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Metabolism of Nucleic Acids during Liver Maturation in the Neonatal Rat

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In a previous paper (Oliver, Ballard, Shield & Bentley, 1962) several biochemical aspects of normal liver growth in postnatal rats were reported. Changes observed in the concentration of liver nucleic acids during the first 25 days after birth suggested that rapid cell division preceded a later phase of cytoplasmic growth. Since these results appeared to be the reverse of the situation

that occurs in regenerating adult rat liver (Hecht & Potter, 1956), observations have now been extended by a study of the rates of incorporation of labelled precursor ($[^{14}\text{C}]\text{orotic acid}$) into liver nucleic acids *in vivo*. In addition, it has been necessary to attempt a definition of the cell types that contribute to changes in nucleic acids, since the rat liver is known to be haematopoietic in early

extrauterine life (see Widdowson & McCance, 1960; Oliver, Blumer & Witham, 1963).

The labelling of nucleic acids was also studied over extended periods after a single injection of the labelled precursor. In principle, such experiments should distinguish between nucleic acid synthesis by hepatic cells and haematopoietic cells, since the DNA of the latter cells is lost during the maturation of the erythrocyte. In addition, techniques have been developed which result in partial separation of haematopoietic cells and liver-cell nuclei, so that some conclusions may be drawn about the contribution of haematopoietic tissue to the labelling of whole liver DNA. Histological studies of liver sections have previously been used to define the cellular composition of the neonatal liver (Oliver *et al.* 1963).

MATERIALS AND METHODS

Incorporation experiments

Albino rats (*Rattus norvegicus*) of the Wistar strain were obtained in litters of eight to twelve animals at known times after birth and weighed. Very young neonatal rats are poikilothermic, and in order that incorporation might be tested at reasonably constant temperature the weighed animals were kept in an incubator at 37.5° for 30 min. before the injection. Immediately after the injection they were returned to the incubator.

Labelled precursor was injected intraperitoneally with a micrometer syringe fitted with a 30-gauge 0.5 in. needle. With very young animals up to 15% of the dose sometimes leaked from the wound immediately after injection. Such animals were rejected, but wherever possible six rats were used in each age group.

At 90 min. after injection the animals were killed by decapitation and exsanguinated, and the whole livers were rapidly dissected out and dropped into ice-cold 0.25 M-sucrose solution containing CaCl_2 (0.18 mM).

Long-term 'decay' experiments

Several litters of four to ten animals were used in each experiment. The animals in each litter were injected with labelled precursor as described above. For incubation times less than 1 day the injected animals were maintained in the incubator at 37.5°; for longer periods they were returned to and maintained by the mothers. Pairs of animals were killed by decapitation and bled. Whole livers were removed and chilled as described above.

Labelled precursors

[^{32}P]Orthophosphate in HCl solution (specific radioactivity 0.72 mc/ml.) was obtained from The Radiochemical Centre, Amersham, Bucks., and diluted before use with an equal volume of 0.27 M-NaCl in 0.2 M- Na_2HPO_4 . The final pH was 7.4. Adult animals used for tests of the method received 0.1 μC of ^{32}P (2.4×10^4 counts/min.)/g. body wt. [^{14}C]Orotic acid (9.0 $\mu\text{C}/\mu\text{mole}$), obtained from The Radiochemical Centre, was dissolved in water and neutralized with ammonia. Each animal received 0.25 μC

of ^{14}C (2.8×10^6 counts/min.)/g. body wt. Stock solutions of each labelled substance were stored at -25° between experiments.

Liver analyses

Separation of cell fractions and nucleic acids. The chilled livers were blotted on filter paper and weighed. Each liver was homogenized in 10 ml. of ice-cold 0.25 M-sucrose containing CaCl_2 (0.18 mM) in Perspex coaxial homogenizers of the Potter-Elvehjem type equipped with an electric-drive motor. The conditions of homogenization were standardized as far as possible by using the same drive speed and same number of strokes of the homogenizer. Examination of resulting homogenates in the phase-contrast microscope revealed very few whole liver cells at any age of the donor animals. In the long-term experiments (see above) a sample of each homogenate was removed for assay of total DNA, total nuclear RNA and total cytoplasmic RNA. The sample was carefully fractionated by the method of Hecht & Potter (1956), but to minimize losses each fraction was acid-washed twice only and then worked up for chemical estimation of nucleic acid by the Schmidt-Thannhauser-Schneider procedure (see Volkin & Cohn, 1954).

