Studies on Enzymes Involved in Nucleic Acid Metabolism in Foetal, Young and Regenerating Rat Liver

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Since 1959 several workers (Bollum & Potter, 1959; Weissman, Smellie & Paul, 1960; Maley & Maley, 1960) have studied the changes in the amount of the enzymes involved in the synthesis of DNA in regenerating liver. Canellakis, Jaffe, Mantsavinos & Krakow (1959) have investigated similarly the enzymes concerned in the catabolism of thymine and of uracil during liver regeneration. The general conclusion is that with the increased rate of mitosis the activities of enzymes involved in DNA synthesis increase, and that the activities of those involved in DNA catabolism decrease. The increases in activities of thymidine kinase, deoxyribonucleic acid polymerase and deoxycytidine monophosphate deaminase can be delayed by giving doses of X-radiation, as low as 500 r., 6 hr. after partial hepatectomy (Bollum, Anderegg, McElya & Potter, 1960; Stevens & Stocken, 1962).

A study of the differences in the enzyme patterns in dividing and resting liver may help to elucidate the factors controlling cell division. We have therefore extended the above observations by following the changes in the enzyme content of foetal and young rat liver with age. Foetal liver has a higher mitotic index than regenerating liver, but cell division is not synchronous and cannot therefore be used to show changes during the cell cycle.

The enzymes examined in foetal and young rat liver were thymidine phosphorylase, uridine phosphorylase, dihydro-orotase and deoxyribonucleic acid polymerase. In regenerating liver, the activities of thymidine phosphorylase and uridine phosphorylase and the effect of X-radiation on the depression of thymine catabolism after partial hepatectomy were studied. The change in the catabolism of thymine and of uracil in young and foetal liver with age has been reported by Stevens & Stocken (1960).

METHODS AND MATERIALS

Chemicals. [2-¹⁴C]Thymine was obtained from The Radiochemical Centre, Amersham, Bucks., and [ureido-¹⁴C]-ureidosuccinic acid from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A., DNA was prepared from rat liver by the method of Kirby (1957).

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Animals. The laboratory strain of Wistar rats was used. Adults were 200 g. males about 3 months old and these were also used for the partial hepatectomies (Higgins & Anderson, 1931). The ages of the fetuses were determined by comparing their weights with those given by Stotsenburg (1915).

Irradiation. X-radiation was carried out at the Churchill Hospital, Oxford. The characteristics of the machine used were as follows: 15 mA, 220 kv, added filters of 1 mm. of aluminium and 0.5 mm. of copper giving a half-value layer of 1.1 mm. of copper. The dose rate calculated to the centre of the body was 61 rads/min.

Enzyme assays. The assays of thymidine phosphorylase and of uridine phosphorylase depend on the differences in extinction coefficient between thymidine and thymine on the one hand and between uridine and uracil on the other at 290 m μ in alkaline solution. They are based on the assay methods of Friedkin & Roberts (1954) and of Canellakis (1957). The incubation mixtures contained: 1.0 ml. of 0.1M-potassium phosphate buffer, pH 6.1, 0.2 ml. of 41.2 mM-thymidine and 1 ml. of supernatant for thymidine phosphorylase; and 1.5 ml. of 0.2M-potassium phosphate buffer, pH 8.0, 0.4 ml. of 0.1M-uridine and 1.0 ml. of supernatant for uridine phosphorylase. Rat liver was homogenized in 9 vol. of 1.15% (w/v) KCl and spun at 105 000g for 60 min. This supernatant was used for all the assays except for that of dihydro-orotase. The incubation time was 20 min. at 37°. After precipitation of protein with an equal volume of N-perchloric acid, the supernatants were made alkaline with 5N-NaOH and the extinctions measured at 290 m μ .

Thymine catabolism was determined as described by Canellakis (1956).

The incorporation *in vitro* of [³H]thymidine into DNA, i.e. the combined assay thymidine kinase, thymidylate kinases and polymerase, was measured as described by Bollum *et al.* (1960).

