The Effect of Whole-Body X-Irradiation of Guinea Pigs on Liver Ribonucleic Acid Synthesis

By E. J. HIDVÉGI, ELISABETH BÖLÖNI, J. HOLLAND, F. ANTONI AND V. VÁRTERÉSZ
Institute for Radiobiological Research, Budapest-Budafok, Hungary

(Received 31 July 1969)

1. Liver RNA synthesis was studied within 24 h after whole-body X-irradiation of guinea pigs that had been starved for 22–24 h. 2. Microsomal RNA was labelled *in vivo* for 3 h with [^{14}C]orotic acid and the isolated labelled RNA was fractionated by sucrose-density-gradient centrifugation. Incorporation was 50–100% higher between 3 and 12 h after 2000 rd X-irradiation and at 22 h was not elevated any further. Whole nuclear RNA was labelled with [^{14}C]orotic acid for 15 min. At 5 h after irradiation the incorporation showed a 50–100% increase. Incorporation increased in all types of RNA studied. 3. The RNA phosphorus/DNA phosphorus ratio of whole liver gradually increased after X-irradiation. Maximal increase was found between 24 and 36 h, which corresponds to a value about 40% above that of the starved control. The RNA phosphorus content of isolated ribonucleoproteins obtained from various cell fractions of the liver was similarly increased after X-irradiation. 4. Liver microsomes were obtained from X-irradiated and control animals. Microsomes were incubated *in vitro* with [^{14}C]phenylalanine in the presence and absence of polyuridylic acid. After the incubation the microsomes were fractionated by sucrose-density-gradient centrifugation. The polyuridylic acid enhancement was twice as great in the microsomes of the control preparation as in the irradiated one. The experiment demonstrated a higher saturation of microsomes by endogenous messenger after X-irradiation. 5. RNA polymerase activity of the purified nuclear preparation was assayed. The activity of the Mg^{2+} -dependent RNA polymerase activity was 50 and 200% respectively above the control values at 6 and 9 h after X-irradiation. 6. Animals were treated with actinomycin D shortly before X-irradiation. This treatment abolished the radiation-induced enrichment of polyribosomes and the increase of protein-synthesizing activity. The effect of X-irradiation on the transcription of the genetic code of the liver is discussed.

Whole-body X-irradiation of animals resulted in an enhanced protein synthesis by the liver and was considered as a consequence of increase of both mRNA and rRNA contents of the cytoplasm (Hidvégi *et al.* 1968). The question to be answered is whether the higher amount of RNA in the cytoplasm after X-irradiation represents an increase in RNA synthesis.

DNA synthesis is depressed in nearly all cell types shortly after irradiation, but the effect of irradiation on RNA synthesis is as yet unclear (Ord & Stocken, 1961; Goutier, 1961). This is especially obvious in radioresistant organs, e.g. the liver. The reports on RNA synthesis in the liver after X-irradiation are contradictory. Some investigators found no change in RNA synthesis in the liver after X-irradiation (Ord & Stocken, 1956), whereas other investigators claimed a decrease (Uchiyama, Fausto & Van Lancker, 1966) and some reported

that the synthesis of various types of RNA of cells was unchanged and that of other types even increased (Payne, Kelly & Entenman, 1952; Klouwen, 1960; Petrovič, 1962). The equivocal data prompted the present authors to study RNA synthesis in liver after X-irradiation.

The purpose of this study was to clarify the role of RNA synthesis relative to the increased protein synthesis after X-irradiation (Hidvégi *et al.* 1968). Results indicate that the increased amounts of rRNA and mRNA in the cytoplasm are the consequence of enhanced RNA synthesis in the liver after whole-body X-irradiation of guinea pigs. Additional evidence suggests that the higher activity of RNA polymerase in nuclei is correlated with the increased RNA synthesis. Moreover, the radiation-induced RNA synthesis appears to be sensitive to actinomycin D. These results could be related to the increased protein synthesis in the cytoplasm

after X-irradiation and provide the functional basis for the enhanced activity of the ribosomal system.

These observations have been reported in part as preliminary accounts (Hidvégi *et al.* 1965; Hidvégi, Holland, Bölöni & Várterész, 1966; Hidvégi, Várterész, Holland & Bölöni, 1966).

METHODS

Animals. Male guinea pigs (380–420 g) starved for 22–24 h before death were used. Each experimental group consisted of three animals. [^{14}C]Orotic acid was administered into the vena saphaena under light ether anaesthesia. Actinomycin D was freshly dissolved in propylene glycol, diluted with 0.14 M-NaCl and administered intraperitoneally.

Irradiation. The animals were exposed to whole-body irradiation as described by Koczkás & Dósay (1966). The controls were anaesthetized and exposed to the same conditions save irradiation. Characteristics of the radiation were: 200 kV, 15 mA, 0.5 mm Cu filter, 81.5 rd/min. The distance between the focus and the middle of the body was 40 cm. A THX-250 instrument (Medicor, Budapest, Hungary) was used.

Preparation of cell nuclei. Purified nuclei were isolated for the determination of RNA P/DNA P ratio and for isolation of rRNA by the method described by Hidvégi, Lónai & Antoni (1963). To assay the RNA polymerase activity the nuclei were purified by the method of Chauveau, Moulé & Rouiller (1956).

Preparation of cytoplasmic cell fractions. The livers were quickly removed, cooled and homogenized in a Teflon-glass homogenizer (ten strokes) in 3 vol. of 0.25 M-sucrose in medium A. Medium A contained (final concns.) 50 mM-KCl, 5 mM-MgCl₂, 6 mM-2-mercaptoethanol and 20 mM-tris buffer, adjusted to pH 7.8 (at 5°C). The homogenate was centrifuged for 15 min at 10000g (all values are g_{av}) in a Spinco L50 preparative ultracentrifuge (rotor no. 30). To sediment the microsomal fraction the postmitochondrial supernatant was centrifuged for 90 min at 78500g (rotor no. 30). When the microsomes were used for isolation of RNA, the KCl was omitted from medium A because of the subsequent treatment with sodium dodecyl sulphate solution.

Total ribosomal particles (Munro, Jackson & Korner, 1964) were obtained from the deoxycholate-treated postmitochondrial supernatant as described by Hidvégi *et al.* (1968).