The homogenates were fractionated at 0-2° by differential centrifugation as described by Hecht & Potter (1956) to yield DNA, nuclear-RNA nucleotides and cytoplasmic RNA. The isolated nucleic acids were dissolved in 0.1 N-ammonia and the nucleotide solutions made 0.1 N with respect to ammonia for determinations of radioactivity and concentration.

Further fractionation of 'nuclear' fraction

Oliver *et al.* (1963) have shown that the homogenization procedure does not rupture haematopoietic cells present in neonatal rat liver. Microscopy shows that these cells are harvested together with liver-cell nuclei in the 'nuclear' fraction. The subfractionation of this fraction was therefore attempted to study the labelling of nucleic acid in liver-cell nuclei and in haematopoietic cells independently.

Phase-contrast microscopy showed that practically all of the nuclei were agglutinated in the nuclear fraction prepared from the livers of very young rats in 0.25 M-sucrose containing CaCl_2 (0.18 mM). When the concentration of CaCl_2 was increased to 0.4 mM or higher, nuclear agglutination was almost completely absent. For the experiments described below, the nuclear fraction was prepared by the following method.

Livers were homogenized in 10 vol. (w/v) of 0.25 M-sucrose containing CaCl_2 (0.4 mM) by the 'standard' procedure. The homogenate was filtered through four layers of coarse surgical gauze to remove connective tissue, and 5.0 ml. was layered over 2.0 ml. of 0.34 M-sucrose containing CaCl_2 (0.4 mM). After centrifugation at 600g for 15 min. at 0°, the supernatant fluid was removed with a Pasteur pipette and the sediment was gently homogenized in 5.0 ml. of 0.25 M-sucrose containing CaCl_2 (0.4 mM), and the layering procedure and centrifugation were repeated. The whole procedure was repeated once more to yield the washed 'nuclear' fraction.

Zone centrifugation on sucrose gradients. The washed nuclear fraction obtained from about 500 mg. of liver was suspended in 0.5 ml. of 0.25 M-sucrose containing CaCl_2 (0.4 mM) and layered over 5 ml. of a sucrose concentration gradient in a Lusteriod centrifuge tube (1.6 cm. $\times$ 7.5 cm.).

The linear gradient was prepared (by the method of Britten & Roberts, 1960) with the sucrose concentration ranging from 2.5 M at the bottom to 0.85 M at the top of the tube; CaCl_2 was also present at a concentration of 0.4 mM. The tube was centrifuged for 30 min. at 1800g in a swing-out rotor (10 ml. buckets) of a bench centrifuge in the cold room at 0°. Three zones were apparent after such treatment. The top zone represented the original zone of application, but contained, of particulate matter, only debris and residual mitochondria. The second zone, 2.5–3.0 cm. from the bottom, contained haematopoietic cells at various stages of maturation, red cells and some nuclei, to judge from microscopy of stained smears. Direct phase-contrast microscopy was insufficiently discriminatory. The third zone, 1.2–1.5 cm. from the bottom, contained mostly liver-cell nuclei and small intact cells possessing intensely acidophilic cytoplasm and 'dumb-bell' nuclei.

The two zones of interest (second and third) appear to be at isopycnic equilibrium since further centrifugation (for up to 2 hr.) did not alter their positions in the gradient. Each zone was collected separately with the aid of the tube slicer manufactured by the Beckman Instrument Co., Spinco division, Palo Alto, Calif., U.S.A. After removal from the tube slicer, the fractions were diluted tenfold with 0.25 M-sucrose containing CaCl_2 (0.4 mM) and centrifuged at 1800g for 15 min. to sediment the particulate matter completely.

