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Hormones and Liver Cytoplasm

4. RIBONUCLEOTIDES, RIBONUCLEIC ACID SYNTHESIS AND PROTEIN SYNTHESIS AFTER ADRENALECTOMY*

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After adrenalectomy, the supernatant fraction obtained from rat liver by differential centrifuging shows increases in the amount of ribonucleic acid (RNA) (Reid, 1955) and in the incorporation of injected [6-¹⁴C]orotic acid into the RNA (Reid, O'Neal, Stevens & Burnop, 1956). On the other hand, the microsomal fraction shows a decreased incorporation of orotic acid. This decrease is in accordance with the postulated role of microsomal RNA in protein synthesis, the rate of which as judged by the incorporation of injected DL-[1-¹⁴C]-leucine appears to be slightly diminished after adrenalectomy (Reid *et al.* 1956). No attempt was made in these studies to ascertain whether adrenalectomy affected the precursor 'pool' or the time course of incorporation. It was not therefore conclusively proved that the increase in the amount of RNA in the supernatant fraction is due to increased synthesis as distinct from decreased catabolism or that this RNA has some function unconnected with protein synthesis.

A closer study has now been made in an endeavour to clarify not only these aspects but also the origin, the fate and the possible metabolic diversity of the RNA of the supernatant fraction.

Liver ribonucleotides have also been investigated, to ascertain whether adrenalectomy affects the ribonucleotide pool and, in particular, the pool of the uridine phosphates into which injected orotic acid is rapidly converted (Hurlbert & Potter, 1952, 1954; Schmitz, Hurlbert & Potter, 1954).

Another aspect which has now been studied, as briefly reported elsewhere (Reid & Stevens, 1956), is the sequence after adrenalectomy of the various changes in the metabolism of RNA and of protein.

* Part 3: Stevens & Reid (1956).

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EXPERIMENTAL

Experimental procedures are described in detail only where these differ from those employed in Part 1 (Reid *et al.* 1956). As previously, the animals were male rats, wt. about 250 g., which had been fed on a protein-rich diet in restricted amount and which were fasted overnight before autopsy. Adrenalectomized rats were maintained with saline.

Chromatography of ribonucleotides

Each experiment was performed with pooled liver samples (total 10–11 g.) from two rats. In the later experiments, as indicated in Table 1, the rats were killed by decapitation, and the liver was ground to a powder in the frozen state (liquid nitrogen) before extraction with perchloric acid solution, as described by Hurlbert & Potter (1954) and Hurlbert, Schmitz, Brumm & Potter (1954) but without perfusion of the liver.

Analysis of the neutralized extracts was performed by anion-exchange chromatography on Dowex 1 (formate) columns, by a gradient-elution procedure with three changes of solvent as described by Hurlbert *et al.* (1954). The pH of the solvents was not adjusted. The mixing flask (containing water initially) was of 500 ml. volume, and the resin column 15 cm. × 0.72 cm.² in size; with a longer column (20 cm.) the resolution was little improved. The effluent was collected in 5 ml. portions (about 300 in each run) which were examined spectrophotometrically at 260 mμ and, in some instances, at 275 mμ. In general, each run was carried out for 3 days without interruption of the flow, the elution pattern being distorted after any interruption. In experiments with radioactive material, performed in a distant laboratory with consequent difficulty in supervision, the runs were shortened by using only two solvents (4N-formic acid followed by 4N-formic acid containing M-ammonium formate), with resulting loss of resolution in the later stages (Expt. 2 of Table 1).

Some of the fractions thus obtained were rechromatographed after removal of water and formic acid by freeze-drying; if the fraction also contained ammonium formate, this was removed by the cautious use of an infrared lamp in

the final stage of drying as in the experiments of Hurlbert *et al.* (1954) with the aid of a mercury-diffusion pump to achieve a higher vacuum. Rechromatography of heterogeneous fractions was carried out by gradient elution with ammonium formate solutions, as described by Hurlbert *et al.* (1954); here the mixing flask was of 250 ml. vol., and the resin column 10 cm $\times$ 0.72 cm.² in size. With careful drying, about 80% of the ultraviolet light-absorbing material eluted in the initial chromatograms could be recovered after the rechromatography.

