The Influence of Age, Sex, Pregnancy, Starvation, and Other Factors on the Cytoplasmic Ribonucleoproteins of Rat Liver*‡

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ABSTRACT

Rat liver was homogenized in 0.88 M sucrose. The DNA and total RNA were determined, and the homogenate was fractionated by differential centrifugation. The pellets obtained between 30 minutes at 20,000 *g* and 180 minutes at 105,000 *g* were analyzed for RNA and nitrogen. The ribonucleoproteins were determined in the analytical ultracentrifuge. The non-pellet RNA was calculated by difference. The results are reported as amounts per 6.7×10^{-9} mg. of DNA.

In young, growing male rats the amounts of microsomal protein and ribonucleoprotein B (83S) increased with age. Non-pregnant adult females showed less non-pellet RNA and much more ribonucleoprotein C (63S) than did adult males.

During pregnancy both of these cell constituents reverted to levels characteristic for male animals. Starvation for 5 days resulted in a reduction in the mass of liver tissue, the non-pellet RNA, the microsomal protein, and ribonucleoproteins B and C. During recovery from starvation the return of the liver to normal paralleled the rate at which body weight was restored. Treatment with cortisone, 25 mg. per rat per day for 5 days, caused an increase in microsomal protein and a decrease in ribonucleoprotein B. Treatment with 6-mercaptopurine, 50 mg. per kilo per day for 5 days, caused little change in liver composition in either males or females.

Although the RNA content of rat liver in a variety of physiological and pathological conditions has been the subject of extensive investigation (1, 2), relatively little is known about the degree to which such conditions modify the relative proportions of the various RNA-containing components. Earlier work in this laboratory has demonstrated changes in the ultracentrifugally determined ribonucleoprotein components (RNP) of the cytoplasm in regenerating liver (3) and azo-dye–induced liver tumors (4). In the control animals an unexpected correlation was observed between the concentration of the principal RNP component, B, and the average cell weight. A variation of 40 per cent in cell weight was accompanied by a variation of 100 per cent in the amount of B per cell (4). These observations suggested that an extension of these experiments might prove profitable. In the present paper the effects of age, sex, pregnancy, starvation, and treatment with cortisone and 6-mercaptopurine are described.

Since the increase in RNP concentration in the larger cells might be related to an increase in polyploidy, the deoxyribonucleic acid (DNA) was determined. The total ribonucleic acid (RNA) was also measured. A cytoplasmic fraction was prepared by differential centrifugation as described under Methods. The pellets included the usual microsomal fraction, which contains fragments of the endoplasmic reticulum with RNP

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particles attached to them (5); they also included the "free" RNP particles, which many workers separate either in a postmicrosomal or in a cell sap fraction (4).

Materials and Methods

The experiments were carried out on male and female Wistar rats maintained on a stock diet of Purina chow.

Each experiment required 9 gm. of liver pulp. With rats that weighed over 70 gm. three livers were pooled for each experiment, while with smaller animals four to six livers were needed. The livers were perfused with saline, removed, chilled, and homogenized (4). Nuclear counts (4) and DNA and RNA determinations (6, 7) were made on the whole homogenate. The nuclei and mitochondria were discarded, and the supernatant, containing the microsomes and free RNP, was centrifuged at 105,000 *g* for 3 hours. The pellets were resuspended in veronal-chloride buffer, ionic strength 0.10 and pH 8.5, and samples were examined in the analytical ultracentrifuge. The pellet nitrogen was determined and the concentration of microsomal protein, exclusive of nucleoprotein, was calculated (4). The pellet RNA was also measured. The analytical values obtained on the pellets were corrected for the percentage of unbroken cells (4). This correction ranged from 4 to 10 per cent. The rest of the liver RNA was estimated by subtracting the pellet RNA from the total and was designated 'non-pellet" RNA. This includes nuclear RNA, mitochondrial RNA, microsomal RNA carried down with the mitochondria, and the final supernatant RNA.

RESULTS AND DISCUSSION

Typical ultracentrifugal patterns obtained on adult male rat liver are shown in Fig. 1. They are very similar to the patterns found in Sherman rats (4). The right-hand picture is incomplete, because of turbidity in the lower half of the cell. The large spreading boundary, M, is caused by the sedimentation of glycogen and microsomal protein. The very sharp boundaries represent the RNP. The middle picture was taken 10 minutes later. The M boundary has disappeared, and the RNP boundaries, A, (120S), B, (83S), C, (63S), and E, (47S), have begun to separate from one another. In the left-hand picture, the A boundary has disappeared, and the RNP boundaries have separated farther. The concentrations of A, B, C, and E were calculated from the areas under the respective curves (3). The concentration of A was usually low, less than 10 per cent that of B. Since A seems to consist of two B particles in association, the sum of A and B has been reported as the total concentration of B (4).