An increase in the concentration of CaCl_2 to 0.6 or 0.8 mM resulted in the formation of only one zone in the centrifugation.

When the livers were obtained from animals given radioactive precursor the fractions were worked up for DNA as described above.

Electrophoretic fractionation. Zone electrophoresis was carried out in a simple vessel constructed as follows. The left-hand limb of a U-tube consists of Perspex tubing (1.9 cm. internal diam. $\times$ 31 cm. long) closed at the bottom by a cemented Perspex plug. A loop of platinum wire, making contact with the outside through a small hole in the wall, is cemented into position 2 cm. from the top. The bottom end of this tube is joined to another similar tube, through a short piece of neoprene tubing (0.8 cm. diam.) tightly fitted in holes drilled through the wall of the tubes. The right-hand arm of the U-tube has the same internal diameter as the left-hand limb but is 15.5 cm. long. The bottom end has a Perspex funnel machined from solid rod and cemented to it. The funnel outlet is closed with a short length of plastic tubing and a screw clip. At the open end of the tube a Perspex collar is cemented which protrudes 1 cm. above the tube end. A further piece of the same tubing (15.5 cm. long) fits closely into the collar. A ring of platinum wire is inserted as in the other arm of the U-tube.

In preparation for use, the connecting tube was closed with a screw clip, and, with the detachable upper tube removed, the right-hand limb was filled with a concentration gradient of sucrose dissolved in 0.4 mM- CaCl_2 buffered with 0.02 M-tris (adjusted to pH 7.5 with $\text{N-H}_3\text{PO}_4$). The concentration of sucrose was 2.0 M at the bottom and decreased in approximately linear fashion to 0.85 M at the top. The tube was filled to within 0.5 cm. of the top. The left-hand limb received a similar gradient solution. The apparatus was equilibrated in the cold room at 0° overnight.

The washed 'nuclear' fraction obtained from up to 2.4 g. of liver was suspended in 1.0 ml. of 0.4 M-sucrose

solution containing CaCl_2 (0.4 mM) and tris (0.02 M), adjusted to pH 7.5 with $\text{N-H}_3\text{PO}_4$. This suspension was layered on the sucrose gradient in the short limb. The upper detached section of this limb contained a fitted piece of plastic sponge loaded with a solution of 0.2 M-sucrose containing CaCl_2 (0.4 mM) and tris-phosphate buffer and flush with the end of the tube. It was inserted into the collar, previously greased with petroleum jelly to ensure a fluid-tight seal, and worked into place. The tube was then filled with the cold buffered 0.2 M-sucrose solution and worked gently so that the solution flowed through the sponge and made contact with the nuclear suspension. By opening the clip on the connecting tube the nuclear zone was then displaced gently downwards about 2 cm. The clip was closed, the left-hand limb filled with the cold buffered 0.2 M-sucrose solution and the clip opened. By judicious addition of sucrose solution to each limb they were then completely filled to contact the electrodes while keeping the nuclear zone stationary.

A potential of 500 v was applied, the right-hand electrode being the anode. The current drawn was 1 mA. After electrophoresis for 16 hr. a clear zone containing haemoglobin remained at the origin, while two cloudy particulate zones appeared just above and below the origin. Further down the limb several poorly resolved zones appeared which are probably a result of slow sedimentation. Up to six fractions were obtained by collection through the funnel at the bottom of the limb. Each fraction was diluted fivefold with 0.25 M-sucrose containing CaCl_2 (0.4 mM) and centrifuged at 1800g for 15 min. The sediments were resuspended in the dilute sucrose solution, and smears were prepared as described by Oliver *et al.* (1963). Fractions 1–4 were mixed fractions consisting of agglutinated liver-cell nuclei and free haematopoietic cells, fraction 5 consisted mainly of haematopoietic cells, and fraction 6 consisted of free liver-cell nuclei.

When electrophoresis of the 'nuclear' fraction from the livers of animals given radioactive precursor was carried out the subfractions were worked up for DNA as described above.