Identification of the nucleotides in the various peaks was based on the data of Hurlbert *et al.* (1954), and was confirmed by chromatographic data obtained with authentic nucleotides (Sigma Chemical Co., St Louis, Mo., U.S.A.) and by examination of the peaks with respect to the ratio of the extinctions at 275 and 260 μ . The chromatograms of the crude tissue extracts showed certain minor qualitative divergences from the data of Hurlbert *et al.* Triphosphopyridine nucleotide (TPN) ran ahead of, rather than with, guanosine 5'-phosphate (GMP). Inosine 5'-phosphate (IMP) and uridine 5'-phosphate (UMP) were not separable without rechromatography (even with the authentic nucleotides in place of the tissue extract), but uridine 5'-pyrophosphate glucose (UDPglucose) was obtained free of guanosine 5'-pyrophosphate (GDP); in these respects the present findings accord with those of Schmitz, Potter, Hurlbert & White (1954). Guanosine triphosphate (GTP) merged with uridine triphosphate (UTP), and UDP and its glucuronic acid derivative merged with adenosine triphosphate (ATP).

Diphosphopyridine nucleotide (DPN) and its reduced form (DPNH) were estimated in freshly excised liver samples by the use of alcohol dehydrogenase according to Spirtes & Eichel (1954). The rats were killed by decapitation, the values tending to be lower if Nembutal (pentobarbital sodium) was used.

Isotopic studies

The number of rats used in each experiment is stated in the legends to the figures; in general each point is derived from data for two to four rats. Adrenalectomized rats were always studied simultaneously with control rats (intact or sham-operated) because of possible day-to-day variations in environment or technique.

In some experiments the interval between adrenalectomy and injection of isotope was deliberately varied. Since these experiments, which are described below, showed that the effects of adrenalectomy were quantitatively dependent on the length of this interval, it was necessary to standardize this where other variables were to be investigated. The length of the interval was 12–15 days unless otherwise stated.

Isolation of fractions. As in the previous study (Reid *et al.* 1956), homogenates of the liver samples removed at autopsy were centrifuged to give nuclear and cytoplasmic fractions.

Further centrifuging of the cytoplasmic fraction gave a mitochondrial fraction (with 'fluffy layer'), which was discarded, a microsomal fraction and a supernatant fraction, i.e. the material un sedimented after 90 min. at 14 500 *g*. In some experiments the latter fraction was centrifuged for 60 min. at 145 000 *g* to sediment an ultracentrifugal fraction.

In some instances supernatant fractions were further fractionated by procedures other than ultracentrifuging; here each point on the figures was obtained not with several

supernatant fractions, but usually with a pooled fraction from a pair of rats which had been similarly treated. Ammonium sulphate precipitation was carried out by addition of saturated (NH₄)₂SO₄ solution at 0–3°, with high-speed centrifuging (10 min. at 14 500 *g*) so as to give a clear supernatant and a compact sediment, which was not washed. Precipitation with Ca²⁺ ions (Schneider, 1946), by addition of CaCl₂ solution to a final concentration of 0.1% CaCl₂, was carried out at room temperature; high-speed centrifuging was not necessary.

As previously (Reid *et al.* 1956), RNA was estimated from the extinction at 260 μ of acidified alkaline digests. The validity of this procedure, as applied to subfractions separated from the supernatant fraction, was confirmed by analyses for RNA phosphate.

In preparing a powder for measurement of radioactivity, the initial removal of acid-soluble and lipid constituents from tissue samples was usually performed with perchloric acid solution followed by treatment at room temperature with ethanol-ether-chloroform (2:2:1, by vol.), as in the experiments of Littlefield, Keller, Gross & Zamecnik (1955). The use of hot ethanolic solvents, as in the previous study (Reid *et al.* 1956), was less convenient and, moreover, might have resulted in some loss of protein (Korner & Debro, 1956). Trichloroacetic acid solution was used in place of perchloric acid solution when it was intended to measure the acid-soluble radioactivity (see below) or when nuclear preparations were being treated; the latter tended to become fibrous if treated with perchloric acid.

Plating of liquid samples. Liquid samples were dried and counted at slightly less than infinite thickness, with a standard amount of carrier sufficient to minimize differences due to any variations in the amount of tissue solid. Hurlbert & Potter (1954) used ammonium trichloroacetate for this purpose; but with this alone, erratic results due to uneven drying have now been encountered, even with nickel planchets in place of polyethylene ones. With BaSO₄ as an additional constituent, as described below, duplicates seldom differed by more than 5%.

With cytoplasmic fractions, the tissue extracts, containing 7% (w/v) trichloroacetic acid, were prepared for counting by pipetting 0.1 ml. into the well of a polyethylene planchet (2 cm.²), followed by 0.13 ml. of 0.5N-Ba(OH)₂ solution and 0.013 ml. of 2N-H₂SO₄ solution. Finally an excess of aq. NH₃ soln. (sp.gr. 0.88) was added, with stirring so as to disperse the precipitate of BaSO₄. The contents of the planchet were carefully taken to dryness by exposure for about 3 hr. to an infrared lamp.