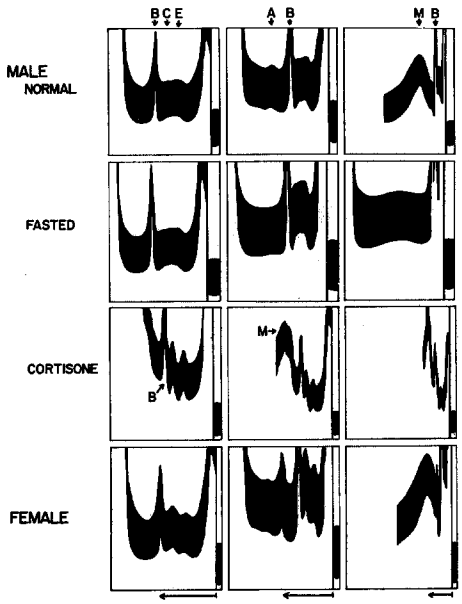

FIG. 1. Ultracentrifugal patterns of liver microsomes and ribonucleoproteins. M, microsomal protein and glycogen; A, B, C, and E are nucleoprotein boundaries. From right to left the pictures were taken at about 4, 12, and 20 minutes after reaching full speed, which was 37,020 R.P.M.

Since the wet weight of a tissue is much too variable to serve as a satisfactory standard of reference in reporting the results of tissue analyses (2, 8–10), nuclear counts were made, with the aim of presenting the results in terms of the average composition per cell (8). When the DNA per nucleus was calculated, however, the results were extremely variable, ranging from the usual diploid amount of DNA, 6.7×10^{-9} mg. per nucleus (11), to values three times as high. While these values gave some indication of the increase in polyploidy with age, it seemed that the nuclear counts were not sufficiently reliable to be used as a basis for reporting the analytical results. The results of these experiments have therefore been reported as amounts per unit of DNA (2). The unit of DNA has been taken as 6.7×10^{-9} mg., the amount per diploid cell in the rat (11). This is equivalent to reporting the amounts per diploid set of chromosomes. The tissue mass per diploid amount of DNA is the mass of a hypothetical liver cell plus its share of extracellular tissue material; it represents the average of the whole cell population, including hepatic parenchymal cells, bile duct cells, etc. Any change in this figure presumably

TABLE I

The Effect of Age, Sex, Pregnancy, and Treatment with 6-Mercaptopurine on the Composition of Rat Liver

Animals	Body weight	No. of experiments	Liver composition in mg. × 10^{-9} per diploid amount of DNA								Pellet RNA / RNP
			Tissue mass	RNA			RNP			Microsomal protein	
				Total	Pellet	Non-pellet	B	C	E		
	gm.										*per cent*
Normal male	51	5	2,380	14.5	3.8	10.7	6.1	1.9	1.8	31	38
	114	4	2,440	14.3	4.3	10.0	6.9	1.7	2.1	39	42
	181	4	2,910	17.4	4.5	12.9	8.2	1.7	2.0	49	39
	254	5	2,940	17.6	5.8	11.8	10.2	2.1	2.2	61	39
Male, 6-MP–treated	213*	1	2,390	18.4	5.2	13.2	9.4	1.4	2.1	49	40
Normal female	241	5	2,580	13.6	6.3	7.3	8.5	3.4	2.1	57	43
Pregnant female	375	5	2,310	19.6	6.8	12.8	7.1	2.0	2.7	57	60
Female, 6-MP–treated	214‡	1	2,390	14.5	4.1	10.4	6.3	2.6	2.6	42	36

* Weight loss, 17 gm.

‡ Weight loss, 6 gm.

reflects a change in the mass of the hepatic parenchymal cells.

The Effect of Age.—The male rats fell into four groups, according to body weight. The mean weights and approximate ages were, 51 gm., about 4 weeks old; 114 gm., 6 weeks; 181 gm., 8 weeks; and 254 gm., 15 weeks. The life span of these animals is 2 to 3 years. From Table I it is apparent that there was a tendency for older and heavier animals to have a greater tissue mass and proportionately greater RNA content than younger and lighter animals. This difference was more marked in the pellet RNA than in the non-pellet RNA. Examination of the three RNP components of the pellet showed that component B was chiefly responsible for the increase in the pellet RNA, components C and E showing very little change. The increase in the pellet RNA was accompanied by a proportionate increase in microsomal protein. The ratio of pellet RNA to RNP remained constant, near 40 per cent, the value found for purified RNP (12). The period of growth covered by these experiments was accompanied by an increase in polyploidy so that although the amount of nucleoproteins C and E did not change with age, the average amounts per cell increased in proportion to the increased polyploidy.