To prepare the 'high-speed supernatant' containing the ribonuclease inhibitor of the cytoplasm, the livers of normal rats were homogenized in 2 vol. of 0.25 M-sucrose in medium A and centrifuged for 10 min at 10000g. The supernatant was centrifuged further for 3 h at 150000g (rotor no. 50). The upper two-thirds of the supernatant was collected and used as the crude fraction containing ribonuclease inhibitor.

Measurement of ribonucleoprotein content of isolated cell fractions. Livers were quickly collected, cooled, weighed and homogenized thoroughly in a Teflon-glass homogenizer in 5–6 vol. of 0.25 M-sucrose in medium A. The homogenate was centrifuged for 10 min at 800g and the supernatant was removed quantitatively and kept. The pellet was washed once, suspended and centrifuged

as described above. The pellet was designated P-1. The two supernatants were combined and a portion was layered on to 10 ml of 0.5 M-sucrose, and plain medium A was layered above to avoid any loss of material. The whole was centrifuged for 15 min at 10000g (rotor no. 30). The supernatant (S-2) was removed quantitatively from the pellet (P-2) and both were kept.

The ribonucleoprotein particles were obtained from fractions P-1, P-2 and S-2 by homogenizing the pellets P-1 and P-2 from 4 g of liver in 30 ml of 'high-speed supernatant' containing the ribonuclease inhibitor and treating with deoxycholate (final concn. 1%, w/v). Portions of the suspension were layered quantitatively over 5 ml of 0.5 M-sucrose in medium A, and plain medium A was layered above. Portions of fraction S-2 were treated directly with deoxycholate and layered as indicated for fractions P-1 and P-2. The tubes containing the suspensions were centrifuged for 4 h at 105000g (rotor no. 40). The RNA content of the ribonucleoprotein pellets was determined as follows. The pellets were suspended and treated with ice-cold trichloroacetic acid, and the final concentration was adjusted to 5% (w/v). The pellets were washed twice with cold 5% trichloroacetic acid, once with ethanol containing potassium acetate (2%, w/v), once with ethanol-ether (3:1, v/v) and finally with ether. The pellets were dissolved in diluted ammonia and the RNA content was assayed by the Ceriotti (1955) orcinol reaction.

Incorporation of ^{14}C -labelled amino acids in vitro. The system for incorporation by total ribosomal particles was as described by Hidvégi *et al.* (1968). Incorporation of [^{14}C]phenylalanine into microsomes was carried out in an identical system but containing microsomes (instead of ribosomes) equivalent to 600 μg of RNA and pH 5 fraction equivalent to 1 mg of protein/ml. This system was divided into two parts: 500 μg of poly U/ml was added to one part and 1 μCi of [^{14}C]phenylalanine/ml to both parts. Incubation was performed at 37°C for 30 min. The mixture was then cooled and a portion (0.3 ml) was layered onto a sucrose density gradient for fractionation.

Sucrose-density-gradient ultracentrifugation. The method for the analysis and the distribution of ribosomes in deoxycholate-treated postmitochondrial supernatant was given in detail by Hidvégi *et al.* (1968). Correction was made for absorption by ferritin by the method of Jackson, Munro & Korner (1964).

Fractionation of microsomes labelled with [^{14}C]phenylalanine *in vitro* was performed in the same way as with ribosomes. The fractions were collected in the cold; portions of each were taken for measurement of extinction and the remainder was precipitated with cold 5% trichloroacetic acid in the presence of 1 mg of albumin as carrier. The precipitate was centrifuged, washed twice with cold 5% trichloroacetic acid, once with 96% ethanol containing potassium acetate (2%) and once again with 96% ethanol. The precipitate was dissolved in conc. formic acid and transferred quantitatively to an aluminium planchet.

The isolated RNA was fractionated by centrifugation in a 5–25% linear sucrose density gradient for 12–14 h at 63500g (rotor SW 25.1). The exact duration of centrifugation is indicated in the legend of each figure (Figs. 1, 2 and 3). The sucrose solution contained 1 mM-EDTA and 10 mM-sodium acetate buffer, pH 5.2. Samples for

determination of E_{260} and for measurement of radioactivity were collected alternately in the cold. Usually one drop was collected for measurement of extinction and 12 drops were collected for measurement of radioactivity. For measurement of radioactivity, the RNA was precipitated with cold 5% (final concn.) trichloroacetic acid in the presence of 1 mg of albumin as carrier. The precipitate was washed once with cold 5% trichloroacetic acid, dissolved in dil. NH_3 and transferred quantitatively to planchets.

Analytical ultracentrifugation. The RNA was dissolved in a medium containing 100mM-NaCl, 1mM-EDTA sodium salt and 50mM-sodium acetate buffer, pH 5.2. The solution was centrifuged with a model G120 ultracentrifuge (Hungarian Optical Works, Budapest, Hungary) at 59960 rev/min at 4°C. The phase-plate angle of the Philpot-Svensson optics was 60°.

Isolation of RNA. The isolation of RNA was performed essentially by the method of DiGirolamo, Henshaw & Hiatt (1964). The microsomes or purified nuclei were suspended with a Teflon-glass homogenizer (ten gentle strokes) in 2% (w/v) sodium dodecyl sulphate dissolved in 0.14M-NaCl-50mM-sodium acetate buffer, pH 5.2, containing 200 μg of polyvinyl sulphate/ml (final concn.). An equal volume of freshly distilled phenol was added and the mixture was homogenized again, stirred at 10°C for 20 min and centrifuged. The phenol solution contained 8-hydroxyquinoline (0.1%, w/v) and was saturated with the sodium dodecyl sulphate buffer. The water phase was decanted off. Where indicated in the Results section, the interphase and pellet were extracted several times with fresh aq. 2% sodium dodecyl sulphate until the interphase was completely dissolved. The combined aqueous phases were deproteinized twice with 0.5 vol. of phenol (saturated with buffer) and the nucleic acids were precipitated from the aqueous phase by the addition of 2.5 vol. of ethanol containing sodium acetate (1% w/v). Finally the RNA was reprecipitated twice.