Radioactivity measurements

Measurement of ^{32}P . Nucleic acid solutions (0.1 ml.) were plated on dimple planchets and dried under a heat lamp. The ^{32}P was measured with an end-window Geiger-Müller counter. Results were corrected for radioactive decay. Control planchets showed that self-absorption was negligible over the range of nucleic acid measured.

Measurement of ^{14}C . The ^{14}C was determined with a counting efficiency of 50% in a liquid-scintillation counter by using the counting solvent Diotol described by Herberg (1960). With the detector used, the addition of 1,4-bis-(5-phenyloxazol-2-yl)benzene was found unnecessary. Samples (0.1 ml.) of nucleic acid solutions were pipetted into 16 ml. clear Pyrex-glass counting vials and 5 ml. of Diotol was added to each. The addition of 1.5 ml. of ethanol as used by Herberg (1960) was not necessary to obtain solution and was omitted. Each vial was counted once until at least 1000 counts were obtained, and, after the addition of an internal standard of [^{14}C]orotic acid (1400 counts/min.), counted again. Quenching corrections were calculated from these results. Slight day-to-day variations in efficiency were established by counting the [^{14}C]orotic acid standard daily, and all counts were corrected to 50% efficiency.

Nucleic acid determinations

Determination of ribonucleic acid. The orcinol procedure for ribose as described by Volkin & Cohn (1954) was found to be unsatisfactory in our hands, as day-to-day standards showed large variations ($\pm 20\%$). A modified procedure was therefore developed.

The stock orcinol reagent was prepared as specified and stored in a dark bottle at 0° . On the day of use a sample was diluted 20-fold with $7.4N$ -HCl, the concentration of which was established by titration with standard NaOH. A 1.0 ml. sample of RNA solution containing $0.80 \mu\text{g.}$ of RNA or its nucleotide equivalent was heated with 3.0 ml. of the working reagent for 1 hr. in a boiling-water bath. The time-course of colour development showed that the reaction was not complete until heating at 100° had proceeded for at least 45 min. The concentration of HCl was also extremely critical for the absolute colour intensity and it was therefore checked by titration. The extinctions of the solutions were determined at $660 \text{ m}\mu$ against a reagent blank prepared in the same way but with 1.0 ml. of water.

Daily standards and duplicates were reproducible to within 2% with this modification. A standard RNA solution was prepared from a commercial preparation of RNA (Sigma Chemical Co., St Louis, Mo., U.S.A.), the purity of which was established by determinations of organic phosphorus (see Oliver *et al.* 1962).

Determination of deoxyribonucleic acid. DNA was determined by the modified diphenylamine reaction for deoxyribose (Burton, 1956). DNA standards were prepared from a commercial sample of DNA (Sigma Chemical Co.), the purity of which was established by determination of organic phosphorus content (Oliver *et al.* 1962).

Calculation of results

Specific radioactivity was calculated as counts/min./mg. of nucleic acid. In the long-term experiments, total nucleic acid of each variety and intracellular origin was calculated as mg./liver and this enabled the specific radioactivity to be converted into total counts/liver found in DNA, nuclear RNA and cytoplasmic RNA.

Histological methods

Nuclear fractions suspended in sucrose- CaCl_2 solution were smeared on glass slides and dried in air at $70\text{--}80^\circ$. They were fixed overnight in Heidenhain's Susa and stained with Harris's haematoxylin and chromotrope 2R.

RESULTS

Fig. 1 shows the rate of incorporation *in vivo* of [^{14}C]orotic acid into DNA, 'nuclear' RNA (isolated as described in the Materials and Methods section) and cytoplasmic RNA, plotted against the age of the animal. Two or more peaks in the rate curve were obtained with all three species of nucleic acid, although the statistical significance of the multiple peaks in the activity of cytoplasmic RNA is doubtful. The first major peak occurs 1–2 days after birth and the second occurs 8–10 days after birth.

