ment, by exposure to mustard gas and by irradiation. One of us (Soares) irradiated a colony of *Apis mellifera* with γ-rays (from a Co^{60} bomb as source). The colony was placed 10 cm distant from the source and received 24 rads per day. One gynandromorph was obtained after 600 rads and were fixed in Dietrich's solution. Detailed analysis was made in the antennal segments by use of a new method.

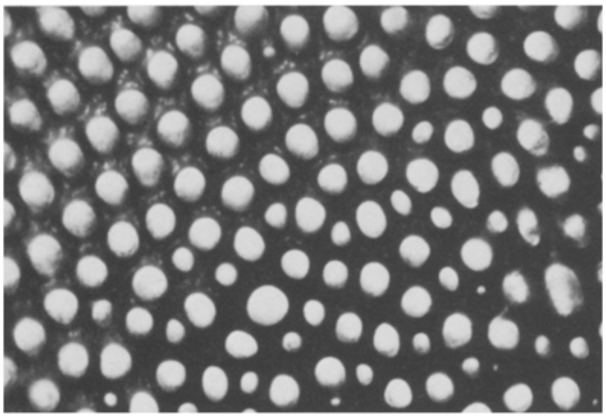

Fig. 3. Photograph of a segment of antennae of a gynandromorph. Male tissue in the left side and female tissue in the right × 500.

Each segment was first separated from the antennae, and, with help of 2 entomological pins, they were opened longitudinally along the back, being distended on a glass. Each segment was covered with Canada balsam and submitted to cover glass pressure. Microscope analysis showed different patterns of mosaicism in the antennae respecting sensorial structures level. Normal honeybee females have 10 segments and normal males have 11 in the flagellum. Females present olfactory plates (sensilla placodea) mixed with sensorial hairs in almost equal proportion (figure 1) whereas the drones have almost only olfactory plates in the segments (figure 2). Normal female segments also show a region without sensorial plates on the back. Nevertheless, drones present all regions of the segments with sensilla placodea. The gynandromorph obtained showed the segments numbers 1–7 typic as in a normal female, and the 8, 9 and 10 with normal male appearance. Microscopical analysis confirmed that the segments 1–7 had the normal female pattern. However, the segments 8–10 presented female and male tissue mixed (figure 3). The back of the segments did not present sensorial plates as occurs with females.

Nucleotide metabolism in the rat liver following whole-body X-irradiation

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Summary. Evidence has been presented to show that, like synthesis of RNA, syntheses of ribonucleotide precursors of RNA in the rat liver were stimulated for 6–18 h following whole-body X-irradiation (1000 R).

Whole-body exposure of mammals to ionizing radiations has been shown to enhance the capacity of liver to synthesize RNA for 4–18 h after irradiation[1-3]. Such stimulated synthesis of RNA may be expected to require increased supply of nucleotide precursors for RNA-polymerization. In this communication, evidence has been presented to show that, like RNA-polymerization, syntheses of liver ribonucleoside phosphates are also stimulated following whole-body X-irradiation of rat. These changes were found to be associated with the increased efficiency of liver high-speed supernatant to catalyze the conversion of orotic acid to pyrimidine nucleotides. The stimulus in synthesis of ribonucleotides was found to be linked to the enhanced rate of RNA-synthesis, since actinomycin D effectively reversed the heightened rate of (^{32}P)-orthophosphate incorporation into nucleotides.

Material and methods. Male albino rats of Wistar strain, each weighing between 150 and 160 g and fed on laboratory stock diet, were used. The animals were exposed (in groups of 4) to whole-body X-irradiation of 1000 R (dose rate 100 R/min) from a Siemens Stabilipan unit operated at *250 kV and 15 mA with a 2-mm Al filter.* The dose was administered from a distance of 62.7 cm. The field size was 26.5 × 26.5 cm². Both control and experimental rats were fasted after irradiation until killed at the time intervals indicated in the text. During fasting period, water was made available ad libitum.

Synthesis of RNA and ribonucleotides in the liver were studied by following incorporation of i.p. injected (^{32}P)-orthophosphate (carrier-free, 2 mCi/100 g b. wt in 0.5 ml of 0.15 M saline) for 1 h. Labelled RNA was isolated from the liver by the method of Munro and Fleck[4]. Labelled ribonucleotides were isolated from the liver acid-soluble pool and fractionated into individual nucleotides by the procedure of Hurlbert[5]. The radioactivities of RNA and ribonucleotides were expressed in terms of relative specific activity (see tables 1 and 3). For this purpose, specific activity of (^{32}P)-orthophosphate from liver homogenate was determined by the method of Ernster et al.[6].

