presence of poly U, as compared with the untreated polyribosomes, was much more pronounced after 30 min. than after 5 min. incubation.

These experiments suggest that during incubation more ribosomal monomers, capable of utilizing poly U as template, are liberated from DMNA-polyribosomes than from controls. Accordingly, polyribosomes (fractions 0-10; Fig.1) from both DMNA-treated and control livers were incubated for 20 min. at 37°, chilled, and analyzed on sucrose gradients. The sedimentation profiles for a typical experiment, reproduced in Fig.2, showed an increased absorbancy in the

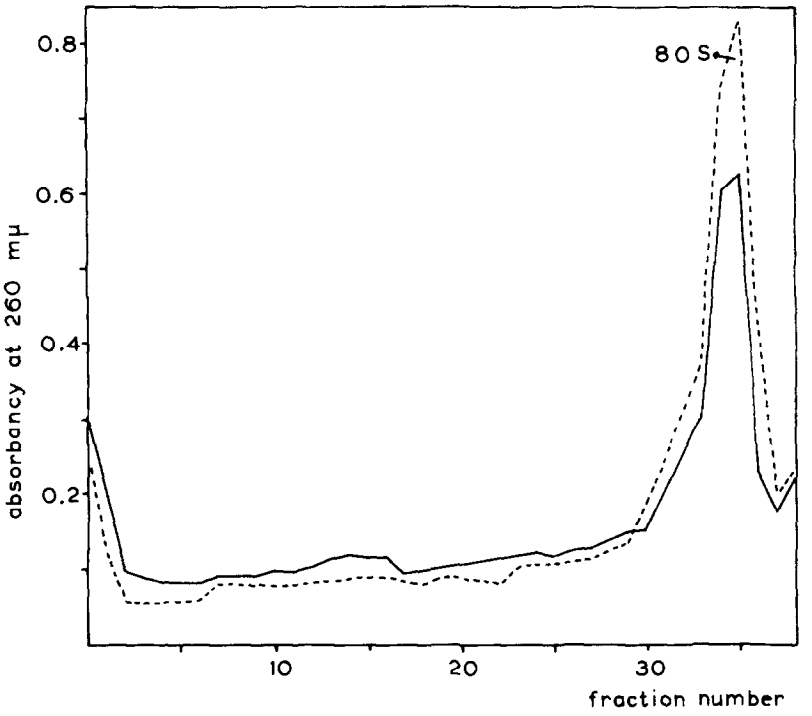


Fig.2. Effect of incubation on polyribosomes separated from normal (solid lines) and DMNA-treated (broken lines) membrane-free RNP particles. The polyribosomes (0.5 mg.protein) were incubated for 20 min. as described for Table I, chilled and analyzed by sucrose-gradient centrifugation.

single ribosome region of the DMNA-treated polyribosomes as compared with the control preparations.

Essentially similar results were obtained with the membrane-bound RNP particles. Both the decrease of amino acid incorporation and the poly U effect observed for these RNP particles, after isolation from DMNA-treated rats, were time-dependent (Table II) and constantly accompanied by the formation of more 80-S particles, as compared with the controls after 15 min. incubation (Fig.3). Since the 80-S peak was missing in both preparations subjected to sucrose-

gradient centrifugation prior to incubation, it may be assumed that the gradual inhibition of amino acid incorporation is due to the release and breakdown of the polyribosomes from the endoplasmic reticulum membranes.

TABLE II - Incorporation of ($^{14}$C)phenylalanine into membrane-bound RNP particles

| | Counts/min./mg. protein | | | | |
|---|---|---|---|---|---|
| Time of incubation | − poly U Control | DMNA | % Inhibition | + poly U Control | DMNA |
| 5 min. | 254 | 229 | 10 | 540(2.1) | 776(3.3) |
| 30 " | 495 | 330 | 33 | 1982(4.0) | 4011(12.1) |

Equal amounts of normal and DMNA-treated preparations corresponding to 0.16 mg. RNA and 2.00 mg. normal 105.000 x g supernatant protein were incubated in standard incorporation mixture containing 0.17 µc (U-$^{14}$C)phenylalanine (300 mc/mM) and 150 µg poly U where indicated. Data are the average of 2 identical experiments. Listed in parentheses: factor stimulation of amino acid incorporation by poly U.