With fractions obtained by anion-exchange chromatography in the formate system, ammonium formate was used as carrier in place of trichloroacetic acid. To 0.15 ml. of the effluent, sufficient 90% (w/w) formic acid solution was added to bring the total amount of formic acid present to 1.15 m-moles (e.g. 0.05 ml. added if none already present; 0.04 ml. was added to effluents containing UMP). After addition of 0.05 ml. of 0.5N-Ba(OH)₂ solution and 0.02 ml. of 2N-H₂SO₄ solution, excess of aq. NH₃ soln. was added with stirring. Even with very careful drying, so as to minimize sublimation of the ammonium formate, the results were less reproducible than with ammonium trichloroacetate as carrier, and it was essential to run several replicates.

Injection of isotope. Injections were given intraperitoneally unless otherwise stated. In general, the dosage chosen was the lowest amount compatible with accuracy in the

final counting, this amount being in the range 2–8 μC , with orotic acid as precursor and with an interval exceeding 1 hr. between injection and autopsy. With a long interval (20 or 30 hr.), a comparison of different doses within the range 0.5–8 μC indicated that the percentage recovery in the RNA of microsomal and supernatant fractions decreased somewhat as the dose was increased. However, the decrease was not marked in relation to day-to-day variations in percentage recovery, and moreover the dosage within each group was the same for adrenalectomized rats as for the controls. Accordingly, no attempt has been made to correct for differences in dosage from one experiment to another. In experiments with an interval of only 40 min. or 1 hr., high doses of orotic acid (up to 40 μC , i.e. 6.3 mg. of orotic acid) were employed so as to ensure measurable labelling of microsomal RNA and of uridine compounds in the acid-soluble fraction; in some of these experiments the dose may have approached 'saturation level'.

Since the incorporation of orotic acid at short time intervals was low but accurately measurable, logarithmic axes have been adopted in plotting the results. These are considered in terms of percentage recovery of injected isotope except where their assessment in terms of specific activity is advantageous; the amounts and specific activities of RNA pyrimidines were not actually determined. It appeared appropriate in experiments requiring a standardized interval to adopt an interval of 135 min. after injection of orotic acid, as previously (Reid *et al.* 1956).

In experiments with leucine the standardized interval was 90 min. Data obtained at shorter time intervals (15 and 40 min.) are also given below: here L-leucine (generally labelled with ^{14}C ; Radiochemical Centre, Amersham) was employed in place of DL-[1- ^{14}C]leucine, usually at a dosage of 0.063 mg. equivalent to 6 μC .

Injection of unlabelled orotic acid. Intact rats were given massive doses of orotic acid (Genatosan Ltd., Loughborough, Leics.) to ascertain the effect upon liver RNA levels. The compound was given as an almost saturated solution in water at 37° and at pH 8.5 (3 mg./ml.); 5 ml. was injected intraperitoneally, followed by four similar injections at hourly intervals, and the rats were killed 2 hr. after the last injection. Control rats were given water containing a trace of sodium bicarbonate.

RESULTS

Liver ribonucleotides

Effects of improvements in technique. Since Hurlbert & Potter (1954) and Hurlbert *et al.* (1954) do not emphasize that replication of their results depends on scrupulous adherence to their techniques, as became evident during the present experiments, the data for control rats (Tables 1 and 2) may usefully be considered from this aspect before the effects of adrenalectomy are discussed. With practice in techniques it became possible to treat the tissue more expeditiously before extraction and, in particular (Expts. 4c *et seq.*), to grind the frozen tissue to a powder before extraction. As is evident from the data for control rats, in the earlier experiments there were post-mortem changes, namely decreases in the levels of triphosphates (ATP, UTP,

GTP) and of certain diphosphates and their derivatives [adenosine diphosphate (ADP), UDPglucose, UDPglucuronic acid and UDP] together with increases in the levels of certain monophosphates [adenosine monophosphate (AMP) and GMP] and possibly of TPN. On the other hand, the levels of DPN, of UMP and of UDPacetylglucosamine were remarkably constant throughout. In general, the patterns obtained in the final experiments quantitatively resembled those of Hurlbert *et al.* (1954) and of Schmitz *et al.* (1954).

At the outset of the study it was hoped that nucleotide levels could be determined in individual fractions isolated from sucrose homogenates. However, it soon became evident that considerable breakdown of nucleotides occurs in the course of separation of nuclei and mitochondria. This conclusion is, of course, in accord with the later findings described above.

