The Effect of Whole-Body X-Irradiation of Guinea Pigs on Liver Ribosomes

By E. J. HIDVÉGI, J. HOLLAND, ELISABETH BÖLÖNI, P. LÓNAI, F. ANTONI
AND V. VÁRTERÉSZ

Institute for Radiobiology, Budapest, Budafok, Hungary

(Received 6 December 1967)

1. The size distribution of aggregates of liver ribosomes and their protein-synthesizing ability *in vitro* were studied shortly after X-irradiation of guinea pigs. 2. Sucrose-density-gradient analysis of the mitochondrial supernatant after treatment with deoxycholate revealed a gradual increase in the number of polysomes, reaching a maximum between 9 and 15 hr. after irradiation. At that period the amount of ribonucleoprotein particles reached a level 25–30% above the control. This finding was confirmed by analytical-ultracentrifugal analysis and electron microscopy. Experiments were conducted to exclude the possibility that the enrichment of polysomes in the irradiated animals had occurred during the isolation procedure. 3. The protein-synthesizing ability of total ribosomal particles was measured *in vitro*. This showed an increase in amino acid incorporation parallel to the progressive enrichment of polysomes. At radiation doses of up to 1000 r. the protein-synthesizing capacity was dependent on the radiation dose: the higher the dose the higher the amino acid incorporation, reaching 40–60% above the control at the period of maximal polysome enrichment. Amino acid incorporation remained at this level after radiation doses of between 1000 and 3000 r. The enhanced protein-synthesizing activity was due solely to the increase in the proportion of polysomes, since irradiation was without effect on the activity of single ribosomes. 4. The results of the experiments are discussed in the light of our knowledge of the effect of radiation on protein synthesis.

Following the demonstration by Hahn & Hevesy (1940) of the high sensitivity of DNA synthesis to X-irradiation, the effect of different types of radiation on the DNA synthesis of various tissues and cells has been reported. Relatively few results are available, however, on the radiosensitivity of RNA metabolism (Goutier, 1961), and even fewer on protein synthesis. In this respect Hevesy (1949*a,b*) was the first to observe that after X-irradiation the incorporation of labelled acetate into the proteins of liver and the small intestine was slightly increased. Though some workers confirmed Hevesy's (1949*a,b*) observation (Richmond, Altman & Salomon, 1951; Butler, Cohn & Crathern, 1957; Kay & Entenman, 1956; Richmond, Ord & Stocken, 1957), others claimed that protein synthesis was either insensitive (Abrams, 1951; Holmes & Mee, 1952) or even decreased after X-irradiation (Hempelmann, Carr, Frantz, Masters & Lamdin, 1950; Richmond *et al.* 1957).

Thus the experimental results on the radiosensitivity of protein synthesis are contradictory. This fact, together with the pronounced ultrastructural changes described in radioresistant organs

shortly after X-irradiation by Braun (1963) and Peters (1963) among others, induced us to study the reaction of the protein-synthesizing ribosomal system to X-irradiation *in vivo*.

The present paper describes the enhanced activity of liver ribosomes in protein synthesis after whole-body X-irradiation. The decrease in the amount of monomeric ribosomes and the increase of polysomes in the cytoplasm furnish the structural basis for such an enhanced activity. A preliminary account of these results has already been published (Hidvégi, Holland, Bölöni & Várterész, 1966*a*).

MATERIALS AND METHODS

Animals. Male guinea pigs (380–420 g.) were used. Since irradiation almost eliminates feeding activity, the animals were starved for 22–24 hr. before being killed. Each experimental group consisted of three animals.

Irradiation. The animals were exposed to whole-body irradiation. Characteristics of the radiation were: 200 kv, 15 mA, 0.5 mm. Cu filter. The distance between focus and the middle of the body was 40 cm. The dose was 81.5 r./min. (Koczkás & Dószay, 1966). The apparatus used was the THX-250 instrument (Medicor, Budapest, Hungary).

Preparation of mitochondrial supernatant and ribosomes. The animals were stunned by a blow on the head and drained of blood by decapitation. Livers were quickly removed, minced with scissors in ice-cold 0.9% NaCl and rinsed several times. The livers were homogenized by ten strokes with a Teflon-glass homogenizer in 3 vol. of 0.25 M-sucrose, dissolved in medium A. Medium A contained (final concentrations) 50 mM-KCl, 5 mM-MgCl₂, 6 mM-2-mercaptoethanol and 20 mM-tris buffer, adjusted to pH 7.8 (at 5°). All procedures were carried out at 0–2°.

Nuclei and debris were removed by centrifugation for 10 min. at 800 g (*g_{av.}*). The supernatant was centrifuged for 15 min. at 15 000 g in a Spinco L 50 preparative ultracentrifuge (rotor no. 30). The upper three-quarters of the postmitochondrial supernatant was recovered, made 1% (w/v) with respect to sodium deoxycholate and after 5 min. used either for sucrose-density-gradient analysis or for sedimentation of the ribosomes.