In Fig. 2 the results of the long-term experiments are summarized. Animals of age 2 days and 8 days were injected with [^{14}C]orotic acid and the total counts found in DNA of the liver are plotted

Fig. 1. Incorporation of [^{14}C]orotic acid *in vivo* into liver nucleic acids during growth of postnatal rats. The nuclear acids examined were: (a) nuclear RNA; (b) cytoplasmic RNA; (c) DNA. Six rats were used for each point. The vertical bars represent $\pm$ s.e. The specific radioactivity is expressed as counts/min./mg. of nucleic acid 90 min. after the injection. Experimental details are given in the text.

Fig. 2. 'Turnover' of liver DNA *in vivo* during growth of postnatal rats. Two rats were used for each point, which represents the average value obtained. Animals were injected with [^{14}C]orotic acid 2 days ($\blacktriangle$) and 8 days ($\bullet$) after birth, and the radioactivities retained in the total DNA of the liver were determined at subsequent times. Experimental details are given in the text.

Table 1. *Incorporation of [¹⁴C]orotic acid into the deoxyribonucleic acid of subfractions of liver 'nuclear' fraction*

Experimental details are given in the text. The specific activity of DNA is expressed as counts/min. incorporated/mg. from [¹⁴C]orotic acid 90 min. after injection. (a) The results are the means of three experiments with zone centrifugation in each of which livers of four 2-day-old rats were pooled for subfractionation of the 'nuclear' fraction as described in the text. (b) The results are from a typical experiment with zone electrophoresis. The 'nuclear' fractions of the livers of four 1.5-day-old rats were pooled for electrophoresis.

(a) Zone centrifugation		
	Low-density zone 2	High-density zone 3
$10^{-3} \times$ Radioactivity (counts/min./mg. of DNA)	17.3	8.3
Percentage of total radioactivity	5	95
Histology	Mainly haematopoietic cells	Liver-cell nuclei plus haematopoietic cells

(b) Zone electrophoresis					
	Zone 1	Zone 2	Zones 3 and 4	Zone 5	Zone 6
$10^{-3} \times$ Radioactivity (counts/min./mg. of DNA)	9.1	14.5	10.5	9.1	7.5
Percentage of total radioactivity	15	69	6	8	1.2
Histology	Mixed	Mixed	Mixed	Mainly haematopoietic cells	Liver-cell nuclei

against the age of the animal. About 50 % of the radioactivity incorporated after injection of the 2-day-old animals was rapidly lost from the liver over the subsequent 3-day period and the radioactivity declined more slowly thereafter. All of the radioactivity incorporated after injection of the 8-day-old animals was subsequently retained in the liver. An initial rapid increase during the first 24 hr. was followed by a slow but steady increase. The radioactivities in RNA of the cytoplasmic and nuclear fractions were also determined. The 'nuclear' RNA was the most rapidly labelled species and the radioactivity reached an apparent maximum 1½ hr. after the injection. The true maximum was probably reached later, but the next point on the curve was obtained 24 hr. after injection. At this time the radioactivity in 'nuclear' RNA had fallen to 30 % of the level at 1.5 hr., but the radioactivity of cytoplasmic RNA was still rising to a maximum, which occurred 24 hr. after injection. Since similar results have been reported by many other authors (see Smellie, 1955) the quantitative results are not reported in detail. The slow phase of incorporation into DNA after the injection of the 8-day-old animals is probably due to reutilization of labelled degradation products of RNA.

Experiments with [³²P]orthophosphate were carried out with adult rats to test the method of the cell-fractionation procedure. In experiments in which nuclear RNA accumulated 1200 counts/min./mg./hr. of incubation *in vivo*, no counts could be detected in DNA. Thus the DNA fraction was obtained free from RNA or other labelled contaminants.

Table 1 gives the results on the incorporation of [¹⁴C]orotic acid into the various subfractions of the 'nuclear' fraction obtained by zone centrifugation and zone electrophoresis. The experiments were conducted in the same general fashion as in the other short-term experiments; animals were killed 1.5 hr. after the injection of [¹⁴C]orotic acid. Although none of the fractions obtained was cytologically pure, haematopoietic cells were obtained as the major component of a low-density centrifugal zone. The centrifugal fractionation indicates that the rate of incorporation into haematopoietic-cell DNA could be twice as high as that found in liver-cell nuclei.