Effects of adrenalectomy. There was a striking decrease in the amounts of uridine nucleotides 2–3 weeks after adrenalectomy (Tables 1 and 2). Rechromatography of fraction (5) of Table 1 showed no change in IMP but a highly significant decrease in UMP ($P < 0.5\%$; Table 2). The results for the labile compound UDPglucose (and possibly those for ADP) suggest that adrenalectomy may decrease its level *in vivo* but minimize its breakdown *post mortem*. The results for UDP, UDPglucuronic acid and UTP (Table 2) indicate marked decreases after adrenalectomy; here there is the difficulty that ribonucleoside diphosphates and their derivatives may undergo some breakdown after the initial isolation (Hurlbert *et al.* 1954). However, the total recovery of uridine nucleotides from fraction (11) showed a significant decrease ($P < 5\%$) after adrenalectomy (Table 2). In general, the decreases in the various uridine nucleotides of the liver appeared to exceed 50% except with UDPacetylglucosamine (29% decrease) and UDPglucose.

The results for UMP and UDPacetylglucosamine in control rats are very consistent, and the single experiment with adrenalectomized rats 3 days after operation permits the conclusion that these compounds are undiminished after only 3 days; it also appears that the amount of UDPglucose is undiminished or even elevated.

The marked decrease in the uridine nucleotide pool is not reflected in the results for adenosine and guanosine nucleotides, with the possible exception of the triphosphates. Indeed, the results for AMP, the amount of which was affected by changes in technique, showed a small but significant increase (7%; $P < 5\%$) when comparison was made between fractions from adrenalectomized rats and fractions from controls studied under similar conditions. No conclusions could be drawn as to cytidine nucleotides because of analytical difficulties.

Table 1. *Chromatography of ribonucleotides in perchloric acid extracts of rat liver*

Control and adrenalectomized rats were studied alternately as indicated by the Expt. nos. In series 1 the rats were killed with Nembutal instead of by decapitation. In series 1-3 the liver was macerated while in a semi-thawed state before extraction, whereas in series 4 and 5 the liver was ground to a powder while frozen (liquid nitrogen), the time between decapitation and freezing of the liver being particularly short (1 min.) in series 5. In view of these improvements in technique, the effects of which are discussed in the text, each run with adrenalectomized rats is, in general, comparable only with the corresponding control run. All quantities refer to an amount of liver corresponding to 100 g. body wt., and are expressed in arbitrary units, namely the calculated optical density (at 260 m μ with water as blank and 1 cm. light path) for the material in the fraction if present in only 1 ml. volume. (Similar units are conventionally used to express the molar extinction coefficients of pure compounds.) Abbreviations used: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; MP, monophosphate; DP, diphosphate; TP, triphosphate; A, adenosine; G, guanosine; I, inosine; C, cytidine; U, uridine. (Thus UDP denotes uridine 5'-pyrophosphate.) These abbreviations, and those for unidentified compounds ('C-3', 'J-2', etc.), are identical with those of Hurlbert *et al.* (1954); 'post-ADP' is a peak immediately following ADP, which was not labelled by these authors. For each fraction, minor constituents are italicized and trace constituents are ignored.

Fraction no.	...	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)*	(12)*	(13)
Expt. no.		DPN, CMP	AMP	TPN	GMP	UMP, 'C-3', IMP, GDP, 'C-4'	Control rats	'Post- ADP'	UDP- acetylglu- cosamine	UDP- glucose	GDP, 'G-2', 'G-3', 'G-4'	UDP- glucuronic acid, UDP, CTP	ATP, 'J-3', 'J-2'	UTP, GTP, 'K-1'
1c		24.4	62.7	6.5	12.0	31.8	22.0	9.6	10.2	7.8	18.4	21.8	—	7.9
2c		23.9	66.8	4.5	11.7	32.3	25.4	—	14.1	—	15.7	16.1	—	—
3c		28.0	73.9	6.4	13.2	35.0	34.5	8.3	11.6	12.9	17.7	8.7	12.2	3.7
4c		23.0	49.5	4.8	8.1	36.8	60.4	9.7	13.5	19.8	22.2	23.6	22.8	14.6
5c		22.5	43.0	3.2	5.0	20.6	51.1	6.0	11.0	ca. 17	—	35.8	23.7	12.2
1a		—	66.6	7.1	11.1	25.4	34.9	14.1	9.3	13.5	19.9	8.1	11.8	7.3
2a		27.8	68.0	6.1	10.6	40.5	30.4	—	29.7	—	11.5	31.0	—	—
3a		33.4	76.8	7.7	13.3	27.4	36.0	10.5	8.9	14.8	—	—	—	—
{4a,		21.4	57.0	2.8	8.1	22.4	—	—	—	—	—	—	—	—
{4a',		25.0	56.8	3.0	7.4	19.5	42.1	4.9	7.0	12.5	19.6	15.3	10.5	10.3
{5a		25.2	45.4	4.2	6.7	25.2	48.8	7.6	8.0	14.7	23.2	36.8	—	9.9
{5a''		—	—	—	—	—	—	—	7.7	12.7	—	17.5	14.1	8.1
5a'		—	—	—	—	28.4	—	—	13.6	34.0	—	—	—	—