Total ribosomal particles were obtained by the modified method of Wettstein, Staehelin & Noll (1963) as described by Munro, Jackson & Korner (1964). The deoxycholate-treated mitochondrial supernatant was layered on a double layer of sucrose; for centrifugation in the Spinco no. 40 rotor this consisted of 4 ml. of 0.5 M-sucrose over 3 ml. of 1.0 M-sucrose, and for Spinco rotor no. 30 10 ml. of 0.5 M-sucrose over 10 ml. of 1.0 M-sucrose. In all cases the sucrose was dissolved in medium A. The ribosomal particles were sedimented by centrifugation at 105 000 g for 4 hr. (rotor no. 40) or 78 500 g for 8 hr. (rotor no. 30). The pellets were gently suspended in 0.25 M-sucrose in medium A to give a concentration of 6–12 mg. of ribonucleoprotein/ml. Any aggregates were removed by centrifugation in the SW 39 rotor at 10 000 g for 10 min. and the supernatant was analysed for ribosome content.

Analysis of ribonucleoprotein particles by sucrose-density-gradient ultracentrifugation. To determine the relative ratio of monomeric ribosomes and polysomes the deoxycholate-treated mitochondrial supernatant was analysed on a special sucrose gradient. The bottom part of the gradient was made convex and the upper part linear (combined convex-linear gradient) (Fig. 1a). This special gradient ensured the accumulation of polysomes in the bottom half, while the lighter ribosomes and monomers separated well in the upper half. The above sucrose solution contained all the components of medium A, except 2-mercaptoethanol. After centrifugation, the bottom of the tube was punctured with a hypodermic needle, drops were collected, and the extinctions at 260, 280 and 320 mμ were measured. Corrections were made for absorption by ferritin (Jackson, Munro & Korner, 1964). The position of monomeric ribosomes in the sucrose-density-gradient pattern was determined by treating the sample with 1 μg. of pancreatic ribonuclease/ml. at 37° for 5 min. The treated sample was cooled and analysed on a sucrose gradient as above. The ratio between monomers and polysomes and their contribution to the total ribonucleoprotein mass in the sample was calculated from the sedimentation patterns by a planimetric method.

Analytical ultracentrifugation. Analytical ultracentrifugal analysis was performed with model G120 ultracentrifuge (Hungarian Optical Works, Budapest, Hungary) operated with a Philpot-Svensson optical system. To determine the sedimentation coefficient, ribosomes suspended in medium A were run at 20° at three concentrations. The *S*_{20,w} values obtained were extrapolated to zero concentration. In two

experiments the *S*_{20,w} values were: monomer, 78 and 81 s; dimer, 113 and 118 s; trimer, 146 and 149 s. These sedimentation coefficients are in good agreement with values obtained by other authors (Sherman & Petermann, 1961; Tashiro & Siekevitz, 1965). Ultracentrifugal analysis of total ribosomal particles was performed at 2–5°, conveniently produced by the direct cooling-heating system of the rotor and vacuum chamber built into the model G120 ultracentrifuge.

Incorporation of ¹⁴C-labelled amino acids in vitro. The pH 5 fraction for the cell-free protein-synthesizing system was prepared from guinea-pig liver by the method of Hoagland, Stephenson, Scott, Hecht & Zamecnik (1958). The complete amino acid-incorporating system consisted of the following components in 1.0 ml.: 250 μmoles of sucrose, 10 μmoles of MgCl₂, 50 μmoles of KCl, 20 μmoles of tris-HCl buffer (pH 7.8 at 37°), 6 μmoles of 2-mercaptoethanol, 0.5 μmole of ATP, 0.05 μmole of GTP, 5 μmoles of phosphoenolpyruvate, 30 μg. of pyruvate kinase, 2–3 mg. of ribonucleoprotein fraction, pH 5 fraction with 1.5–2.5 mg. of protein and 1 μC of ¹⁴C-labelled amino acid. The RNA/protein ratio of ribosomes was 1.20 ± 0.05 for both control and irradiated specimens and only ribosomal preparations whose composition lay within these limits were used for incorporation experiments. The concentration of the ribonucleoprotein and pH 5 fractions varied in different experiments. However, for each individual experiment the same concentrations were used throughout. Incubation was carried out at 37° with shaking (100–120 oscillations/min.).

To stop the reaction, the mixture was precipitated in ice-cold 5% (w/v) trichloroacetic acid. The proteins were isolated by a modification of the method of Siekevitz (1952). The trichloroacetic acid precipitate was washed with cold 5% trichloroacetic acid and extracted twice with 5% trichloroacetic acid at 90° for 15 min. This was followed by washing with 96% (v/v) ethanol containing potassium acetate (2%, w/v), ethanol-ether (3:1, v/v) and again with 96% ethanol containing potassium acetate (2%). The precipitate was dissolved in concentrated formic acid, diluted and centrifuged. Samples of the supernatant were used for measuring radioactivity and protein content.