The electrophoretic separation yields rather arbitrary fractions, especially in zones 1-4 in which the various components were always agglutinated. These fractions were of mixed cytology and appear to arise by slow sedimentation of large aggregates. Their behaviour was probably uninfluenced by the electric field. In zone 6, the liver-cell nuclei were not agglutinated and there were few haematopoietic cells present, whereas zone 5 contained mainly dispersed haematopoietic cells. There appears to be little difference in the rate of labelling of DNA isolated from these two fractions.

DISCUSSION

At first sight, the incorporation results of Fig. 1 are difficult to compare with previous findings obtained on nucleic acid concentration (Oliver *et al.* 1962). However, the amount of DNA synthesized/day/mg. of liver DNA can be calculated from our previous results and such values may be

compared directly with the results of incorporation experiments (Fig. 1). Such calculations cannot profitably be made for RNA which turns over rapidly. Such a calculation (see Table 2) shows two peaks, the first at about 1 day after birth, the second at about 11 days after birth, which is in reasonable agreement with the results obtained with [^{14}C]orotic acid (Fig. 1). The appearance of two maxima in the rate of labelling of liver DNA is also reflected somewhat imperfectly in the labelling of RNA during growth.

The neonatal rat liver contains transient haematopoietic tissue, and quantitative estimates by Oliver *et al.* (1963) indicate that the proportion of the organ occupied by such tissue falls from 30 % in the newborn animals to zero at 5 days after birth. The results on long-term labelling of nucleic acids presented in Fig. 2 indicate that some metabolically unstable or transient DNA is synthesized in the liver of 2-day-old animals, whereas the DNA synthesized at 8 days after birth is stable. Attempts were therefore made to determine the distribution of label between the DNA of haematopoietic cells and liver-cell nuclei in the 2-day-old animals. The techniques that were finally developed for the separation of these two components do not result in cytologically pure fractions, but the enrichment is such that the results of Table 1 allow some assessment of the contribution made by each component to the total labelling of DNA found in liver of 2-day-old animals.

In the 2-day-old animals about 18 % of the liver volume is accounted for by haematopoietic tissue (Oliver *et al.* 1963). The proportion of total liver DNA contained in this fraction is difficult to assess accurately, but the centrifugal separation yields only 5 % of the total DNA in the 'haematopoietic-cell' zone. The upper limit for the DNA of this fraction is thus unlikely to be more than 18 % of the total, and may well be considerably less, since the haematopoietic cells are in various stages of maturation. The rate of labelling of the haematopoietic-cell fraction is about twice that of the zone

of liver-cell nuclei (Table 1). Hence it can be calculated that the total haematopoietic-cell fraction of the liver of the 2-day-old animal (i.e. 18 % of total DNA) would contribute not more than 33 % of the total radioactivity accumulated in liver DNA after incorporation for 90 min. *in vivo*. A substantial proportion of the total labelled DNA must then be due to incorporation of radioactivity into liver-cell DNA, and the rate of labelling of this DNA is about 6×10^3 counts/min./mg./90 min. in the liver of the 2-day-old animal (from Fig. 1). The electrophoretic separations, which yield a nearly pure sample of liver-cell nuclei, also show that the rate of labelling of the liver-cell DNA is of a similar order of magnitude (Table 1).

The metabolically unstable DNA, whose presence is demonstrated in Fig. 2, cannot be rigorously proven to be due only to haematopoietic cells, since the cells continuously disappear from the liver whereas some labelling remains. Further, the techniques used for separation do not allow the preparation of a pure liver nuclear fraction in sufficient yield to allow assessment of the metabolic stability of the liver-cell DNA which is apparently labelled during this period. However, the distribution of radioactivity between the liver-cell DNA and haematopoietic-cell DNA indicates that the first peak of labelling shown in Fig. 1 is substantially due to the synthesis of liver-cell DNA, and that not more than 33 % of this radioactivity could be due to synthesis of haematopoietic-cell DNA. Thus there are two periods during the postnatal development of the rat liver in which DNA is replicated by the liver cells. These periods of DNA synthesis are also reflected by increases in the rate of RNA synthesis.