* These fractions were never well separated, and the values given in some instances for the two fractions separately may not be individually accurate.

Diphosphopyridine and reduced diphosphopyridine nucleotides. Separate analyses for these compounds were performed with five pairs of rats, each pair consisting of an intact rat and a rat which had been adrenalectomized 2–3 weeks previously. The mean values for DPN and DPNH in the controls were 1.06 and 0.46 mg./100 g. body wt. respectively (1 mg. of DPN is equivalent to 27.7 'units' as defined in Table 1). The mean changes (with standard error) after adrenalectomy were $+0.04 \pm 0.142$ and -0.05 ± 0.072 respectively; these changes are insignificant.

Incorporation of orotic acid in relation to time after injection of isotope

In presenting their data for the time course of labelling of the various RNA fractions, Hurlbert & Potter (1954) remark: 'The data do not offer an accurate kinetic record because too few animals were used to offset the large variations in utilization of the dose in these experiments. The reason for these variations has not been determined....' The more extensive data now obtained show not only quite consistent changes with time in spite of day-to-day variations, but also good agreement with the data given by Hurlbert & Potter (1952, 1954) for the

labelling of microsomal, supernatant, nuclear and acid-soluble fractions analogous to those now prepared.

Microsomal and supernatant fractions. In control rats (Fig. 1), maximal labelling occurred at about 30 hr. with the supernatant fraction, and at about 40 hr. with the microsomal fraction, the labelling of the latter being low in the first few hours after the injection. The labelling of both fractions remained high for at least the next 3 days.

If the RNA of the supernatant fraction were the precursor of microsomal RNA, the descending portion of the specific-activity curve for the former should intersect that for the latter at its maximum (Zilversmit, Entenman & Fishler, 1943). The data for control rats in Fig. 1 are compatible with this possibility, the curves for percentage recovery being also a rough measure of specific activity since microsomal and supernatant fractions as now prepared contain similar amounts of RNA (about 8 mg./100 g. body wt.; cf. Reid, 1956; Reid *et al.* 1956).^{*} Consideration is given below to the apparent plateau in the ascending portion of the curve for the supernatant fraction.

^{*} In Table 3 of Part 3 (Stevens & Reid, 1956), the values refer, not to RNA, as incorrectly stated, but to RNAP.

Table 2. *Rechromatography of heterogeneous ribonucleotide fractions*

See Table 1 for abbreviations and for method of expressing results. Fraction nos. are those indicated in Table 1.

Fraction no.	Expt. no.	UMP	IMP	UDP	UDP-glucuronic acid, CTP	UTP	GDP	GTP
Control rats								
(5)	3c	9.0	5.4	—	—	—	—	—
	4c	12.9	8.5	—	—	—	—	—
	5c	9.5	4.5	—	—	—	—	—
	Adrenalectomized rats (2–3 weeks after operation)							
	3a	5.2	4.6	—	—	—	—	—
	4a	4.6	6.5	—	—	—	—	—
	4a'	4.7	4.7	—	—	—	—	—
	5a	4.2	8.2	—	—	—	—	—
	Adrenalectomized rats (3 days after operation)							
	5a'	12.2	—	—	—	—	—	—
Control rats								
(11)	3c*	1.3	—	0.2	6.0	—	—	—
	4c	0.9	—	2.0	11.7	—	—	—
	5c	0.4	—	2.5	17.5	—	—	—
	Adrenalectomized rats (2–3 weeks after operation)							
	4a'	0	—	0.9	3.8	—	—	—
	5a''	1.8	—	0.6	2.0	—	—	—
Control rats								
(13)	5c	0	—	0.3	—	3.5	1.5	0.9
	Adrenalectomized rats (2–3 weeks after operation)							
	5a	0	—	0.3	—	0.9	0.8	—
	5a''	0	—	0.4	—	0.9	<0.2	0.5

^{*} This experiment, in which the conditions were not optimum, has been disregarded in assessing the effect of adrenalectomy on UDP and UDPglucuronic acid.