The assay of dihydro-orotase depends on the fact that dihydro-orotic acid and orotic acid are stable towards nitrous acid, whereas ureidosuccinic acid is decomposed by nitrous acid to give malic acid. The incubation mixture was: 0.5 ml. of 0.1M-potassium phosphate buffer, pH 6.1, 0.1 ml. of 72 μ M-[ureido-¹⁴C]ureidosuccinic acid (specific activity 3.5 mc/m-mole) and 0.1 ml. of 2% (w/v) homogenate. After incubation for 20 min. at 37°, samples (0.25 ml.) of the reaction mixture were each added to 0.25 ml. of 0.5N-HCl and 0.05 ml. of 20% (w/v) NaNO₂. The mixture was allowed to stand at room temperature for 15 min. The protein precipitate was removed by centrifuging and the radioactivity of the supernatant measured. Samples were evaporated on planchets under an infrared lamp and counted in a Nuclear-Chicago gas-flow counter.

Table 1. *Change in activities of thymidine phosphorylase and uridine phosphorylase with age in rat-liver supernatant*

The values given are means, together with the number of determinations and the range in parentheses. Details of the assay methods are given in the text.

Age (days)	Thymidine phosphorylase (μ moles of thymine formed/20 min.)		Uridine phosphorylase (μ moles of uracil formed/23 min.)	
	Per mg. of DNA phosphorus	Per g. of supernatant protein	Per mg. of DNA phosphorus	Per g. of supernatant protein
Foetus (19-20 days)	0.0 (3)	0.0 (3)	0.30 (2) (0.14-0.46)	1.4 (2) (1.3-1.5)
New-born	4.5 (3) (3.5-5.1)	26 (3) (19-30)	0.31 (3) (0.22-0.40)	0.62 (3) (0.52-0.76)
3	5.5 (3) (4.1-7.3)	52 (3) (44-63)	0.66 (3) (0.33-1.10)	2.1 (3) (1.2-3.2)
7	12.6 (3) (11.3-14.3)	68 (3) (64-70)	0.81 (3) (0.66-0.86)	—
14	12.1 (3) (9.3-14.1)	95 (3) (86-101)	1.80 (3) (1.70-1.98)	—
Adult	34.0 (3) (33.4-35.0)	104 (3) (99-111)	5.32 (4) (4.76-6.30)	7.1 (4) (6.6-8.2)

The counts were compared with those obtained from a sample of the same incubation mixture that had not been treated with 0.05 ml. of 20% (w/v) NaNO_3 , i.e. in which the counts from ureidosuccinic acid had not been released. The difference between the two was equal to the amount of ureidosuccinic acid remaining after incubation.

Determinations of deoxyribonucleic acid and of protein. Determinations of DNA were carried out on the original homogenates (Burton, 1956), and protein was determined (Layne, 1957) in the supernatants after centrifuging the homogenates for 1 hr. at 105 000g.

RESULTS

Thymidine-phosphorylase and uridine-phosphorylase activities were determined in the livers of rats from 2 days before birth until 2 weeks after birth, and these were compared with those present in the liver of the adult rat. Thymidine-phosphorylase activity (Table 1) was not detectable until birth, after which it increased gradually during the period of development under study. A similar change with age occurred with uridine phosphorylase (Table 1), though this enzyme was already detectable in foetal liver. By contrast, the rate of incorporation of thymidine into DNA (Table 2) fluctuated with age, being maximal 3 days after birth and minimal in adult liver. Dihydro-orotase activity also fluctuated with age (Table 2), increasing to a maximum at birth; thereafter it fell to less than half the value at birth and then remained fairly constant. The results shown in Tables 1 and 2 were obtained with livers taken from different rats.

The changes in thymidine-phosphorylase and uridine-phosphorylase activities during liver regeneration were less marked than those occurring

Table 2. *Dihydro-orotase activity and the incorporation of thymidine into deoxyribonucleic acid in young, foetal and adult liver*

The values given are means, together with the number of determinations and the range in parentheses. Details of the assay methods are given in the text.