The capacity of high speed supernatant (105,000 × g) of rat liver to catalyze the conversion of orotic acid to pyrimidine ribonucleotides was assayed using (^{3}H)-orotic acid (1 mCi/mole) essentially by the procedure of Hurlbert and Kammen[7]. The labelled nucleotides (cytidine and uridine mono-, di- and triphosphates) formed after 30-min-incubation of the liver supernatant with (^{3}H)-orotic

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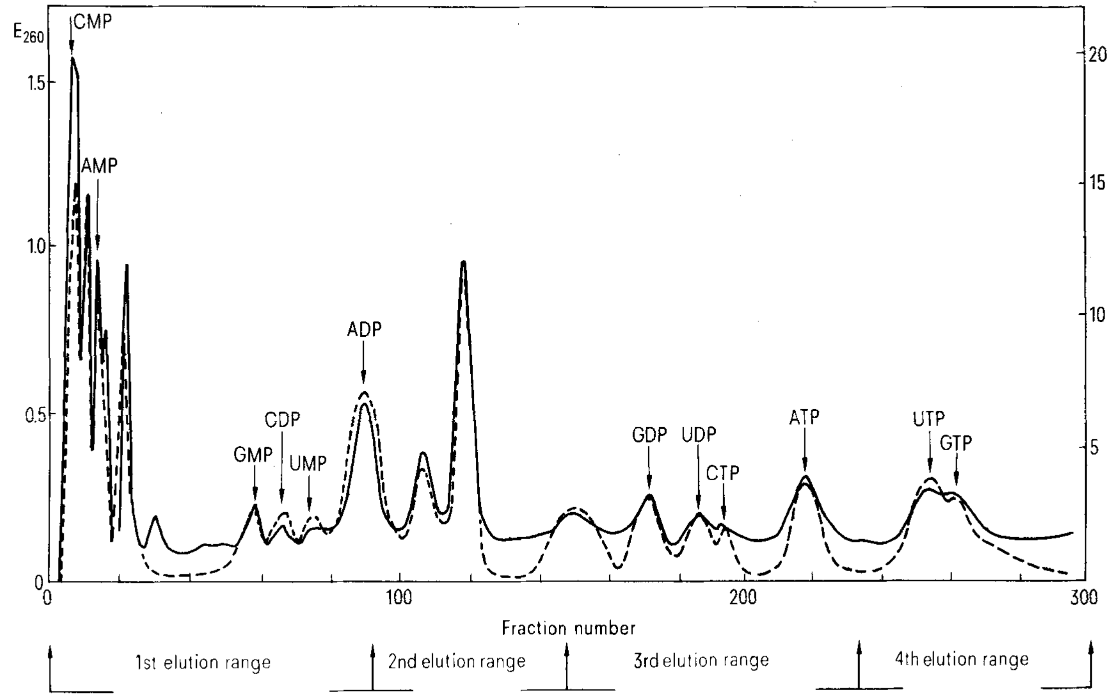

Fig. 1. Separation of (^{32}P)-labelled rat liver ribonucleotides on Dowex-1-formate. Rats were administered (^{32}P)-orthophosphate (carrier-free, 2 mCi/100 g b.wt) 1 h prior to sacrifice. Livers from 4 rats were pooled, homogenized and the acid-soluble nucleotides were prepared according to Hurlbert[5]. An aliquot of acid-soluble nucleotides equivalent to 300 E$_{260}$ units was applied to Dowex-1-formate column (0.8 cm × 22.0 cm). Elution of nucleotides was carried out by solutions containing formic acid and ammonium formate withgraded changes in their proportion using assembly for gradient elution consisting of a mixer flask of 500 ml capacity in conjunction with 1000 ml reservoir flask. The mixer flask contained the fixed volume of 300 ml solution (initially 300 ml of water). The concentration range of eluent solution was shifted 4 times by filling the reservoir flask serially with the solutions of increasing ionic strength and acidity as described by Hurlbert[5]. Fractions of 6 ml were collected and the E$_{260}$ was determined using water as blank. Aliquots of 0.1 ml were withdrawn from each fraction for determination of radioactivity. Nucleotides were identified by UV-absorption at 250, 260, 280 and 290 nm[5]. Further confirmation was carried out by paper chromatography using the solvents, a) isopropanol : conc. HCl : water (65 : 16.7 : 18.3), and b) isobutyric acid : conc. ammonium hydroxide : water (66 : 1 : 33). E 260, (—); radioactivity, (----).