The nucleic acid preparation extracted from the cell nuclei contained some DNA, which was removed by treatment with deoxyribonuclease. The precipitate containing RNA and DNA was dissolved in 10mM-tris-HCl buffer, pH 7.4, containing 1mM- MgCl_2 . To the solution containing about 200 μg of DNA/ml, 5 μg of deoxyribonuclease/ml (final concn.) was added and digestion was carried out at 10°C for 30 min. The mixture was deproteinized with phenol and the RNA precipitated and reprecipitated twice more.

Assay of RNA polymerase activity. The RNA polymerase was measured essentially by the method of Widnell & Tata (1964). The system contained the following components in a volume of 1.0 ml: 5 μmol of MgCl_2 , 6 μmol of 2-mercaptoethanol, 5 μmol of phosphoenolpyruvate, 10 μg of pyruvate kinase, 0.1 mol of tris-HCl buffer (pH 8.5 at 37°C), 0.6 μmol of each of CTP, UTP and GTP, 0.72 μCi of [^{14}C]ATP and nuclei equivalent to 0.6 mg of DNA. Incubation was carried out at 37°C for 15 min with shaking. The RNA was precipitated, extracted and the enzymic activity expressed as described by Widnell & Tata (1964).

Chemical assays. To measure the amount of RNA, DNA or both in homogenates or cell fractions, the RNA, DNA and proteins were separated by slight modification of the Schmidt & Thannhauser method (Hidvégi *et al.*

1963). RNA was measured by the orcinol method (Ceriotti, 1955), with ribose as standard and DNA was measured by Burton's (1956) method with DNA from chicken erythrocytes as standard. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystallized bovine serum albumin as standard.

Measurement of radioactivity. The preparation of samples for measurement of radioactivity was as described above for the sucrose-density-gradient fractions. For incorporation of [^{14}C]labelled amino acids *in vitro*, preparation was as described by Hidvégi *et al.* (1968). The radioactivity of infinitely thin samples was measured in all experiments on a Frieske-Hoepfner methane gas-flow counter (type FH 51) (Hidvégi *et al.* 1968).

Materials. [6- ^{14}C]Orotic acid (44 mCi/mmol), L-[U- ^{14}C]phenylalanine (222 mCi/mmol) and L-[U- ^{14}C]lysine (136 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The actinomycin D preparation was produced by the Pharmaceutical Research Institute, Budapest, Hungary. According to their analysis it contained 90–92% of component IV (actinomycin D), 6–8% of component V and 1–2% of components I and II. Other compounds used were: polyvinyl sulphate potassium salt (from Serva Entwicklungslabor, Heidelberg, Germany), disodium ATP (from Reanal, Budapest, Hungary), GTP, CTP, UTP, pyruvate kinase, phosphoenolpyruvic acid, sodium dodecyl sulphate (all from Sigma Chemical Co., St Louis, Mo., U.S.A.), poly U (from Calbiochem, Los Angeles, Calif., U.S.A.) and deoxyribonuclease I, ribonuclease-free (from Worthington Biochemical Co., Freehold, N.J., U.S.A.).

RESULTS

The microsomal RNA preparations were checked by analytical ultracentrifugation (not shown). The RNA preparations obtained from microsomes of X-irradiated animals were composed of the same main components that are found in the normal liver cytoplasm (28S, 18S and a more heterogeneous 4–6S component) (Gierer, 1958). No major unique components or degradation products have been detected.

Incorporation of [^{14}C]orotic acid into RNA. The microsomal RNA, labelled with [^{14}C]orotic acid for 3 h *in vivo*, was fractionated by sucrose-density-gradient ultracentrifugation. Fig. 1(a) shows that at least 3 h labelling time was required to give sufficiently labelled rRNA. The radioactivity profile coincides with the E_{260} profile. Figs. 1(b) and 1(c) show that at 9 and 12 h respectively, after 2000 rd irradiation the incorporation into 18S rRNA and 28S rRNA was twice that in the control. The radioactivity, however, was not higher than the control 22 h after irradiation (Fig. 1d).

The next experiment showed that repeated extractions of microsomes with aq. sodium dodecyl sulphate released more of the rapidly labelled RNA that sedimented heterogeneously between low-molecular-weight RNA and rRNA (Fig. 2a).

Fig. 1. Sucrose-density-gradient centrifugation of [^{14}C]orotic acid-labelled microsomal RNA. Each animal was injected intravenously with $5\mu\text{Ci}$ of orotic acid/100 g body wt. and killed 3 h later. The microsomes were isolated and the RNA extracted as described in the Methods section. One phenol-sodium dodecyl sulphate extraction was used in this experiment. The RNA was centrifuged for 13 h. The sedimentation is from right to left. (a) Control; the other groups of animals were irradiated with 2000rd and killed (b) 9 h; (c) 12 h; (d) 24 h later. —, E_{260} ; ----, radioactivity. The arrow indicates the top of the gradient.

Several investigators contend that this kind of cytoplasmic RNA could be considered to contain mRNA (Georgiev, Samarina, Lerman, Smirnov & Severtzov, 1963; Staehelin, Wettstein, Oura & Noll, 1964; Munro & Korner, 1964; DiGirolamo *et al.* 1964). At 3 h after 2000rd irradiation the labelling of this type of RNA more than doubled, whereas the labelling of rRNA was enhanced only slightly (Fig. 2b). The experiment in Fig. 2(c) shows that at 6 h after irradiation the incorporation was about double in all types of microsomal RNA.

Whole nRNA was labelled *in vivo* with orotic acid. The sucrose-density-gradient pattern of control animals showed that 15 min labelling time was sufficient to label the rapidly synthesized RNA, which sedimented between the low-molecular-weight RNA and the rRNA (Fig. 3a). The labelling increased in all RNA fractions 5 h after X-irradiation (Fig. 3b). The labelling was 1.5 times the control value in the fractions sedimenting between the low-molecular-weight RNA and the rRNA and was about double in the fractions sedimenting more rapidly than the 28S rRNA. In all repeated experiments the incorporation into whole nRNA was 50–100% above the control 2–6 h after irradiation.

After 8 h the labelling of nRNA was not enhanced but remained at the control value (not shown).

In Figs. 1–3 it should be noted that the labelling of the low-molecular-weight RNA was also increased. The RNA sedimenting at 4–8S was found to be heterogeneous (Prestayko & Busch, 1968), but was not investigated in detail by the present authors because primary attention was given to the rRNA and mRNA of liver after irradiation.