The labelling of the supernatant fraction was higher with adrenalectomized rats than with control rats at all times up to 13 hr., and lower at all subsequent times. However, the labelling of the microsomal fraction was lower in adrenalectomized rats at all times, and especially after 13 hr. or longer. Even if allowance is made for the previous finding (Reid, 1956; Stevens & Reid, 1956) that supernatant fractions from adrenalectomized rats contain somewhat more RNA than those from controls, the curves for adrenalectomized rats are also compatible with the hypothesis that the RNA of the supernatant fraction is the precursor of microsomal RNA.

One assumption underlying this postulate is that the microsomal and supernatant fractions are each homogeneous with respect to RNA. It appears from the following experiments that the supernatant fraction is not entirely homogeneous in this respect.

Subfractions obtained from supernatant fraction. The results for subfractions obtained by ultracentrifuging are shown in Fig. 2. It is evident that there is no striking difference in the time course of labelling between the sedimented ('pellet') RNA (this being about one-half of the original RNA) and the non-sedimented ('supernatant') RNA, and that the subfractions are equally affected by adrenalectomy except, perhaps, with respect to the labelling at 20 hr.

In the next experiment (Fig. 3), $(\text{NH}_4)_2\text{SO}_4$ was added to 35% of saturation, and then to 55% of saturation. The results were somewhat variable, but

it does appear that at short time intervals, in contrast with long time intervals, the precipitate obtained at 35% of saturation becomes labelled as rapidly as the other subfractions and has a higher specific activity (cf. RNA values in Table 3). There is no indication that adrenalectomy affects one of the fractions selectively with respect to labelling. However, the precipitate obtained between 35 and 55% of saturation may be particularly affected with

Fig. 2. Incorporation of injected orotic acid into the RNA of subfractions separated from supernatant fraction, namely ultracentrifugal pellet (●) and ultracentrifugal supernatant (▲), in control rats (—) and adrenalectomized rats (---). Total no. of rats, 22.

Fig. 3. Incorporation of injected orotic acid into the RNA of subfractions separated from supernatant fraction, namely precipitate obtained by ammonium sulphate at 35% of saturation (●), precipitate obtained from 35 to 55% (▲), and supernatant (■), in control rats (—) and adrenalectomized rats (---). Each point represents one experiment; total no. of rats used in preparation of supernatant fraction 'pools', 28.

Fig. 1. Incorporation of injected orotic acid into the RNA of the microsomal fraction (○) and supernatant fraction (△) in control rats (—) and adrenalectomized rats (---). Total no. of rats, 59.

respect to the amount of RNA (Table 3), this being increased by 16 %, whereas the average increases for the first precipitate and for the final supernatant were 10 and 2.5 % respectively.

Other experiments (not illustrated) were performed with three concentrations of $(\text{NH}_4)_2\text{SO}_4$, namely 33, 38 and 55 % saturation. The activity values showed the same trends as in the above experiments, the second precipitate being intermediate in behaviour between the first and third precipitates.

Barnum & Huseby (1950) mention preliminary experiments in which the RNA of the supernatant fraction, from mice given ^{32}P , was fractionated by CaCl_2 (0.4 %) or by isoelectric precipitation: 'It was invariably possible to obtain a small non-precipitable fraction with a comparatively high

relative specific activity... The reproducibility of these fractions was poor, but it is possible to conclude that the supernatant PNA [i.e. RNA] contains a small fraction whose relative specific activity initially increases much more rapidly than does that of the microsomal PNA.' Accordingly, CaCl_2 has now been employed, with the results shown in Fig. 4. Since little more than one-half of the original RNA was precipitated (Table 3), presumably less than in the experiments mentioned by Barnum & Huseby, the curves representing percentage recovery are also a rough measure of specific activity. The data for both adrenalectomized and control rats do in fact show a difference between the precipitable RNA and the non-precipitable RNA, the specific activity of the latter being about double that of the former at the shortest time interval (40 min.) and half that of the former at long time intervals (20 and 42 hr.). Since, however, the peak of labelling occurred at the same time for both subfractions, as for the precipitates obtained with $(\text{NH}_4)_2\text{SO}_4$, it cannot be concluded that one is the direct precursor of the other.

The data of Table 3 show that an increase in the amount of RNA after adrenalectomy occurs more consistently with the precipitable subfraction than with the non-precipitable subfraction.