Chemical assays. Protein, RNA and DNA were separated by a slight modification of the Schmidt-Thannhauser method (Antoni, Hidvégi & Lónai, 1962; Hidvégi, Lónai & Antoni, 1963). RNA was measured by the orcinol reaction of Ceriotti (1955), DNA by Burton's (1956) method and the protein content by the method of Lowry, Rosebrough, Farr & Randall (1951). The following solutions were used as standards: ribose for orcinol reaction, DNA from chicken erythrocytes for Burton's reaction and crystallized bovine serum albumin for proteins.

Measurement of radioactivity. The radioactivity of infinitely thin samples of ¹⁴C-labelled protein was determined on a Frieske-Hoepfner methane gas-flow counter.

For measurements of the radioactivity of ribonucleoprotein particles, labelled in the RNA component *in vivo* with ¹⁴C- or ³²P-labelled precursors or both, fractions were collected from sucrose gradients. The ribonucleoprotein was precipitated with cold 5% trichloroacetic acid in the presence of 1 mg. of albumin as carrier. The precipitate was washed twice with cold 5% trichloroacetic acid, once with 96% ethanol containing potassium acetate (2%), once with ethanol-ether (3:1, v/v) and again with 96% ethanol

containing potassium acetate (2%). The precipitate was dissolved in concentrated formic acid and transferred quantitatively to an aluminium planchet. The radioactivity was measured on the methane gas-flow counter with and without a 0.02 mm. aluminium filter. This filter eliminates 96% of the ^{14}C counts but only 17% of the ^{32}P counts.

Similar results were obtained for the radioactive profile in parallel experiments if the washed precipitates were dissolved in Hyamine and measured in a Packard Tri-Carb type 3154 scintillation counter. The scintillation fluid contained 2,5-diphenyloxazole (4 g./l.) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (0.30 g./l.) in toluene. Quenching of the samples was taken into account by means of the channels-ratio method.

Materials. All sucrose solutions were treated with purified bentonite. Carrier-free inorganic ^{32}P phosphate was used (from the Isotope Institute, Budapest, Hungary). $[6\text{-}^{14}\text{C}]\text{Orotic acid}$ (40.2 mc/m-mole) (from The Radiochemical Centre, Amersham, Bucks.) was dissolved in dilute NaOH. The following labelled amino acids were used: L- $[U\text{-}^{14}\text{C}]\text{phenylalanine}$ (7.8 mc/m-mole), L- $[U\text{-}^{14}\text{C}]\text{lysine}$ (136 mc/m-mole) (both from The Radiochemical Centre) and L- $[U\text{-}^{14}\text{C}]\text{lysine}$ (112 mc/m-mole) (from UVVVR, Prague, Czechoslovakia). The compounds used were:

disodium ATP (from Reanal, Budapest, Hungary), disodium GTP, pyruvate kinase, phosphoenolpyruvic acid (all from Sigma Chemical Co., St Louis, Mo., U.S.A.) and polyuridylic acid (from Calbiochem, Los Angeles, Calif., U.S.A.).

RESULTS

Time-course of decrease of monomeric ribosomes after irradiation: sucrose-density-gradient analysis. Guinea pigs were killed at different times after whole-body irradiation with 3000 r. The mitochondrial supernatant obtained from the liver was treated with deoxycholate and analysed by sucrose-density-gradient ultracentrifugation to establish the distribution of ribosomes.

In some 30 experiments the monomers constituted about 30–40% of the total ribosomes in the liver of control animals that had been starved for 24 hr. (Fig. 1a). The monomers gradually decreased in the cytoplasm from 2–5 hr. after irradiation (Figs. 1b and 1c). After 9–24 hr. they had almost disappeared (Figs. 1d–1g) and reappeared again about 36 hr. (Fig. 1h).

Fig. 1. Changes in ribosome distribution at various times after irradiation with 3000 r.: sucrose-density-gradient analysis. A 1.0–1.2 ml. sample of deoxycholate-treated mitochondrial supernatant was layered over a convex-linear sucrose gradient. Centrifugation was carried out in the SW 25.1 rotor for 3.5 hr. at 61 500 g. Further details are given in the Materials and Methods section. The direction of sedimentation is from right to left. (a) Control animals. The density gradient of sucrose throughout the centrifuge tube is indicated on this Figure. The other Figures show the distribution of ribosomes obtained from animals killed: (b) 2 hr., (c) 5 hr., (d) 9 hr., (e) 12 hr., (f) 15 hr., (g) 24 hr. and (h) 36 hr. after irradiation.

Fig. 2. Changes in ribosome distribution 13 hr. after various doses of irradiation: sucrose-density-gradient analysis. Experimental details are identical with those in Fig. 1. (a) Control animals. Other animals were irradiated with (b) 300 r., (c) 600 r., (d) 1000 r., (e) 2000 r. and (f) 4000 r.