Effects of Sex and Pregnancy.—The ultracentrifugal pattern of female liver is shown in Fig. 1. The principal differences from the normal male pattern are in the increased size of the A and C boundaries. The differences in composition between the livers of adult male and female rats are shown in Table I. Tissue mass was slightly lower in the female, and total RNA was definitely reduced. The amounts of pellet RNA, of components B and E of the RNP, and of microsomal protein were the same for both sexes, as was the ratio of pellet RNA to RNP. Component C of the RNP was almost twice as high in the female as in the male. On the other hand, non-pellet RNA was about 40 per cent lower in the female.

The pregnant animals were studied on the 18th day, when the increase in the liver RNA should be maximal (13, 14). The results are shown in Table I. Pregnancy had tended to abolish some of the differences between female and male liver. Total RNA and non-pellet RNA were increased to about the male level. This increase in non-pellet RNA must have been due to an increase in nuclear and mitochondrial RNA, since the RNA in the final supernatant was the same, 2×10^{-9} mg., in both pregnant and non-pregnant females. Component C of the RNP also fell to the male level. The amounts of components B and E of the RNP, and of microsomal protein, which are the same for both sexes, were unaffected by pregnancy, and the pellet RNA was increased about 10 per cent. Pregnancy did, however, increase the ratio of pellet RNA to RNP to a value half as great again as that found in males and non-pregnant females.

Effects of Starvation.—The results of a study on the effects of starvation are shown in Table II. Twelve rats were fasted for 5 days, with water

TABLE II

The Effect of Starvation and of Treatment with Cortisone on the Composition of the Liver in the Adult Male Rat

Animals	Body weight		No. of experiments	Liver composition in mg. × 10^{-3} per diploid amount of DNA								Pellet RNA / RNP
	Initial	Final		Tissue mass	RNA			RNP			Microsomal protein	
					Total	Pellet	Non-pellet	B	C	E		
	gm.	*gm.*										*per cent*
Normal control	—	181	4	2,910	17.4	4.5	12.9	8.2	1.7	2.0	49	39
Fasted 5 days	195	137	1	1,680	7.7	4.4	3.3	5.0	1.1	2.0	27	54
Fasted 5 days, fed 2 days	208	190*	1	3,350	14.1	6.7	7.4	11.6	2.6	3.2	53	39
Fasted 5 days, fed 5 days	184	138‡	1	2,680	14.7	6.5	8.2	6.6	1.7	2.2	28	62
Treated with cortisone	160	151	1	3,350	21.1	6.1	15.0	5.8	2.4	1.3	76	64

* Weight after fast, 153 gm.

‡ Weight after fast, 125 gm.

ad libitum. Three died. Of the survivors, the three that appeared to be in the best physical condition were sacrificed immediately; the second best three were given the stock diet *ad libitum* for 2 days before being sacrificed; and the three in the worst condition were maintained on the stock diet for 5 days before being sacrificed.

The animals sacrificed at the end of the 5 day fast showed a sharply diminished tissue mass and total RNA. The fall in RNA was limited to the non-pellet RNA, which was only 25 per cent of the normal value, whereas the pellet RNA was apparently unaffected. The ultracentrifugal pattern (Fig. 1) showed very little turbidity. The M boundary was very small, and the amount of material that piled up on the bottom of the cell was greatly reduced. The A boundary had disappeared. Components B and C were reduced (see also Table II). The amount of microsomal protein was only half the normal value, whereas the ratio of pellet RNA to RNP was substantially above the control level.

Electron micrographs of the hepatic parenchymal cells of fasting rats show decreased amounts of both endoplasmic reticulum (15, 16) and nucleoprotein granules (16). Since the endoplasmic reticulum accounts for most of the microsomal protein found on fractionation of homogenates (5), the results of electron microscopy agree with the decreases in microsomal protein and nucleoprotein reported in this paper.

The animals which were fed for 2 days after being fasted recovered about two-thirds of the body weight which they had lost during the fast. This recovery of body weight was accompanied by a partial return to normal in the composition of the liver. Thus the tissue mass had returned to the normal value and the total RNA had risen to 80 per cent of the control level. The non-pellet RNA, however, was still only 60 per cent of the control value, while the pellet RNA was 50 per cent above the normal figure and all the RNP components were increased proportionately. The ultracentrifugal pattern showed a restoration of the turbidity, the M boundary, and the A boundary. The microsomal protein and the ratio of pellet RNA to RNP had returned to normal.

The group of animals fed for 5 days after the fast were still in poor condition and had recovered only 25 per cent of the body weight they had lost. Nonetheless, as in the preceding group, the tissue mass and the total RNA had returned to about the control levels, and the ultracentrifugal pattern had returned to normal. The RNP components, however, had not increased above normal, and the microsomal protein and the ratio of RNA to RNP were still at the fasting levels. It would therefore appear that recovery from starvation was less complete than in the preceding group. The composition of rat liver during recovery from starvation has been described in detail (17).