The specific activity of the non-precipitable RNA at 40 min. after the injection was little altered by the use of lower final concentrations of CaCl_2 (0.06 or 0.075 % in place of 0.1 %). Concentrations higher than 0.1 % did not increase the amount of RNA precipitated.

Isoelectric precipitation was performed in one experiment, with a short interval after isotope injection, but the specific activity of the non-precipitable RNA was no higher than that of the precipitable RNA, in contrast with the results mentioned by Barnum & Huseby (1950). The trial of alumina gel (C_γ) as an alternative means of fractionation was abandoned when similar values were found, at long or short intervals after isotope in-

Fig. 4. Incorporation of injected orotic acid into the RNA of subfractions separated from supernatant fraction, namely precipitate (●) and supernatant (▲) obtained by addition of CaCl_2 to 0.1 % (w/v), in control rats (—) and adrenalectomized rats (---). Each point at 13 hr. represents two experiments, and the other points each represent one experiment; total no. of rats used in preparation of supernatant fraction 'pools', 25.

Table 3. *Ribonucleic acid in subfractions separated from supernatant fraction of rat liver*

Values are expressed as mg. of RNA/100 g. body wt. The last column gives the mean differences (adrenalectomized rats minus controls) $\pm$ s.e. of differences (with no. of degrees of freedom in parentheses).

	Control rats: mean value	Adrenalectomized rats: mean difference from controls
$(\text{NH}_4)_2\text{SO}_4$ fractionation		
1st precipitate (35% of saturation)	2.7	+0.27 $\pm$ 0.135 (6)
2nd precipitate (35% to 55% of saturation)	4.9	+0.79 $\pm$ 0.291 (7)*
Supernatant	1.9	+0.05 $\pm$ 0.123 (6)
CaCl_2 fractionation		
Precipitate (0.1 % CaCl_2)	6.0	+0.82 $\pm$ 0.339 (8)†
Supernatant	4.0	+0.53 $\pm$ 0.459 (8)

* $P < 5\%$.

† $P < 10\%$.

jection, for the ratio of the specific activity of the non-adsorbed RNA to that of the RNA eluted from the gel by 0.15M- Na_2HPO_4 solution.

Nuclear fraction. Data for nuclear fractions, containing any cells left unbroken after the two treatments in the homogenizer, are given in Fig. 5. In view of the heterogeneity of the RNA in this crude fraction, no conclusions can be drawn from the recovery values, or even from specific activity values, concerning the possible role of nuclear RNA as the precursor of cytoplasmic RNA. It can, however, be concluded that the increase in the labelling of supernatant-fraction RNA after adrenalectomy is not attributable to more rapid entry of label into nuclear RNA with consequent faster transfer to the supernatant fraction.

In a few experiments, crude nuclear fractions were treated repeatedly with citric acid solution as in the experiments of McIndoe & Davidson (1952). With these partially purified nuclei, as with the crude fractions, there was no indication of an increase in activity after adrenalectomy at the times studied (1, 4 and 20 hr. after injection).

Acid-soluble fraction. The recovery of activity in this fraction, as shown in Fig. 5, was not consistently higher after adrenalectomy. Evidently adrenalectomy does not increase the proportion of injected orotic acid which enters the liver. It was of interest to know whether the pattern of labelling of the various constituents of the acid-soluble fraction is altered by adrenalectomy. Anion-exchange chromatography was therefore carried out with tissue excised 40 min. after injection of orotic acid in high dosage (Table 4). At this time the labelling of cytidine derivatives, which are present in relatively small amounts (Hurlbert & Potter, 1954), is low in comparison with that of uridine derivatives (Hecht & Potter, 1956). From the few experiments performed, it appears that the rate of conversion of orotic acid into uridine derivatives is unchanged or possibly somewhat decreased after adrenalectomy. Orotic acid given in low dosage is not detectable in the liver even 30 min. after injection, its conversion into uridine compounds being very rapid (Hurlbert & Potter, 1952, 1954). Its identification in the liver extracts now studied was based on its position

(judged by the radioactivity and compared with that of authentic material) on the anion-exchange chromatogram and on a subsequent paper chromatogram with the propan-2-ol-water-HCl solvent of Wyatt (1951). In the former system orotic acid was not separable from ADP.

Labelling of liver deoxyribonucleic acid and of kidney ribonucleic acid. With the short time interval (135 min.) employed in the previous study (Reid *et al.* 1956), the labelling of liver deoxyribonucleic acid (DNA) was very low, and that of kidney RNA was too variable for any effect of adrenalectomy to be manifest. These aspects have now been reinvestigated, with the same dose (8 μC) but with a longer time interval (20 hr.). It was again found that the labelling of DNA was barely measurable, as also found by Hurlbert & Potter (1954), and that the labelling of the RNA in the whole kidney or in the supernatant fraction was very variable.