Dose-dependence of the decrease of monomeric ribosomes: sucrose-density-gradient analysis. These experiments were performed 13 hr. after irradiation when, according to the time-dependence shown in Fig. 1, the amount of monomers was very low.

Guinea pigs were irradiated with various doses and the deoxycholate-treated mitochondrial supernatant was analysed. Fig. 2 shows the liver ribosomal pattern of animals irradiated with increasing doses. After low doses, such as 300 and 600 r. (Figs. 2b and 2c), the peak of ribosomal monomers was slightly decreased. After doses of 1000, 2000 and 4000 r. (Figs. 2d–2f) the monomer peak was very low and had practically disappeared.

Effect of starvation and feeding on the ribosome pattern. Since irradiated animals do not all feed to the same extent, the animals were starved. Therefore the influence of starvation and feeding on the ribosomal pattern in control unirradiated animals was checked.

Experiments revealed that the amount of monomeric ribosomes was low in the liver of well-fed animals (Fig. 3b). This is in agreement with the results of Wilson & Hoagland (1965) and Sox & Hoagland (1966). The amount of monomers increased after starvation for 18 hr. (Fig. 3a).

Fig. 3. Effect of starvation and feeding on ribosome distribution: sucrose-density-gradient analysis. Experimental details are as given in Fig. 1. Sedimentation patterns are shown for (a) starved (18 hr.) and (b) well-fed animals.

Fig. 4. Analytical ultracentrifugation of total ribosomal particles. The upper photographs represent the control. The lower photographs show a ribosomal preparation obtained from animals killed 12 hr. after irradiation with 600 r. The ribosomal particles were suspended in medium A and centrifuged in the model G 120 ultracentrifuge at 35040 rev./min. (temperature 5-8°). The phase-plate angle of the schlieren optics was 24° in both cases. Photographs were taken at the following times after the maximum centrifugal force had been reached: (control) (a) 1 min., (b) 3 min. and (c) 5 min.; (irradiated) (d) 1 min., (e) 2 min. and (f) 4-5 min. Sedimentation is from right to left.

Comparison of Fig. 3 with the ribosomal profiles obtained from irradiated animals 9-24 hr. after 3000 r. (Fig. 1) or 13 hr. after irradiation at dosages larger than 300 r. (Fig. 2) shows that the content of monomeric ribosomes was lower in the irradiated animals than in the control animals that had been fed up to the time of their being killed. Since starvation results in an increase in the monomeric ribosome content the decrease in monomers in irradiated animals cannot be attributed to their being deprived of food.

Analytical ultracentrifugation. The total ribosomal particles of liver were isolated and subjected to analytical ultracentrifugation. As shown in the upper part of Fig. 4 ribosomal preparations from control animals could be resolved into three main components, particles larger than trimers remaining unresolved. Heavier components were also observed in ribosomal preparations from animals killed 12 hr. after irradiation with 600 r. In addition, an appreciable amount of polysomes sediments between 200 and 700 s (Fig. 4). From this it was

Table 1. Yield of ribosomes obtained from mitochondrial supernatant after treatment with deoxycholate

The RNA content of ribosomes was related to the DNA P content of the homogenates from which they were derived. Homogenization was performed thoroughly in 5 vol. of 0.25 M-sucrose in medium A. The animals were X-irradiated with 3000 r. The RNA P/DNA P ratio of the homogenate is also given.

Time after irradiation (hr.)	Ribosomal-RNA P (μg/100 μg. of DNA P)	RNA P/DNA P ratio
Control	94.3 (100%)	2.3
15	120 (127%)	2.8
18	118 (125%)	3.1

concluded that the ratio of polysomes to monomers increases on irradiation.

Ribosome content of mitochondrial supernatant. Since the relative amount of monomeric ribosomes

decreased in favour of polysomes after irradiation, the question arose as to whether the total amount of ribosomes (monomeric ribosomes and polysomes together) remained unchanged.

The amount of ribonucleoprotein particles sedimented from deoxycholate-treated mitochondrial supernatant was determined and related to the DNA content of the homogenate. The DNA content is a suitable reference basis, since it does

not change significantly on irradiation (Butler & Laurence, 1960; Ernst, 1962). According to the data in Table 1, the amount of ribonucleoprotein particles in guinea pig liver 15–18 hr. after irradiation with 3000 r. was increased by about 25–28%.