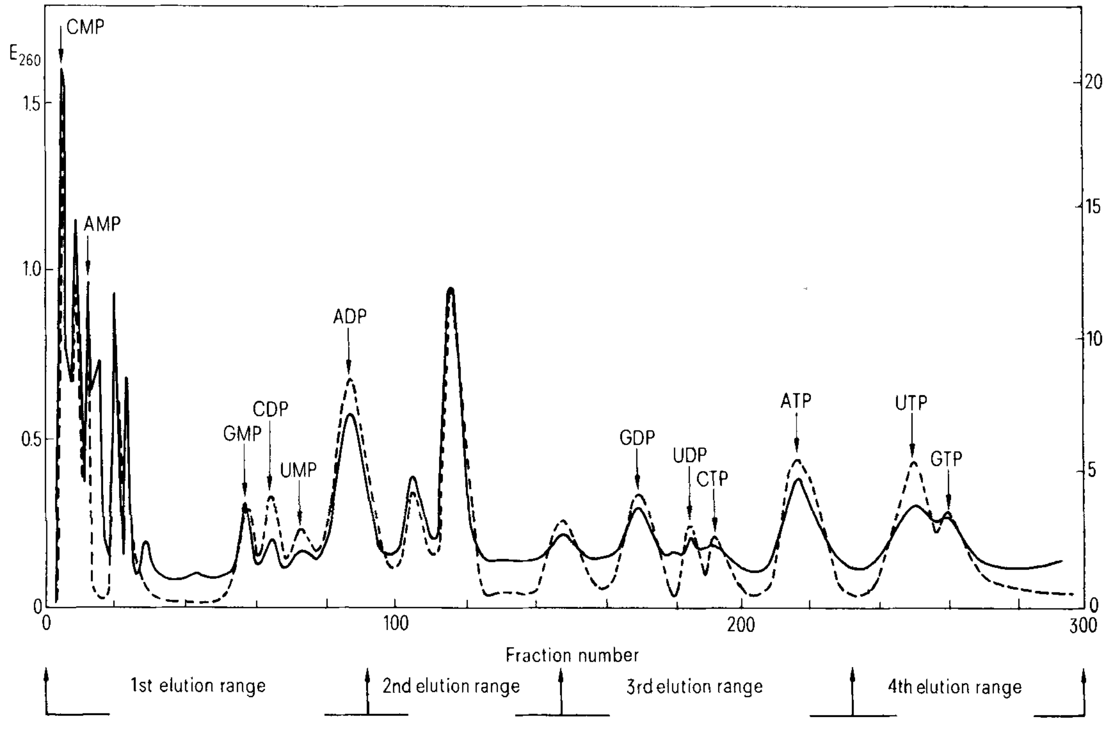

Fig. 2. Effect of whole-body X-irradiation on levels and synthetic patterns of liver ribonucleotides in rat. Groups of rats were administered with (^{32}P)-orthophosphate (carrier-free, 2 mCi/100 g b.wt) at 6 h post-irradiation (1000 R) and sacrificed 1 h later. Other details are as described in figure 1. E 260, (—); radioactivity, (---).

acid at 37 °C, were hydrolyzed to nucleoside monophosphates and separated by chromatography on Dowex-1-Cl and Dowex-50 according to Katz and Comb[8].

Protein was estimated by the method of Lowry et al.[9] using bovine serum albumin as standard. RNA was determined by the orcinol reaction[10] using yeast RNA as standard.

Table 1. Incorporation of (^{32}P)-orthophosphate into ribonucleoside triphosphates in the liver of rats following whole-body X-irradiation.

	ATP	GTP	UTP	CTP
	(relative specific activity)*			
Unirradiated				
Experiment I	65	72	69	75
Experiment II	70	73	76	74
Irradiated (1000 R)				
After 6 h	82	86	88	84
After 18 h				
Experiment I	90	102	98	96
Experiment II	95	109	105	104

$$^*\frac{\text{cpm/nmole of nucleoside triphosphate}}{\text{cpm/pmole of inorganic phosphate}}.$$

Groups of rats were administered with (^{32}P)-orthophosphate (carrier-free, 2 mCi/100 g b.wt) at 6 and 18 h post-irradiation and sacrificed 1 h later. The acid-soluble nucleotides were isolated and separated as described in figure 1. For each experiment livers from 4 rats in the respective groups were pooled.