The labelling of the acid-soluble nucleotide pool of whole liver was also checked. In all of these experiments there was no marked change in the specific radioactivity of that pool 3–22 h after irradiation. This supports the thesis that the increased labelling of RNA was due to the increased synthesis of RNA and not to the increased labelling of precursors. There are also some indications in the literature that the amount (Maass & Timmis, 1964) and labelling (Ord & Stocken, 1956) of acid-soluble nucleotide pools in liver do not change after irradiation.

RNA content. At various times after 3000rd irradiation the RNA and DNA contents of whole liver cells were measured. The results in Table 1 demonstrate that the RNA P/DNA P ratio gradu-

Fig. 2. Sucrose-density-gradient centrifugation of [^{14}C]orotic acid-labelled microsomal RNA. The procedures were identical with those described in Fig. 1 except that the microsomal RNA was extracted three times with phenol-sodium dodecyl sulphate. The RNA was centrifuged for 15.5 h. The sedimentation is from right to left. (a) Control; the other groups of animals were irradiated with 2000rd and killed (b) 3 h; (c) 6 h later. —, E_{260} ; ----, radioactivity. The arrow indicates the top of the gradient.

ally increased after irradiation. The maximal increase, which corresponds to about 40% above the value for starved controls, was found between 24 and 36 h. Several investigators (Butler & Laurence, 1960; Ernst, 1962) as well as the present authors find that the DNA content of liver cells does not change significantly on irradiation and therefore may serve as a suitable reference basis. Consequently the rise in the RNA P/DNA P ratio indi-

Fig. 3. Sucrose-density-gradient centrifugation of [^{14}C]orotic acid-labelled nRNA. Each animal was injected intravenously with $10\mu\text{Ci}$ of [^{14}C]orotic acid/100 g body wt. and killed 15 min later. The purification of nuclei and the isolation of RNA from the nuclei are described in the Methods section. The RNA was extracted three times with phenol-sodium dodecyl sulphate. The RNA was centrifuged for 12 h. The sedimentation is from right to left. (a) Control; (b) the other group of animals, irradiated with 2000rd and killed 5 h later. —, E_{260} ; ----, radioactivity. The arrow indicates the top of the gradient.

cates the increase in RNA content of whole liver cells after irradiation.

The liver homogenate was separated into cell fractions by differential centrifugation and ribonucleoprotein particles were obtained from each fraction. Ribonucleoprotein content was determined by measurement of RNA in each ribonucleoprotein fraction. In Table 1 fraction P-1 corresponds to the ribonucleoproteins obtained from the crude once-washed nuclear pellet. Fraction P-2 contained mostly the ribonucleoproteins of mitochondria and the heavier fragments of endoplasmic reticulum. Fraction S-2 contained the ribonucleoproteins of supernatant after centrifugation for 15 min at $10000g$ (see the Methods section). The results given in Table 1 demonstrate that the RNA content of each fraction increased after irradiation. In fractions S-2 and P-1 the RNA content increased to 28–34% above the control at 18, 24 and 36 h after irradiation. The RNA content was highest in fraction P-2 and its maximal value was exhibited later (at 24 and 36 h) than for

Table 1. *RNA P/DNA P ratio of whole cells and the RNA P content of various ribonucleoprotein fractions of the cells*

Experiments were performed at various times after 3000rd X-irradiation. RNA P/DNA P ratio was determined as described in the Methods section. Ribonucleoproteins were separated from P-1, P-2 and S-2 fractions and the RNA P values were referred to the amount of the respective fraction derived from an amount of homogenate containing 100 μ g of DNA P. Fraction P-1 corresponds to the first pellet obtained by centrifugation for 10 min at 800g. Fraction P-2 was obtained from the supernatant from the preparation of fraction P-1 by centrifugation for 15 min at 10000g. Fraction S-2 was the supernatant from the preparation of fraction P-2. Further details are given in the Methods section. The results are given as means $\pm$ S.D. of the numbers of separate experiments indicated.

	No. of expts.	RNA P/DNA P ratio for whole cells	μ g of RNA P		
			P-1	P-2	S-2
Control	8	2.55 $\pm$ 0.26	65.0 $\pm$ 5.8	55.0 $\pm$ 12.5	98.2 $\pm$ 7.5
10h	3	2.82 $\pm$ 0.25	78.3 $\pm$ 6.1	63.0 $\pm$ 9.4	115.0 $\pm$ 9.8
15h	4	3.12 $\pm$ 0.28	82.7 $\pm$ 8.1	81.5 $\pm$ 8.5	123.8 $\pm$ 7.2
18h	3	3.35 $\pm$ 0.37	85.2 $\pm$ 9.1	80.5 $\pm$ 12.3	129.3 $\pm$ 11.5
24h	4	3.58 $\pm$ 0.21	87.6 $\pm$ 6.5	88.3 $\pm$ 12.8	128.2 $\pm$ 8.7
36h	3	3.62 $\pm$ 0.44	84.1 $\pm$ 9.3	92.5 $\pm$ 14.2	127.5 $\pm$ 14.1

the other two fractions. This suggests a gradual increase of heavy endoplasmic reticulum after irradiation.

The RNA P/DNA P ratio of purified nuclei was also measured in three experiments. Table 2 shows that at 4h after irradiation a slight increase could be seen. The increment was greater at 6 and 8h, reaching a value double that of the control. This ratio did not change remarkably up to 12h, which was the latest time investigated.

The higher RNA content of all investigated cell fractions could be interpreted as the result of increased RNA synthesis after whole-body X-irradiation.

Protein-synthesizing activity of microsomes in the presence and absence of poly U. Microsomes obtained from control and irradiated animals were incubated with [14 C]phenylalanine in the presence and absence of poly U as the synthetic messenger. After incubation the microsomes were fractionated by sucrose-density-gradient centrifugation (Fig. 4). Figs. 4(a) and 4(b) indicate that the control samples contained more monomers, whereas the irradiated samples contained relatively more heavier particles. Most of the radioactivity incorporated by endogenous messenger was found in the heaviest fractions. No remarkable differences were observed between the radioactivity profiles of control and irradiated samples, which agrees with the earlier results, i.e. that single ribosomes display no change in their functional activity on irradiation (Hidvégi *et al.* 1968). Figs. 4(c) and 4(d) show the profiles when the same microsomes were incubated in the presence of poly U. The curves of radioactivity and specific radioactivity demonstrate that the enhancement by the synthetic messenger was highest in the fractions containing the monomeric ribosomes and lighter

Table 2. *RNA P/DNA P ratio of purified cell nuclei*

Nuclei were isolated at various times after 3000rd X-irradiation. Purification of nuclei and the determination of RNA and DNA are described in the Methods section. The results are given as means $\pm$ S.D. of the numbers of separate experiments indicated.