Effect of the isolation procedure on the polysome profiles. The possibility that the differences in the ribosomal patterns obtained from liver of irradiated and of control animals was a consequence of the

Fig. 5. Sucrose-density-gradient profile of ribosomes obtained from mixed liver homogenates of irradiated and control animals. Each control animal was given 1 mc of [^{32}P]phosphate intraperitoneally 36 hr. and again 18 hr. before being killed. Similarly each animal that was to be irradiated was given 50 μC of [^{14}C]orotic acid. The experimental animals were irradiated with 4000 r. 14 hr. before being killed. Samples of each homogenate were mixed in equal parts. Deoxycholate-treated mitochondrial supernatants were prepared from original homogenates and the mixture. A 1.2 ml. sample of deoxycholate-treated mitochondrial supernatant was layered over a combined convex-linear sucrose density gradient (as in Fig. 1a) and centrifuged in rotor SW 25.1 for 4 hr. at 61 500 g. Samples for determination of extinction and radioactivity were collected alternately, 3 drops for extinction measurements and 10 drops for radioactivity determinations. Further details are given in the Materials and Methods section. In this experiment ^{14}C and ^{32}P radioactivities were differentiated by a filter absorption technique. —, E_{260} (corr.); ●—●, ^{32}P radioactivity; ▽.....▽, ^{14}C radioactivity. (a) Control; (c) irradiated preparations; (b) mixed homogenates.

Fig. 6. Effect of irradiation on the incorporation of $[^{14}\text{C}]$ lysine by total ribosomal particles of liver *in vitro*. In experiment (a) the pH5 fraction was derived from the liver of control animals and the total ribosomal particles were obtained from deoxycholate-treated mitochondrial supernatant from the following sources: $\bullet$ — $\bullet$, control; $\circ$ — $\circ$, animals killed 12 hr. after 3000 r.; $\circ$ — $\circ$, animals killed 18 hr. after 3000 r. In experiment (b) pH5 fraction derived from liver of homologous animal: $\bullet$ — $\bullet$, ribosomes and pH5 fraction from control animals; $\circ$ — $\circ$, ribosomes and pH5 fraction from animals killed 13 hr. after 3000 r. Details of the complete incorporation system are given in the Materials and Methods section.

experimental procedure had to be excluded. To this end, liver homogenates of control and irradiated animals were mixed and the size distribution of the two ribonucleoprotein populations was determined.

The RNA components of the ribosomes were labelled *in vivo* by incorporation of $[^{32}\text{P}]$ phosphate into the control animals and $[^{14}\text{C}]$ orotic acid into the irradiated animals. Samples of the original homogenates were analysed separately (Figs. 5a and 5c). As revealed by the sucrose-density-gradient analysis, the ribonucleoprotein of the mixed homogenates (Fig. 5b) showed the distribution one would expect from a summation of the individual contents: about half of the material was rich in ^{14}C -labelled polysomes and the other half rich in ^{32}P -labelled monomers. Hence, no essential transformation of polysomes into monomers occurred during the isolation procedure. This experiment supports the view that the differences in the ribosomal patterns of irradiated and control liver already exist *in vivo* and do not develop during the isolation process.

Incorporation of amino acids *in vitro*. The amino acid-incorporation activity of total ribosomal particles sedimented from deoxycholate-treated mitochondrial supernatant was measured *in vitro*.

When ribosomes of different origins were incubated with a pH5 fraction isolated from the liver of control animals, incorporation of ^{14}C -labelled amino

acid was higher in ribosomes from irradiated animals (Fig. 6a). If the ribosomes were incubated with the homologous pH5 fraction, an increased amino acid incorporation was found in the preparation obtained from irradiated animals (Fig. 6b). Thus ribosomes originating from irradiated animals and incubated with pH5 fraction taken from liver of either irradiated or unirradiated animals showed an elevated amino acid-incorporating activity. Consequently, it is the ribosomes themselves and not the pH5 fraction that are responsible for the increased incorporation of amino acids.

A number of experiments confirmed that the maximum increase in the amino acid-incorporating ability of ribosomes from irradiated liver (some 40–60% above the sample from unirradiated animals) was observed 12–15 hr. after irradiation, i.e. the protein-synthesizing ability of the liver ribosomal system increased parallel to polysome enrichment after X-irradiation.

Dose-dependence of amino acid incorporation *in vitro*. The effect of the dose on the amino acid-incorporation activity of ribosomes was studied. Fig. 7 represents a typical experiment. The ribosomes were isolated 15 hr. after irradiation with different doses and incubated with pH5 fraction obtained from control liver. The amino acid-incorporating activity of the ribosomes increased with increasing dosage. After irradiation at 500 r.

Fig. 7. Dose-dependence of incorporation of [^{14}C]lysine by total ribosomal particles of liver *in vitro*. Total ribosomal particles were prepared from the deoxycholate-treated mitochondrial supernatant of animals killed 15 hr. after various doses of X-irradiation. The ribosomes were incubated with pH5 fraction from control liver. The composition of the complete incorporation system is given in the Materials and Methods section. ●—●, Control; △—△, 500 r.; ○—○, 1000 r.; ■—■, 4000 r.

the amino acid incorporation of the ribosomes exceeded that of the control ribosomes by 18% and after irradiation at 1000 r. this difference increased to 50%. On the other hand, after irradiation at 4000 r. amino acid incorporation increased to only 22% above the controls.