Effects of Cortisone.—Table II also shows the effect of cortisone on the composition of the liver. The hormone was given intraperitoneally at a level of 25 mg. per rat per day for 5 consecutive days, the animals being sacrificed on the 6th day (18).

The treatment caused slight increases in the tissue mass, and in both the pellet and non-pellet RNA. Of the RNP components, B and E were somewhat reduced while C was elevated. The microsomal protein was 50 per cent above the control level, and the ultracentrifugal patterns (Fig. 1) showed a great increase in the M boundary and in the amount of material piled up on the bottom of the cell. The ratio of RNA to RNP was half as great again as in normal liver.

The cortisone experiment was undertaken because of the early report that after cortisone treatment the RNA was found chiefly in the final supernatant of rat liver fractionated in saline (18). In a preliminary experiment in this laboratory the livers of cortisone-treated rats were separated into nuclear, mitochondrial, microsomal, and supernatant fractions in 0.88 M sucrose. No abnormality in RNA distribution was observed; only 9 per cent of the RNA remained in the final supernatant after 2 hours at 150,000 *g*. It has recently been reported (19) that cortisone caused no change in the total RNA of the whole liver. The RNA and nitrogen in the microsomal and ultracentrifugal fractions obtained in 0.25 M sucrose decreased somewhat, but the supernatant RNA was less than 10 per cent of the total. On the basis of stability studies, the authors suggested that there were at least two structurally distinct types of polymerized RNA in the liver, and that the second variety, which does not stain with basic dyes, accumulated in cortisone-treated animals. Such a hypothesis might possibly explain the increase in the ratio of pellet RNA to nucleoprotein found in this laboratory, in male animals treated with cortisone or fasted, or in pregnant females; but the alternative, that the nucleoproteins of these animals were less stable *in vitro*, must first be eliminated. No unusual breakdown to small fragments occurred prior to preparative ultracentrifugation, since the RNA was found in the pellets and the supernatant RNA was not elevated. When the pellets were resuspended in veronal-chloride buffer the nucleoprotein bound to the endoplasmic reticulum was freed. In preparations from normal male liver this release appeared to be fairly complete, since the ratio of total RNA, as chemically determined, to free RNP, as measured in the ultracentrifuge, was close to 40 per cent, the value found for purified RNP (12). When the ratio of pellet RNA to free RNP was high the release of RNP may have been incomplete, or the released RNP may have aggregated or dissociated into forms which would not be recorded in the ultracentrifugal analyses. There exists a further possibility, that the RNP from these livers might have a higher RNA content than RNP from normal male rat liver. This seems most unlikely, since no difference has ever been observed in its sedimentation behavior or other physical properties.

Two laboratories (20, 21) have recently described microsomal subfractions which were rich in RNA but showed relatively few dense 150 A granules on electron micrography. Whether these findings also represent RNA that was originally in some form other than the macromolecular ribonucleoproteins, or merely reflect the instability of these substances, remains to be decided.

The marked decrease in non-pellet RNA found after starvation may also represent a change in some other type of RNA; but since the microsomal protein and non-pellet RNA rose and fell in a roughly parallel fashion, the possibility exists that the primary change was a decrease in the amount of endoplasmic reticulum, which resulted in a decrease in the amount of particulate ribonucleoprotein trapped in the mitochondrial fraction.

Effects of 6-Mercaptopurine.—Table I shows the effects of 6-mercaptopurine on the livers of both male and female rats. The drug was injected intraperitoneally at a level of 50 mg. per kilo per day for 5 consecutive days, the animals being sacrificed on the 6th day. This dose is well below the acutely lethal level, though still quite toxic (22, 23). No consistent effects were found. In the males the only change was a decrease in component C of the RNP. In the females this component was relatively low, but still within the normal range; instead there was a fall in component B and a rise in non-pellet RNA. These observations are in agreement with the view that 6-mercaptopurine exerts its toxic action by interfering with some metabolic activity other than nucleic acid synthesis (24).

The variations in the RNA content of the liver associated with such factors as age, sex, and nutritional status represent the sum of complex variations in the different RNA-containing components of the tissue. In view of the role played by the RNP in protein synthesis (25), two generalizations may be made.

1. The distinctive pattern given by the livers

of young and growing animals, in which component B of the RNP, unlike components C and E, has not yet reached the adult level, may perhaps be related to the hypothesis (4) that components C and E are concerned with synthesis of the proteins of the liver itself, whereas component B is concerned with the synthesis of protein for export.

2. The differences in liver composition between the sexes and the fact that these differences are abolished in pregnancy might indicate that the liver of the non-pregnant female has a capacity for further growth which is only realized during pregnancy. The liver of the male, which does not have to cope with such an additional load, may be regarded as already fully developed and lacking in this special potentiality.

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