	No. of expts.	RNA P/DNA P ratio
Control	6	0.21 $\pm$ 0.025
4h	3	0.25 $\pm$ 0.045
6h	3	0.32 $\pm$ 0.029
8h	3	0.48 $\pm$ 0.023
12h	3	0.52 $\pm$ 0.031

polyribosomes. The heavier the microsomes, the smaller was the effect of poly U enhancement, owing to the smaller number of longer chains of poly U in the preparation used. By comparison of the radioactivity curves of Figs. 4(c) and 4(d) it is obvious that the radioactivity is much lower in the preparation originating from irradiated animals.

Calculations were made for both control and irradiated samples by subtracting the specific radioactivity of each fraction of the density gradient obtained in the absence of poly U from those values obtained in its presence. The results indicate the enhancement of [14 C]phenylalanine incorporation in microsomal preparations by the addition of poly U. Fig. 5 shows the curves calculated for both preparations and plotted as a function of density-gradient fractions. Fig. 5 shows that the poly U enhancement was twice as great in the microsomes of control preparations as in the irradiated ones. The results support the concept that the liver microsomal preparation of irradiated animals contained more natural mRNA, and that therefore

Fig. 4. Incorporation of [^{14}C]phenylalanine in the presence and absence of poly U by the microsomes of control and X-irradiated animals *in vitro*. Microsomes were prepared from control animals (a and c) and from animals killed 13 h after X-irradiation with 2000rd (b and d). Incorporation experiments were performed by using the amino acid-incorporation system described in the Methods section in both the absence (a and b) and presence (c and d) of poly U. At the end of incubation the suspension was cooled in ice, a portion (0.3 ml) was layered over a linear 20–50% (w/v) sucrose density gradient and centrifuged for 70 min at 204 000g (rotor SW50L). Sedimentation is from right to left. Samples for the determination of E_{260} and for radioactivity were collected as described in the Methods section. —, E_{260} (corr.); Δ . . . Δ , radioactivity; $\bullet$ — $\bullet$ — $\bullet$, specific radioactivity expressed in c.p.m./ E_{260} unit. M, monomer peak; the other arrow indicates the top of the gradient.

the preparation was less able to accept the exogenous messenger, poly U. The interpretation of these results is similar to the interpretation made by other investigators (Campbell, Serck-Hanssen & Lowe, 1965; Mager, Bornstein & Halbreich, 1965).

RNA polymerase activity of nuclei. The RNA polymerase activity of whole nuclei was measured after whole-body X-irradiation of animals. Expt. 1 in Table 3 shows that the RNA polymerase activity in the control preparation was dependent on the presence of all four nucleotide triphosphates and was sensitive to elimination of the template by deoxyribonuclease. The control preparation was inhibited by the addition of actinomycin D. The results of both experiments in Table 3 demonstrate that at 6 and 9 h after irradiation RNA polymerase activity was increased by about 50% and 127% respectively.

Effect of actinomycin D pretreatment on the activity of the ribosomal system. In this experiment liver RNA synthesis was inhibited by blocking DNA-directed transcription with actinomycin D. Distribution of ribosomes and the protein-synthesizing

capacity of isolated ribosomes *in vitro* were investigated after actinomycin D treatment of both control and X-irradiated animals.

Fig. 6 shows the distribution of ribosomes obtained from the post-mitochondrial supernatant, treated with deoxycholate and analysed by sucrose-density-gradient ultracentrifugation. The control animals (Fig. 6a) contained 30–40% of monomeric ribosomes after 24 h starvation, in agreement with results obtained by Hidvégi *et al.* (1968). Figs. 6(b)–6(d) show the patterns of non-irradiated animals treated with increasing amounts of actinomycin D and analysed for the distribution of ribosomes 13 h later. Actinomycin D at 0.3 mg/kg (Fig. 6b) did not change the pattern obtained with the untreated group and treatment with 0.5 mg/kg changed the pattern only slightly (Fig. 6c). During this 13 h period, 1.0 mg of actinomycin D/kg decreased the amount of polyribosomes remarkably and increased the amount of monomers (Fig. 6d). This finding is in agreement with work of Staehelin, Wettstein & Noll (1963). Figs. 6(e)–6(h) show the patterns of irradiated animals. The irradiation alone caused

Fig. 5. Enhancement of [^{14}C]phenylalanine incorporation by the addition of poly U. The specific-radioactivity values shown in Fig. 4 were used for calculation of the enhancement of [^{14}C]phenylalanine incorporation in each fraction of the density-gradient pattern. The specific-radioactivity values of each fraction in Fig. 4(a) were subtracted from the corresponding values in Fig. 4(c), thus giving a value for the enhancement of amino acid incorporation of control microsomes. The enhancement of amino acid incorporation for microsomes of X-irradiated animals was obtained in the same way from the values of Figs. 4(b) and 4(d). —, Difference of specific-radioactivity values of control;, difference of specific-radioactivity values of irradiated animals. The areas under the curves for control and X-irradiated animals, marked by two vertical dashed lines cutting the abscissa, were measured with a planimeter. The size of the area proportional to poly U enhancement was taken as 100% in the case of the control. In this case the value for the X-irradiated animals was 49%. M, monomer.

the nearly complete disappearance of monomeric ribosomes and accumulation of polyribosomes (Fig. 6e), as reported by Hidvégi *et al.* (1968). If the animals were treated with actinomycin D 30 min before irradiation, 0.3 mg/kg did not interfere with the accumulation of polyribosomes after irradiation (Fig. 6f). At 0.5 mg/kg, however, actinomycin D inhibited the accumulation of polyribosomes completely (Fig. 6g). Treatment with 0.5 mg of actinomycin D/kg (Fig. 6g) and with 1.0 mg/kg (Fig. 6h) gave rise to patterns that were similar to those obtained with the non-irradiated animals treated with the same amount of actinomycin D (Figs. 6c and 6d). The experiment with 1.0 mg of actinomycin D/kg demonstrated (Fig. 6h) that, in ad-

Table 3. RNA polymerase activity of nuclei

RNA polymerase activity was measured as described in the Methods section, and was expressed as c.p.m. of [^{14}C]ATP incorporated into RNA during 15 min and related to the amount of nuclei in the suspension containing 100 μg of DNA P.