Five experiments were performed to reveal the dose-dependence of amino acid incorporation. Up to a dose of 1000 r. incorporation gradually increased in all experiments. Between 1000 and 3000 r. it did not, on the whole, increase any further. However, when the dose was increased to more than 3000 r. incorporation by ribosomes from irradiated animals exceeded the control values by only 20–40%.

Comparative study of the effect of polyuridylic acid on the incorporation of [^{14}C]phenylalanine into the ribosomes. The enhanced activity *in vitro* could be due to the higher content of polysomes, or an increased functional activity of the single ribosomes could also play a role. To clarify this point, the incorporation of [^{14}C]phenylalanine into ribosomal preparations both from control and irradiated animals was compared with polyuridylic acid as synthetic messenger. Endogenous messenger-RNA content was decreased to a minimum by preincubation of the ribosomal preparations for 60 min. As shown in Fig. 8 the incorporation of

Fig. 8. Incorporation of [^{14}C]phenylalanine into preincubated total ribosomal particles from control and irradiated animals, in the presence or absence of polyuridylic acid. Total ribosomal particles were prepared from controls and from animals killed 12 hr. after irradiation with 600 r. Preincubation was performed at 37° for 60 min. in a complete incorporation system. At the end of preincubation ATP and GTP were added to the system in the same quantities as in the original incubation mixture. The system was then divided: 100 μg . of polyuridylic acid/ml. (final concentration) was added to the one half and 1 μC of [^{14}C]phenylalanine/ml. to both parts. Incubation was continued at 37°. ○—○, Control in the absence of polyuridylic acid; ●—●, irradiated in the absence of polyuridylic acid; ▲—▲, control in the presence of polyuridylic acid; △—△, irradiated in the presence of polyuridylic acid.

phenylalanine was enhanced to a similar extent in preparations from both control and irradiated animals after the addition of polyuridylic acid. Thus the ribosomes in the two preparations did not show any difference in their ability to utilize the synthetic messenger. On the basis of the foregoing it may be concluded that the increased amino acid incorporation observed in the presence of endogenous messenger after irradiation is exclusively the result of the higher polysome content of the irradiated system.

DISCUSSION

According to our results the liver displayed a very sensitive reaction to X-irradiation of whole animals. An enrichment of the high-molecular-weight

aggregates was observed in ribonucleoprotein preparations obtained from cytoplasm of irradiated animals. The maximum accumulation of the aggregates occurred between 12 and 18 hr. after irradiation. These aggregates, both from the control and irradiated animals, proved to be sensitive to endogenous or to low concentrations of exogenous ribonuclease (cf. the Materials and Methods section), indicating that the aggregates are polysomes. In addition to this, a definite increase in the amount of ribonucleoprotein particles was observed in the mitochondrial supernatant after irradiation.

The nature of the ribosomal preparations was also studied by electron microscopy (not shown). The preparations consisted of ribosomal particles and were found to be free from membranes and other contaminants. The size distribution of the particles was determined from electron micrographs. A shift in the ribonucleoprotein pattern from lighter to heavier components was observed after irradiation.

The capacity for protein synthesis in the cytoplasm increased parallel to the enrichment of polysomes after irradiation. The assay for protein synthesis was carried out *in vitro* rather than *in vivo* for the following reasons: the system *in vitro* eliminated the variations due to the changes in the amino acid precursor pools and changes in membrane permeability, known to occur after X-irradiation. In addition, by varying the components in the system *in vitro* it was possible to show that the origin of the pH5 fraction did not affect the reaction and that the increase in the protein-synthesizing activity of ribonucleoprotein preparations from irradiated animals was due to their increased messenger-RNA content. In a few experiments the incorporation of ^{14}C -labelled amino acids was measured also *in vivo* (not shown). It was established that after 12 hr. at 2000 r. irradiation the incorporation into the proteins of various cell fractions of liver was increased (1 hr. incorporation time).

The results may be correlated with the investigations of other authors. In addition to Hevesy (1949a,b), other workers reported an increase in amino acid incorporation into liver cells or cell fractions *in vivo* after whole-body X-irradiation of rats (Payne, Kelly & Entenman, 1952; Richmond *et al.* 1957; Butler *et al.* 1957; Lauenstein, Haberland, Hempelman & Altman, 1957; Haberland, Schreier, Altman & Hempelman, 1957). Moreover, Hultin, Decken, Arrhenius & Morgan (1961) incubated liver mitochondrial supernatant 24 hr. after irradiation *in vivo* at 2500 r. and observed a significant increase in amino acid incorporation. A similar result was obtained by Taketa, Leon, Castle, Howard & Daligcon (1965). All the quoted results on the liver seem to agree with the experi-

ments reported here and might be interpreted according to the concept that the messenger-RNA content in the liver increases as an early radiation response.