Table 2. Conversion of (^{3}H)-orotic acid to uridine and cytidine nucleotide by 105,000 × g supernatants of the livers from unirradiated and irradiated rats

	UMP (cpm)	CMP (cpm)
Non-irradiated	180 000 ± 12 000	23 000 ± 2122
Irradiated (1000 R)		
After 18 h	328 000 ± 18 750	34 000 ± 2765

Each reaction mixture (2 ml final volume) contained 105,000 × g supernatant fraction (20 mg protein), 0.5 µmole of (^{3}H)-orotic acid (1 mCi/mole) and other components as described by Hurlbert and Kammen[7]. The incubation was at 37 °C for 30 min. Each value represents average of 3 independent experiments (3 animals) ± SEM.

Results and discussion. The optical density profiles and the synthetic patterns of liver acid-soluble ribonucleotides from non-irradiated and irradiated rats are depicted in figures 1 and 2 respectively. It can be seen from these figures that the optical density profiles of liver ribonucleotides in non-irradiated and irradiated rats are similar. However, the radioactivity distribution patterns from the 2 groups of rats show distinct differences. This indicates that irradiation has significantly elevated (^{32}P) incorporation into ribonucleoside di- and triphosphates in the liver. The relative specific activities of the 4 ribonucleoside triphosphates from non-irradiated and irradiated rats are given in table 1. Stimulation in the rate of incorporation of (^{32}P)-othophosphate into ribonucleoside triphosphates is seen at 6 h post-irradiation, and this is much more pronounced at 18 h. It was ascertained that the observed increase is not due to enhanced labelling of inorganic phosphate in the acid-soluble pool since (^{32}P)-orthophosphate specific activities practically remained unchanged, being 1.5 and 1.7 cpm/pmole of inorganic phosphate for non-irradiated and irradiated rats respectively.

To see whether the heightened rates of incorporation were due to increased activation of the enzyme involved in the synthesis of nucleotides, experiments were carried out to assess the extent of synthesis of nucleotides catalyzed by liver enzymes in vitro. Results in table 2 indicate that the liver supernatant fraction obtained from rats at 18 h post-irradiation has much higher efficiency for conversion of orotic acid to pyrimidine nucleotides than that obtained from unirradiated control. This would mean that at least some of the limiting enzymes in the synthetic pathway of pyrimidine nucleotides could have been activated in response to radiation stress in the early post-irradiation periods.

Results from table 3 show that actinomycin D, besides inhibiting RNA-synthesis, also retards the phosphorylation of nucleotides in rat liver. However, the enzymatic activity in the liver high-speed supernatant responsible

Table 3. Effect of actinomycin D on (^{32}P)-orthophosphate incorporation into RNA, UTP, CTP and on enzymatic conversion of orotic acid to pyrimidine nucleotides in the liver of irradiated rat

	RNA	UTP	CTP	Enzymatic conversion of orotic acid into	
	(relative specific activity)*	(relative specific activity)**		UMP (cpm)	CMP (cpm)
Irradiated (1000 R)					
After 18 h	10 750 ± 976	98	99	334 000 ± 45 000	35 000 ± 6 000
Irradiated (1000 R)					
After 18 h + actinomycin D	8200 ± 383	69	65	329 000 ± 64 000	37 000 ± 5 800

$$^*\frac{\text{cpm/mg RNA}}{\text{cpm/pmole of inorganic phosphate in liver homogenate}},$$

$$^{**}\frac{\text{cpm/nmole nucleoside triphosphate}}{\text{cpm/pmole of inorganic phosphate in liver homogenate}}.$$

Actinomycin D (150 µg/100 g b. wt) and (^{32}P)-orthophosphate (carrier-free, 2 mCi/100 g b. wt) were administered 1 h and 30 min prior to sacrifice, respectively. Each value for (^{32}P) labelling of RNA and for enzymatic conversion to pyrimidine nucleotides, represents average of 3 independent experiments (3 animals) ± SEM. For UTP and CTP, livers from 4 rats were pooled and the relative specific activity was determined.