Expt. no.	System	Radioactivity in RNA	
		(units)	(% of control)
1	Control (complete)	283	100
	GTP, CTP and UTP omitted	46	17
	20 μg of deoxyribonuclease added	52	18
	60 μg of actinomycin D added	158	59
	Complete: 6 h, 2000 rd.	417	147
	Complete: 9 h, 2000 rd.	645	227
2	Control (complete)	265	100
	Complete: 6 h, 2000 rd.	420	158

dition to the abolition of radiation-induced enrichment of polyribosomes, the amount of monomers increased to a value above that of the control (cf. Figs. 6h and 6a). This finding, the increase of monomers and decrease of polyribosomes on treatment with large amounts of actinomycin D (Figs. 6d and 6h), is in agreement with work of Staehelin *et al.* (1963) and could be explained by the inhibition of mRNA synthesis.

Fig. 7 shows the kinetics of incorporation of ^{14}C -labelled amino acids by isolated ribosomes *in vitro*. In this experiment irradiation alone increased the amino acid incorporation by about 45% above the control, in agreement with the earlier experiments of Hidvégi *et al.* (1968). There was a critical amount of actinomycin D, namely 0.4–0.6 mg/kg, that inhibited the irradiation-induced increase of protein-synthesizing activity of total ribosomal particles.

These experiments demonstrate that both the enrichment of polyribosomes and the enhanced protein-synthesizing activity after X-irradiation (Hidvégi *et al.* 1968) were abolished if the animals were treated with actinomycin D before irradiation. It may be concluded that the increase in RNA synthesis is responsible for the higher protein-synthesizing activity of the cytoplasm after whole-body irradiation of animals.

DISCUSSION

The experiments described in this paper showed that liver RNA synthesis increased after whole-body X-irradiation. The synthesis of all types of RNA increased and hence this effect appears to be a general and probably unspecific change in liver RNA synthesis.

Fig. 6. Effect of actinomycin D pretreatment on the X-irradiation-induced enrichment of polyribosomes. Samples (1.0 ml) of deoxycholate-treated postmitochondrial supernatant were layered over a convex-linear sucrose density gradient and centrifuged for 3.5 h at 61 500g (rotor SW 25.1). The percentage of sucrose (w/v) through the centrifuge tube is indicated by a dashed line in (a). Sedimentation is from right to left. (a)–(d) Control animals; (e)–(h) animals irradiated with 3000rd and killed 13 h later. Groups of animals in (a) and (e) were not treated with actinomycin D, whereas those in groups (b)–(d) and (f)–(h) were treated, 30 min before X-irradiation, with the following amounts of actinomycin D: (b) and (f) 0.3 mg/kg, (c) and (g) 0.5 mg/kg; (d) and (h) 1.0 mg/kg. M, monomer peak; the other arrow indicates the top of the gradient.

With regard to rRNA synthesis, Payne *et al.* (1952) first reported the increase of [^{32}P]P_i incorporation into rat liver cytoplasmic RNA 20 h after X-irradiation. The incorporation into the nRNA, however, was not increased at the same time. This dissimilarity was interpreted by Hevesy & Forssberg (1955) as the 'blockage of nuclear reactions to irradiation'. The authors meant this discrepancy to be explained by considering the fact that the incorporation increased into both cytoplasmic RNA and nRNA as a first reaction to X-irradiation. The enhancement of nRNA synthesis, however, ceased 7–9 h after irradiation, earlier than the incorporation of the precursors into cytoplasmic RNA. Klouwen (1960) found also a slight increase of liver nRNA synthesis shortly after irradiation. Petrovič (1962) reported an increase of incorpor-

ation into RNA of some cell fractions of liver, whereas the incorporation into the RNA of some other fractions remained unchanged. Ord & Stocken (1956) and Uchiyama *et al.* (1966), however, did not find any change in liver RNA synthesis on X-irradiation. The difference in species, in methods of isolation of RNA and in the mode of administration of radioactive label may account for this divergence of results. With regard to the results obtained by the administration of labelled precursors, note should be made of the present authors' observation that only intravenous administration of radioactive label gave comparable results. This is understandable if one considers the great change in absorption after irradiation.

The increased rRNA synthesis is supported by the finding that the RNA/DNA ratio of whole liver

Fig. 7. Effect of actinomycin D pretreatment on the X-irradiation-induced enrichment of amino acid incorporation by liver ribosomes *in vitro*. Ribosomal particles of various origins were incubated with [^{14}C]lysine and with the pH5 fraction derived from the liver of non-irradiated control animals. The details of the incorporation system were as given by Hidvégi *et al.* (1968). The X-irradiated animals were killed 13h after 3000rd X-irradiation. Actinomycin D treatment was performed 30min before irradiation for the irradiated animals and 13h before killing for the non-irradiated animals. ○, Control (i.e. non-irradiated and not treated with actinomycin D); △, X-irradiated; ●, irradiated and pretreated with 0.2mg of actinomycin D/kg; ▲, irradiated and pretreated with 0.4mg of actinomycin D/kg; □, non-irradiated and treated with 0.6mg of actinomycin D/kg; ■, irradiated and pretreated with 0.6mg of actinomycin D/kg.

cells and the ribonucleoprotein content of some cell fractions increased after irradiation. Both increments could be ascribed largely to the increase in rRNA content. Related results are also reported on the increase in RNA content of liver after X-irradiation *in vivo* (Caldera, Cozzani & Moruzzi, 1966; Cima, Fassina & Pozza, 1959; Mitchel, 1942a,b,c). The RNA/DNA ratio of the nucleus also shows a large increase in nRNA after irradiation. These findings, together with the results of precursor-incorporation experiments, support the idea that enough nRNA is synthesized and accumulated in the nucleus after X-irradiation to elevate, by its transport from the nucleus, the cytoplasmic RNA content. Experiments on the fractionation of RNA obtained from the subfractions of the nucleus (e.g. nucleolus, chromatin) should

reveal the types of RNA that have been predominantly synthesized on X-irradiation. In relation to this enhanced function of the nucleus, hypertrophy of liver nucleus and nucleolus was described after X-irradiation and this phenomenon was considered as a sign of higher synthetic activity (Scherer, Ringleb & Ventzke, 1953; Peters, 1963). On the basis of these and other morphological studies (Braun, 1961) and on the basis of the few available biochemical observations (Ernst, 1962), Scherer (1956) suggested that enhanced migration of nuclear material into the cytoplasm followed X-irradiation.