Some experiments by other workers do not agree with the results cited above. Logan (1959) described a decrease in amino acid uptake by the liver after irradiation. However, the decrease was measured by radioautography on a nuclear suspension *in vitro* and could not be confirmed by biochemical methods. Moreover, in another case the radioautographic studies (Gerbaulet, Maurer & Brückner, 1963) were carried out too soon after irradiation to detect any effect on the liver.

Two crucial points in the evaluation of the radiosensitivity of protein synthesis are the time-dependence and dose-dependence of the phenomenon. The effect of irradiation on protein synthesis cannot be determined precisely merely by studying one or a few points of time after irradiation. The present results show that, if measurements are performed at times either before or after the maximal effect occurs, one might conclude that the protein synthesis is not sensitive to radiation. On the other hand the dose-dependence of protein synthesis showed that at the time of maximum effect the amino acid incorporation by ribosomes was proportional to the radiation dose up to 1000 r. and between 1000 and 3000 r. remained at the same high level. In some cases, however, after irradiation at 3000 r. or higher doses the incorporation failed to reach this maximum. This suggests that irradiation at these very high doses also affected some other processes that resulted in decreased protein synthesis. The observed plateau of amino acid incorporation after the doses between about 1000 and 3000 r. may be the net result of two opposing effects.

At least two possibilities may be proposed to explain the increased protein synthesis of the liver cytoplasm after irradiation. One possibility is that irradiated cells contain more messenger RNA and ribonucleoprotein as a result of increased synthesis of these components. Such an increase in the messenger-RNA and ribonucleoprotein content of these cells would account for their greater protein-synthesizing capacity. An increase in the amount of ribonucleoprotein in irradiated cells has been reported in this paper (Table 1), and it has previously been demonstrated (Hidvégi *et al.* 1965; Hidvégi, Holland, Bölöni & Várterész, 1966b) that shortly after X-irradiation the ribosomal-RNA and the messenger-RNA synthesis in liver increased both in the cytoplasm and in the nucleus. These data support the above hypothesis.

The other possibility that has to be taken into account is that X-irradiation results in increased stability of the polysomes already present. This

may be due to certain factors that maintain the polysome integrity or to the decreased activity of enzymes involved in the catabolism of polysomes. The authors have established that starved animals contain more monomeric ribosomes than do well-fed animals, in agreement with the results of Sox & Hoagland (1966). However, if the starved animals are irradiated they are subsequently found to contain fewer monomeric ribosomes than the non-irradiated well-fed animals. Since starvation causes depletion of the polysomes (Sox & Hoagland, 1966) it is reasonable to assume that X-irradiation somehow counteracts the processes involved in the starvation-induced breakdown. It has been shown previously that X-irradiation counteracts the effect of starvation on protein synthesis in the liver (Kay & Entenman, 1956). It might therefore be suggested that the increased messenger-RNA content of the cytoplasm in the starved-irradiated animals is due, not merely to an enhanced RNA synthesis, but also to the preservation of polysomes against the starvation-induced breakdown. The decreased endogenous ribonuclease activity of the liver microsomes after X-irradiation may account for the better preservation of polysomes (Hidvégi, Bölöni, Holland, Szabó, Antoni & Várterész, 1967).

The results obtained clearly suggest that the biochemical mechanisms studied here show a high sensitivity to X-irradiation. The effects of irradiation on liver protein synthesis resemble those observed when animals are re-fed after starvation or are deprived of amino acids; treatment with certain hormones or an increase in precursor pools also has the same effect. Probably irradiation acts in a similar and non-specific manner. The reaction observed in the liver may result from the response of the animal as a whole to X-irradiation rather than being a direct effect of irradiation of the liver.

The authors are indebted to the International Atomic Energy Agency for supporting part of this work. They gratefully recall the late Professor G. Hevesy, who, after having read their preliminary report on the subject, encouraged them to go on studying this problem. The authors are most indebted to Dr P. N. Campbell, Dr L. G. Lajtha and Dr G. J. Köteles for their useful suggestions on the manuscript. They thank the Hungarian Optical Works, Budapest, for putting an analytical ultracentrifuge at their disposal at the beginning of the experiments and Ing. I. Jaczyna for his valuable assistance. They also thank Mrs Hanna Sinay and Mrs Klára Szilvássy for their excellent technical assistance.