Age (days)	Dihydro-orotase (μ moles of dihydro-orotate formed/15 min./mg. of DNA phosphorus)	Incorporation of thymidine into DNA (μ g. of thymidine incorporated into DNA/hr./mg. of DNA phosphorus)
Foetus (17-18 days)	6.9 (3) (5.5-8.5)	0.5 (2) (0.45-0.54)
New-born	23.5 (3) (21.9-26.0)	0.39 (2) (0.35-0.42)
3	7.1 (3) (5.9-9.2)	1.14 (2) (1.10-1.17)
7	11.6 (3) (11.0-12.6)	0.56 (2) (0.53-0.59)
14	6.5 (3) (4.0-8.9)	1.09 (2) (1.07-1.10)
Adult	12.1 (3) (10.9-13.5)	0.03 (4) (0.007-0.07)

in foetal and young rat livers. There is little change in the activity of uridine phosphorylase (Table 3) during regeneration though there is a decrease in that of thymidine phosphorylase to one-third of its original value after 72 hr., measured on a DNA basis (Table 3). Janion & Shugar (1961) have also shown a decrease in thymidine-phosphorylase activity during regeneration, but by a much smaller decrement than we have found.

Table 4 shows that X-radiation delays the decrease in thymine catabolism which normally occurs during liver regeneration. In the control

Table 3. *Effect of partial hepatectomy on the activities of thymidine phosphorylase and uridine phosphorylase in rat-liver supernatant*

The values given are means, together with the number of determinations and the range in parentheses. Details of the assay methods are given in the text.

Time after partial hepatectomy (hr.)	Thymidine phosphorylase (μ moles of thymine formed/20 min./ mg. of DNA phosphorus)	Uridine phosphorylase (μ moles of uracil formed/20 min.)	
		Per mg. of DNA phosphorus	Per g. of supernatant protein
Normal resting liver	34.0 (3) (33.4-35.0)	5.32 (4) (4.76-6.30)	7.10 (4) (6.6-8.15)
4	31.4 (3) (28.3-34.7)	5.72 (3) (4.5-7.0)	7.8 (3) (6.1-9.7)
12	29.6 (3) (26.7-31.6)	6.72 (3) (6.15-7.70)	9.6 (3) (7.6-10.9)
24	23.3 (3) (20.5-26.0)	4.25 (3) (3.65-4.76)	6.72 (3) (4.35-9.4)
48	14.5 (3) (7.4-21.0)	5.79 (3) (4.32-6.90)	9.45 (3) (7.45-11.1)
72	10.4 (3) (9.8-11.6)	5.55 (3) (4.86-5.90)	11.8 (3) (10.9-12.2)

Table 4. *Effect of X-radiation on the enzymes catabolizing thymine in regenerating rat liver*

Total-body radiation (400r.) was given 6 hr. after hepatectomy. The values given are means, together with the number of determinations and the range in parentheses. With the irradiated population the s.e.m. is given. Details of the assay method are given in the text.

Time after partial hepatectomy (hr.)	Thymine catabolized (μ moles/30 min./mg. of DNA phosphorus)	
	Control rats	Irradiated rats
Normal liver	2.54 (4) (1.59-3.58)	—
18	2.40 (4) (1.04-3.69)	—
24	0.83 (4) (0.79-0.91)	2.25 (8) $\pm$ 0.38
36	0.79 (2) (0.62-0.96)	
48	0.56 (2) (0.53-0.58)	1.65 (8) $\pm$ 0.19