The present experiments demonstrated the increased activity, amount, or both, of RNA polymerase in whole nucleus at 6 and 9h after irradiation. The enhanced activity of Mg^{2+} -activated RNA polymerase was observed by Barnabei, Romano, Bitonto, Tomasi & Sereni (1966) at 48h after irradiation and by Omata, Ichii & Yago (1968) at 24h. It was proposed that the RNA polymerase activity, measured in the presence of Mg^{2+} , corresponds to the enzyme responsible for rRNA synthesis (Widnell & Tata, 1966). It should be noted that DNA-dependent nucleic acid synthesis was considered to be highly sensitive to irradiation (Harrington, 1964). Most investigators, however, have reported a failure of X-irradiation to inhibit DNA replication (Smellie, McArdle, Keir & Davidson, 1958; Beltz, Van Lancker & Potter, 1957) and RNA transcription from DNA (Chambon, Mandel, Weill & Busch, 1962; Pitot, Peraino, Lamar & Leshner, 1965).

According to our experiments actinomycin D prevented the development of radiation-induced enrichment of polyribosomes and the increase in protein synthesis. This effect should be considered as one of the principal pieces of evidence that whole-body X-irradiation of animals affected transcription of the genetic code in liver. The dependence of inhibition on the amount of actinomycin D administered proved that the increment of protein synthesis was abolished by 0.5mg of actinomycin D/kg body weight. Under the experimental conditions (13h after X-irradiation, with animals starved for 24h) the same amount of actinomycin D did not influence protein synthesis in the non-irradiated control animals. This finding suggests that the radiation-induced increment in protein synthesis is more sensitive to actinomycin D treatment than the overall protein synthesis in the liver of control animals. Revel & Hiatt (1964) showed that the liver mRNA species have various half-lives. Wilson & Hoagland (1967) suggested that liver mRNA species fall into two large classes with respect to stability and it was proposed that the more stable polyribosomes served for synthesis of 'export' proteins in the liver (Wilson, Hill &

Hoagland, 1967). From these results it might be concluded that the actinomycin D-sensitive class of mRNA, whose synthesis is increased on irradiation, probably belongs to the less stable class of liver mRNA and that it might be responsible for the synthesis of non-'export' proteins in the liver cells. Experiments by John & Miller (1968) proved that albumin synthesis in isolated liver of X-irradiated animals was unchanged and α_1 acidic glycoprotein and α_2 (acute-phase) globulin syntheses were increased. The experiments with actinomycin D, however, allow only the conclusion that DNA-dependent RNA synthesis increased on X-irradiation and that synthesis of this type of RNA is more sensitive to the antibiotic than the overall normal RNA synthesis of the liver. Indeed, results are available that suggest that rRNA synthesis is more sensitive to low concentrations of actinomycin D than is mRNA synthesis (Perry, Srinivasan & Kelly, 1964; Georgiev *et al.* 1963) and that the synthesis of ribosomal precursor RNA in the nucleolus is especially sensitive (Muramatsu, Hodnett, Steele & Busch, 1966).

The present results suggest that whole-body X-irradiation of animals increased DNA-dependent RNA synthesis in the liver. This is a sufficient reason for the explanation of the higher protein synthesis in the cytoplasm described by Hidvégi *et al.* (1968). The present authors do not consider the increase of DNA-dependent RNA synthesis to be a direct consequence of X-irradiation on the liver. Experiments were performed when the liver was shielded during the irradiation (Várterész, Hidvégi, Holland, Szabó & Antoni, 1967) and even so RNA and protein synthesis in the liver increased in the same way as when the whole body was X-irradiated, as shown in this paper and as described by Hidvégi *et al.* (1968).

The authors are indebted to the International Atomic Energy Agency for supporting part of this work. They thank the Hungarian Optical Works, Budapest, for putting an analytical ultracentrifuge at their disposal, and Ing. I. Jaczyna for his valuable assistance. The authors thank Professor Gy. Koczkás and his colleagues for the measurement of the radiation dose and for the irradiation of the animals. They are indebted to the Worthington Biochemical Co. for the free sample of their ribonuclease-free deoxyribonuclease I. They thank Mrs Klára Szilvássy, Miss Mária Frigyesi and Mrs Hanna Sinay for their excellent technical assistance.