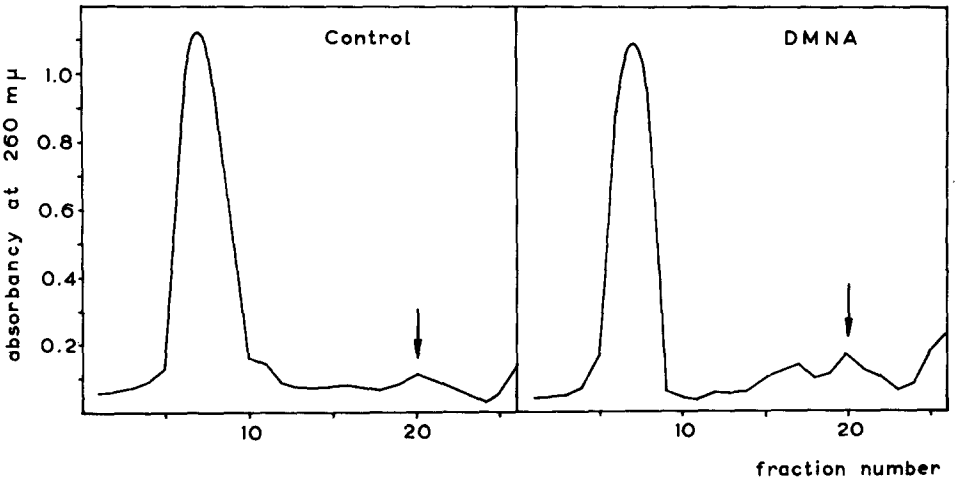


Fig.3. Sucrose-gradient analysis of the membrane-bound RNP particles from normal and DMNA-treated livers 15 min. after incubation in the incubation mixture as described for Table II. Aliquots of 1.0 ml. (0.32 mg.RNA input) were layered on 5-20 % linear sucrose-gradient with 50 % at the bottom of the tube. Direction of sedimentation from right to left. The light peak (arrow) contains free ribosomes (Henshaw et al.,1963).

The observations reported here, and the fact that no significant differences were detected in the rate of incorporation of $^{32}P$-inorganic phosphate into nuclear and cytoplasmic RNA isolated from normal and DMNA-treated liver, indicated that the loss of incorporation activity is not the result of an impairment of the synthesis of rapidly labeled RNA. The latter phenomenon has been reported for ethionine, which was also shown to inhibit protein synthesis (Farber et al.,1964) and to alkylate nucleic acids in rat liver (Farber and Magee,1960). Our data rather show that the defect in protein synthesis following DMNA treatment is due to an accelerated breakdown of polyribosomes, which may be the result of methylation of template RNA (Mizrahi and Emmelot,1962,1964).

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References

Benedetti,E.L., Bloemendal,H. and Bont,W.S., C.R.Acad.Sci. Paris,259,1353(1964).
Bloemendal,H., Bont,W.S. and Benedetti,E.L., Biochim. Biophys.Acta,87,177(1964).
Farber,E. and Magee,P.N., Biochem.J.,76,58P(1960)
Farber,E., Schull,K.H., Villa-Trevino,S., Lombardi,B. and Thomas,N., Nature,203,34(1964).
Henshaw,E.C., Bojarski,T.B. and Hiatt,H., J.Mol.Biol.,7, 122(1963).
Mizrahi,I.J., Biochim.Biophys.Acta,108,419(1965).
Mizrahi,I.J. and Emmelot,P., Cancer Res.,22,339(1962).
Mizrahi,I.J. and Emmelot,P., Biochim.Biophys.Acta,91,362 (1964).