DISCUSSION

The results of the determinations of uridine-phosphorylase and thymidine-phosphorylase activities and of the incorporation of thymidine into DNA indicate that the inverse relationship between catabolism and synthesis, which Canellakis *et al.* (1959) have shown to occur in regenerating liver, also occurs in foetal and young liver. With increase in age the rate of growth declines and the enzymes concerned with catabolism become more active, whereas the rate of DNA synthesis, as measured by thymidine incorporation, decreases. The activities of thymidine phosphorylase and uridine phosphorylase increase with age, as do those of the enzymes responsible for the breakdown of uracil and thymine (Stevens & Stocken, 1960). However, thymidine phosphorylase and uridine phosphorylase are detectable at birth, whereas dihydrothymine dehydrogenase and dihydrouracil dehydrogenase do not appear until about 12 days after birth. It is possible that thymine and uracil formed by the phosphorylases may cause the induction of the dehydrogenases, but, in experiments in which we tried to induce dihydrothymine dehydrogenase by injecting rats daily from birth with 0.3 mg. of thymine, there was no premature appearance of the enzyme (L. Stevens & L. A. Stocken, unpublished work).

In regenerating liver thymine catabolism is depressed to one-quarter of its original level after 48 hr., whereas in foetal liver thymine catabolism is not detectable (Stevens & Stocken, 1960). The changes in thymidine-phosphorylase and uridine-phosphorylase activities during liver regeneration

animals after 24 hr. the enzyme activity is reduced to about one-third of its original value, and after 48 hr. to about one-quarter. In the irradiated animals the activity is not decreased after 24 hr., and after 48 hr. is still two-thirds of the original value. The *t* test was applied collectively to the results obtained after 24 and 48 hr. and it gave a *P* value of between 0.001 and 0.01. X-radiation therefore seems to produce a significant effect on thymine catabolism in regenerating liver at short intervals after partial hepatectomy, though the extent of regeneration after 1 month is the same in both control and irradiated rats (L. Stevens & L. A. Stocken, unpublished work).

are not so marked as those occurring in young and foetal liver. This is to be expected by analogy with thymine catabolism.

The effect of X-radiation on thymine catabolism in regenerating liver shows that, under conditions in which the formation of thymidine kinase is delayed, a delay in the depression of thymine catabolism also occurs. It is possible that the depression in thymine catabolism may be influenced by the appearance of thymidine kinase which occurs at about 18 hr. after partial hepatectomy (Bollum & Potter, 1959). When the formation of thymidine kinase is inhibited by X-radiation, the depression in thymine catabolism is delayed.

SUMMARY

1. The activities of enzymes involved in nucleic acid metabolism in foetal, young and regenerating rat liver were compared with those in normal rat liver.
2. Thymidine phosphorylase and uridine phosphorylase were less active in foetal and young liver than in adult liver. Thymidine-phosphorylase activity was also reduced in regenerating liver.
3. Thymidine was incorporated into DNA *in vitro* more rapidly in the liver of foetal and young rats than in adult rats.
4. Dihydro-orotase activity was maximal in the liver at birth when it was twice as active as in the adult.
5. Total-body X-radiation (400 r.), when given to rats 6 hr. after partial hepatectomy, delayed the decrease in thymine catabolism.

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The Association of Protein Synthesis with the Formation of Pigments in some Photosynthetic Bacteria

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The formation of the photosynthetic pigments, bacteriochlorophyll and carotenoids, by *Rhodospseudomonas spheroides* and related organisms is closely associated with protein synthesis under a variety of conditions (Lascelles, 1959; Cohen-Bazire & Kunisawa, 1960; Gibson, Neuberger & Tait, 1962a; Sistrom, 1962). This association also occurs in suspensions of non-pigmented organisms which form bacteriochlorophyll and carotenoids when incubated at a low pressure of oxygen. The

inhibition of protein synthesis by the addition of chloramphenicol, 8-azaguanine or *p*-fluorophenylalanine, or by omitting a nitrogen source, also inhibits pigment synthesis (Lascelles, 1959). During incubation of the organisms under these conditions there is a rise in the activity of enzymes required in the early stages of bacteriochlorophyll synthesis, namely δ -aminolaevulate synthetase and δ -aminolaevulate dehydratase; this increase precedes bacteriochlorophyll formation, suggesting