REFERENCES

- Barnabei, O., Romano, B., Bitonto, G., Tomasi, V. & Sereni, F. (1966). *Archs Biochem. Biophys.* **113**, 487.
 Beltz, R. E., Van Lancker, J. L. & Potter, V. R. (1957). *Cancer Res.* **17**, 688.
 Braun, H. (1961). *Naturwissenschaften*, **48**, 679.
 Burton, K. (1956). *Biochem. J.* **62**, 315.
 Butler, J. A. V. & Laurence, D. J. R. (1960). *Int. J. Radiat. Biol.* **2**, 331.
 Caldera, C. M., Cozzani, F. & Moruzzi, M. S. (1966). *Experientia*, **22**, 579.
 Campbell, P. N., Serck-Hanssen, G. & Lowe, E. (1965). *Biochem. J.* **97**, 422.
 Ceriotti, G. (1955). *J. biol. Chem.* **214**, 59.
 Chambon, P., Mandel, P., Weill, J. D. & Busch, S. (1962). *Life Sci.* **5**, 167.
 Chauveau, J., Moulé, Y. & Rouiller, C. L. (1956). *Exp. Cell Res.* **11**, 317.
 Cima, L., Fassina, G. & Pozza, F. (1959). *Exp. Cell Res.* **17**, 1.
 DiGirolamo, A., Henshaw, E. C. & Hiatt, H. H. (1964). *J. molec. Biol.* **8**, 479.
 Ernst, H. (1962). *Z. Naturf.* **17b**, 300.
 Georgiev, G. P., Samarina, O. P., Lerman, M. L., Smirnov, M. N. & Severtzov, A. N. (1963). *Nature, Lond.*, **200**, 1292.
 Gierer, A. Z. (1958). *Z. Naturf.* **13b**, 788.
 Goutier, R. (1961). In *Progress in Biophysics*, vol. 11, p. 53. Ed. by Butler, J. A. V., Katz, B. & Zirkle, R. E. Oxford: Pergamon Press Ltd.
 Harrington, H. (1964). *Proc. natn. Acad. Sci. U.S.A.* **51**, 59.
 Hevesy, G. & Forssberg, A. (1955). *Proc. 3rd int. Congr. Biochem., Brussels*, p. 479. New York: Academic Press Inc.
 Hidvégi, E. J., Holland, J., Bölöni, E., Lónai, P., Antoni, F. & Várterész, V. (1968). *Biochem. J.* **109**, 495.
 Hidvégi, E. J., Holland, J., Bölöni, E. & Várterész, V. (1966). *Proc. FEBS 3rd Meet., Warsaw*, p. 208. London: Academic Press Inc.; Warsaw: P.W.N.
 Hidvégi, E. J., Kálmán, E., Lónai, P., Bölöni, E., Holland, J. & Várterész, V. (1965). *Proc. FEBS 2nd Meet., Vienna*, p. 324. Vienna: Verlag der Wiener Medizinischen Akademie.
 Hidvégi, E. J., Lónai, P. & Antoni, F. (1963). *Z. Krebsforsch.* **65**, 471.
 Hidvégi, E. J., Várterész, V., Holland, J. & Bölöni, E. (1966). *Proc. 3rd int. Congr. Radiat. Biol., Cortina d'Ampezzo*, p. 109, abstr. no. 429.
 Jackson, R. J., Munro, A. J. & Korner, A. (1964). *Biochim. biophys. Acta*, **91**, 666.
 John, D. W. & Miller, L. L. (1968). *J. biol. Chem.* **243**, 268.
 Klouwen, H. M. (1960). *Biochim. biophys. Acta*, **42**, 366.
 Koczkás, Gy. & Dósay, K. (1966). *Acta biochim. biophys. hung.* **1**, 305.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 Maass, H. & Timmis, M. (1964). *Strahlentherapie*, **123**, 64.
 Mager, J., Bornstein, S. & Halbreich, A. (1965). *Biochim. biophys. Acta*, **95**, 682.
 Mitchel, J. S. (1942a). *Br. J. exp. Path.* **23**, 285.
 Mitchel, J. S. (1942b). *Br. J. exp. Path.* **23**, 296.
 Mitchel, J. S. (1942c). *Br. J. exp. Path.* **23**, 309.
 Munro, A. J., Jackson, R. J. & Korner, A. (1964). *Biochem. J.* **92**, 289.
 Munro, A. J. & Korner, A. (1964). *Nature, Lond.*, **201**, 1194.
 Muramatsu, M., Hodnett, J. L., Steele, W. J. & Busch, H. (1966). *Biochim. biophys. Acta*, **123**, 116.

Bioch. 1970, 116

- Omata, S., Ichii, S. & Yago, N. (1968). *J. Biochem., Tokyo*, **63**, 695.
- Ord, M. G. & Stocken, L. A. (1956). *Biochem. J.* **63**, 3.
- Ord, M. G. & Stocken, L. A. (1961). In *Mechanism in Radiobiology*, vol. 1, p. 259. Ed. by Errera, M. & Forssberg, A. London and New York: Academic Press Inc.
- Payne, A. H., Kelly, L. S. & Entenman, C. (1952). *Proc. Soc. exp. Biol. Med.* **81**, 698.
- Perry, R. P., Srinivasan, P. R. & Kelly, D. (1964). *Science, N.Y.*, **145**, 504.
- Peters, K. (1963). In *Strahlenpathologie der Zelle*, p. 32. Ed. by Scherer, E. & Stender, H. S. Stuttgart: Georg Thieme.
- Petrovič, S. (1962). *Biochim. biophys. Acta*, **61**, 842.
- Pitot, H. C., Peraino, C., Lamar, C., jun. & Lesher, S. (1965). *Science, N.Y.*, **150**, 901.
- Prestayko, A. W. & Busch, H. (1968). *Biochim. biophys. Acta*, **169**, 339.
- Revel, M. & Hiatt, H. H. (1964). *Proc. natn. Acad. Sci. U.S.A.* **51**, 810.
- Scherer, E. (1956). *Strahlentherapie*, **100**, 86.
- Scherer, E., Ringleb, D. & Ventzke, L. P. (1953). *Strahlentherapie*, **90**, 41.
- Smellie, R. M. S., McArdle, A. H., Keir, H. M. & Davidson, J. N. (1958). *Biochem. J.* **69**, 37p.
- Staehelin, T., Wettstein, F. O. & Noll, H. (1963). *Science, N.Y.* **140**, 180.
- Staehelin, T., Wettstein, F. O., Oura, H. & Noll, H. (1964). *Nature, Lond.*, **201**, 264.
- Uchiyama, T., Fausto, N. & Van Lancker, J. L. (1966). *J. biol. Chem.* **241**, 991.
- Várterész, V., Hidvégi, E. J., Holland, J., Szabó, L. D. & Antoni, F. (1967). *Proc. 5th A. Meet. Eur. Soc. Radiat. Biol., Spindleruv Mlyn, Czechoslovakia*, p. 237.
- Widnell, C. C. & Tata, J. R. (1964). *Biochem. J.* **92**, 313.
- Widnell, C. C. & Tata, J. R. (1966). *Biochim. biophys. Acta*, **123**, 478.
- Wilson, S. H., Hill, H. Z. & Hoagland, M. B. (1967). *Biochem. J.* **103**, 567.
- Wilson, S. H. & Hoagland, M. B. (1967). *Biochem. J.* **103**, 556.