REFERENCES

- Abrams, R. (1951). *Arch. Biochem.* **30**, 91.
 Antoni, F., Hidvégi, E. J. & Lónai, P. (1962). *Acta physiol. Acad. sci. hung.* **21**, 325.
 Braun, H. (1963). In *Strahlenpathologie der Zelle*, p. 233. Ed. by Scherer, E. & Stender, H. S. Stuttgart: George Thieme.
 Burton, K. (1956). *Biochem. J.* **62**, 315.
 Butler, J. A. V., Cohn, P. & Crathern, A. R. (1957). In *Advances in Radiobiology*, p. 33. Ed. by Hevesy, G., Frossberg, A. & Abatt, J. D. Edinburgh: Oliver and Boyd.
 Butler, J. A. V. & Laurence, D. J. R. (1960). *Int. J. Radiat. Biol.* **2**, 331.
 Ceriotti, G. (1955). *J. biol. Chem.* **214**, 59.
 Ernst, H. (1962). *Z. Naturf.* **17b**, 300.
 Gerbaulet, K., Maurer, W. & Brückner, J. (1963). *Biochim. biophys. Acta*, **68**, 462.
 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.
 Haberland, G. L., Schreier, K., Altman, K. I. & Hempelman, L. H. (1957). *Biochim. biophys. Acta*, **25**, 237.
 Hahn, L. & Hevesy, G. (1940). *Nature, Lond.*, **140**, 549.
 Hempelman, L. J., Carr, S., Frantz, I. D., Masters, R. & Lamin, E. (1950). *Fed. Proc.* **9**, 183.
 Hevesy, G. (1949a). *Nature, Lond.*, **163**, 869.
 Hevesy, G. (1949b). *Nature, Lond.*, **164**, 269.
 Hidvégi, E. J., Bölöni, E., Holland, J., Szabó, L. D., Antoni, F. & Várterész, V. (1967). *Proc. 5th Symp. Fed. Europ. radiobiol. Soc., Spindleruv Mlyn, Czechoslovakia*.
 Hidvégi, E. J., Holland, J., Bölöni, E. & Várterész, V. (1966a). *Acta biochim. biophys. hung.* **1**, 107.
 Hidvégi, E. J., Holland, J., Bölöni, E. & Várterész, V. (1966b). *Proc. 3rd Meet. Fed. Europ. biochem. Soc., 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. 2nd Meet. Fed. Europ. biochem. Soc., Vienna*, p. 324. Vienna: Verlag der Wiener Medizinischen Akademie.
 Hidvégi, E. J., Lónai, P. & Antoni, F. (1963). *Z. Krebsforsch.* **65**, 471.
 Hoagland, M. B., Stephenson, J. F., Scott, L. O., Hecht, P. C. & Zamecnik, J. (1958). *J. biol. Chem.* **231**, 241.
 Holmes, B. E. & Mee, L. K. (1952). *Brit. J. Radiol.* **25**, 273.
 Hultin, H., Decken, A. von der, Arrhenius, E. & Morgan, W. S. (1961). In *Biological Structure and Function*, vol. 1, p. 222. Ed. by Goodwin, T. W. & Lindberg, O. London and New York: Academic Press Inc.
 Jackson, R. J., Munro, A. J. & Korner, A. (1964). *Biochim. biophys. Acta*, **91**, 666.
 Kay, R. E. & Entenman, C. (1956). *Arch. Biophys. Biochem.* **62**, 419.
 Koczás, Gy. & Dósay, K. (1966). *Acta biochim. biophys. hung.* **1**, 305.
 Lauenstein, K., Haberland, G. L., Hempelman, L. H. & Altman, K. I. (1957). *Biochim. biophys. Acta*, **26**, 421.
 Logan, R. (1959). *Biochim. biophys. Acta*, **35**, 251.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 Munro, A. J., Jackson, R. J. & Korner, A. (1964). *Biochem. J.* **92**, 289.
 Payne, A. H., Kelly, L. S. & Entenman, C. (1952). *Proc. Soc. exp. Biol., N.Y.*, **81**, 698.
 Peters, K. (1963). In *Strahlenpathologie der Zelle*, p. 32. Ed. by Scherer, E. & Stender, H. S. Stuttgart: George Thieme.
 Richmond, J. E., Altman, K. I. & Salomon, K. (1951). *J. biol. Chem.* **197**, 817.

- Richmond, J. E., Ord, M. G. & Stocken, L. A. (1957). *Biochem. J.* **66**, 123.
- Sherman, J. H. & Petermann, M. L. (1961). *Biochim. biophys. Acta*, **47**, 188.
- Siekevitz, P. (1952). *J. biol. Chem.* **149**, 549.
- Sox, H. C., jun. & Hoagland, M. B. (1966). *J. molec. Biol.* **20**, 113.
- Taketa, S. T., Leon, H. A., Castle, B. L., Howard, W. H. & Daligeeon, S. (1965). *Radiat. Res.* **25**, 247.
- Tashiro, Y. & Siekevitz, P. (1965). *J. molec. Biol.* **11**, 149.
- Wettstein, F. O., Staehelin, T. S. & Noll, H. (1963). *Nature, Lond.*, **197**, 430.
- Wilson, S. H. & Hoagland, M. B. (1965). *Proc. nat. Acad. Sci., Wash.*, **54**, 600.