It was suggested by Oliver *et al.* (1962) that the growth of postnatal rat liver was characterized by a period of rapid DNA synthesis and cell division followed by synthesis of RNA and cytoplasmic growth. The results presented above indicate that there are two periods of rapid DNA synthesis, but these results are consistent with the previously reported findings on changes in the concentration of DNA.

The rapid formation of stable DNA 8–10 days after birth can be correlated with the sharp increase in the weight of the liver that occurs about this time, but the significance of the rapid synthesis of liver-cell DNA 2 days after birth is not known.

SUMMARY

1. The metabolism of nucleic acids during the maturation of neonatal rat liver has been followed by measuring the rate of incorporation of [^{14}C]orotic acid into nucleic acids over short time-periods *in vivo*.

Table 2. *Synthesis of deoxyribonucleic acid in neonatal rat liver*

The values were calculated from the results of Oliver *et al.* (1962), as indicated in the Discussion section.

Animal age (days)	DNA synthesized ($\mu\text{g. of DNA/day/}$ mg. of liver DNA)
1	186
5	64
7	5
9	57
11	178
15	122
20	26

2. There are two periods in which liver nucleic acids (DNA, cytoplasmic RNA and nuclear RNA) show maximal rates of labelling. The first occurs at 2 days after birth and the second period at 8–10 days after birth.

3. Long-term experiments on the retention of [^{14}C]orotic acid in DNA after a single injection show that some of the DNA initially labelled at 2 days is transient, whereas all the radioactivity incorporated at 8 days after birth is retained for long periods.

4. Zone electrophoresis and zone centrifugation have resulted in a crude fractionation of liver of the 2-day-old animal into haematopoietic cells and liver-cell nuclei, and the labelling of the DNA of these fractions shows that about 70 % is accounted for by liver-cell DNA. The haematopoietic-cell DNA is more highly labelled but accounts for the minor proportion of the total labelled DNA, and for the transient material.

5. There are two periods during growth of the neonatal rat liver in which DNA and RNA are rapidly synthesized.

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Effect of Growth Hormone on Ribonucleic Acid Metabolism

3. NATURE AND CHARACTERISTICS OF NUCLEAR SUBFRACTIONS STIMULATED BY HORMONE TREATMENT*

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Talwar, Panda, Sarin & Tolani (1962) reported that growth hormone causes a significant increase in the incorporation *in vivo* of [^{32}P]orthophosphate into the nuclear and microsomal fractions of rat-liver RNA.

Experimental results from various sources indicate that most, if not all, of the cellular RNA is synthesized in the nucleus. A fraction of RNA (messenger RNA), having a rapid turnover rate and having the biological properties of 'transcribing' the genetic information and the base ratios corresponding to the species DNA, has been described in bacteria (Gros *et al.* 1961; Brenner, Jacob & Meselson, 1961). Hiatt (1962) has isolated a rapidly labelled RNA from the rat-liver nuclei.

* Part 2: Panda, Goel, Mansoor & Talwar (1962).

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Similarly, ribosomal RNA–DNA hybrid fractions have been described (Yankofsky & Spiegelman, 1962*a, b*), implying the nuclear synthesis of ribosomal RNA. In higher organisms, Sibatani, de Kloet, Allfrey & Mirsky (1962) reported the isolation of four fractions of RNA from thymus nuclei. Besides the nuclear transfer RNA and ribosomal RNA, they were able to separate three other RNA fractions from the animal-tissue nuclei. A fraction of RNA that is extractable in 1M-sodium chloride is linked to the DNA strands. Sibatani *et al.* (1962) also described the isolation of a nucleolar fraction having the same base ratios as the DNA.

Since in our studies growth hormone has been shown to stimulate the synthesis of nuclear RNA, it was decided to determine the type of fraction concerned in this effect, and more particularly to discover if it can be defined as a messenger RNA.