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for conversion of orotic acid to pyrimidines remains unaffected. This observation implies that the depression of RNA-synthesis in irradiated rat receiving actinomycin D might be associated with inhibition of yet another crucial enzyme functioning in this pathway.

Thus the present findings suggest that, besides changes at the transcriptional level[1-3], whole-body exposure of rats to X-rays also causes a significant stimulation in the synthsis of ribonucleotides in liver. Increase an RNA-synthesis and accelerated rates of formation of ribonucleoside precursors could probably be interlinked as can be conjectured from the above findings and also other reports[11,12]. The levels of ribonucleotides are not elevated significantly in the liver of irradiated rat as compared to those from non-irradiated ones. This could possibly be due to increased demand for nucleotides to meet the enhanced rate of RNA-synthesis. Mandel and coworkers[13] have shown that in regenerating rat liver, although there is no increase in the levels of ribonucleoside precursors, the enzymes responsible for the synthesis of ribonucleosides are significantly activated.

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Orientation of *Papilio demoleus* larvae to coloured solutions

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Summary. The present work compares the taxis of *Papilio demoleus* larvae to different coloured solutions. The larval positive taxis, i.e., attraction, is maximum for yellow colour and declines with the increase or decrease in the wavelength of maximum light transmission. Red and bluish-green colours repel the larvae.

Several insects have been reported to show different orientational responses to different colours (Mazokhin-Porshnyakov[1]). But there are very few reports on this with respect to lepidopterous larvae. Götz[2] and Hundertmark[3] studied the orientational responses of the larvae of certain lepidopterans to different colours. Meisner and Ascher[4], though reporting attraction of the larvae of *Spodoptera littoralis* to various colours, studied their role in the larval feeding. Electrophysiological study of colour perception by *Bombyx mori* larvae was undertaken by Ishikawa[5]. In the course of our study of the role of visual stimuli in determining the relationships between various plants and *Papilio demoleus* larvae, their orientational responses to different colours have been tested and the results are given here.

Material and methods. Newly emerged last (fifth) instar larvae of *Papilio demoleus* were taken from culture, maintained as described before[6], and starved for 4–6 h before testing their orientational responses to different colours. The sources of stimuli used were 0.1% aqueous solutions of certain dyes mixed in different proportions to give different colours (table). Each solution was filled in a thin-walled, rectangular glass trough which was 75 mm long, 20 mm high and 5 mm in depth. Its 1 wall (75×20 mm) was externally lined with a white filter paper to form a reflecting surface for the light incident through the opposite transparent wall.

The orientational response of the larvae was studied on a grid by the method developed by Saxena et al[7]. The grid consisted of 13 longitudinal ribs intersecting 17 transverse ribs at right angles at about 2 cm distance. The grid was placed flat on a horizontal fluorescent white screen, uniformly illuminated from underneath by 2 20 W fluorescent lights. The glass trough containing the desired coloured solution was placed vertically on 1 side of the central transverse rib, parallel to it, at 1 cm distance from its central intersection with the central longitudinal rib. The centre of the glass trough was held at the level of the central longitudinal rib and the colour of its solution could be seen through its transparent wall facing the central intersection. Each larva was allowed to move on the central transverse rib towards the central intersection from the preceding intersection. On reaching the intersection, the larva might continue to move forward or turn and move on the central longitudinal rib towards the coloured solution on one side or towards the opposite blank side. The next larva was then tested from the opposite direction. On the basis of 10 tests in each replicate, the percentages of the larvae moving forward (F), turning towards the stimulus source (S) and towards the blank side (B) were recorded. The difference (S–B) between the percentages of the larvae turning towards the stimulus source and the blank side would show their orientational preference, its positive values indicating the attraction and negative values repulsion of the larvae by the coloured solution. Each experiment was repeated five times.

Results and discussion. The maximum percentage (92%) of the larvae turned towards the yellow coloured solution, the opposite blank side drawing only 4% larvae and the remaining ones moving forward on the grid (table). Consequently, the orientational preference of the larvae for the yellow solution was the highest (88%). With the decrease in the range of wavelengths for maximum transmission of light through different coloured solutions, the percentage of larvae turning and showing orientational preference towards the solutions declined. Thus, the larval preference for the yellowish-green solution was $^{7}/_{8}$, for the greenish-yellow $^{2}/_{3}$, for the green $^{2}/_{5}$ and for the greenish-blue $^{1}/_{3}$ of that for the yellow. A further decrease in the wavelength for maximum light transmission resulted

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