Fig. 5. Incorporation of injected orotic acid into the RNA of the nuclear fraction (O), and recovery of radioactivity in the acid-soluble fraction (Δ), in control rats (—) and adrenalectomized rats (---). Total no. of rats, 16 (nuclear fraction) or 30 (acid-soluble fraction).

Table 4. *Radioactivity of constituents of acid-soluble fraction of rat liver after large doses of orotic acid*

Rats were killed 40 min. after injection of $[6-^{14}\text{C}]$ orotic acid. Techniques used for chromatography and for measuring radioactivity are described in the text; techniques in Expts. C and A differed in minor respects from those in C' and A'. Values for the constituents (see Table 1 for abbreviations) represent percentage recovery of injected isotope.

	Expt.	UMP	Orotic acid	UDPacetylglucosamine, UDPglucose	UDP, UDPglucuronic acid
Control rats	C	2.4	0.4	2.6	—
	C'	1.45	1.5	1.6	0.8
Adrenalectomized rats	A	1.25	2.4	2.1	0.3
	A'	1.45	1.75	1.5	—

Incorporation of leucine in relation to time after injection of isotope

Whole cytoplasmic fraction. It was conceivable that a delayed absorption of injected DL-leucine after adrenalectomy was responsible for the decrease previously found (Reid *et al.* 1956) in the labelling of cytoplasmic protein. Since each of the cytoplasmic elements shared in this decrease, only the whole cytoplasmic fraction has now been reinvestigated. In the previous experiments, with an interval of 90 min. between injection and autopsy, the recovery of isotope (corrected for the presence of the 'inert' D isomer) in cytoplasmic protein was 9% in rats adrenalectomized 2 weeks previously, as compared with 10.2% in corresponding controls. With L-leucine, mean values of 7.1 and 7.25% respectively have now been found 40 min. after injection, and values of 9.2 and 10.7% have been found 15 min. after injection, the latter high values being perhaps a reflexion of day-to-day variation. It was anticipated that liver excised 15 min. after the injection would contain an appreciable amount of radioactivity in the form of free leucine; but the amount thus recovered was only about 0.1%, this amount being unaffected by adrenalectomy. It is, however, evident that the slight decrease in protein labelling after adrenalectomy is manifest even at relatively short time intervals.

Subfractions from microsomal fraction. Littlefield *et al.* (1955) have claimed that in the first few minutes after intravenous injection of a labelled amino acid, the activity of the microsomal protein which remains insoluble after deoxycholate treatment and which is rich in RNA is markedly higher than that of the solubilized protein. An attempt has now been made to ascertain whether adrenalectomy affects this initial labelling of the insoluble protein.

In preliminary experiments it was confirmed that a pellet consistently rich in RNA could be obtained by deoxycholate treatment of microsomal fractions, provided that the sedimented fractions were dispersed in a small volume of water before addition of the deoxycholate solution rather than dispersed directly in the latter. In the next experiment, liver was excised from control and adrenalectomized rats 3 min. after intravenous injection of labelled leucine, and microsomal fractions were rapidly isolated and treated with deoxycholate as in the experiments of Littlefield *et al.* (1955). In agreement with these authors, the specific activity of each pellet was higher than that of the corresponding supernatant (Table 5). However, the individual rats varied so widely that it was impossible to assess whether adrenalectomy had any effect on the labelling. There was no consistent effect of adrenalectomy on the yield or RNA content of the pellet or supernatant.

Incorporation of orotic acid and of leucine in relation to time after adrenalectomy

In the following experiments, adrenalectomized rats were studied at different times after operation; in isotopic experiments the time after injection of isotope was standardized as stated in the Experimental section.

Amount and labelling of ribonucleic acid. It is evident from Fig. 6 that the effect of adrenalectomy in increasing the amount of RNA in the supernatant fraction is not manifest 1 day after operation, but is fully manifest at 3 days. The increase in the incorporation of orotic acid into the RNA of the supernatant fraction is already manifest at 3 days, but continues to increase with time. On the other hand,

Table 5. *Labelling of microsomal subfractions 3 min. after intravenous injection of leucine*

Values represent counts/min./[100 - (% of RNA)] after injection of 12 μ C of DL-[1- 14 C]leucine/250 g. body wt., and are an arbitrary measure of the specific activity of the protein (the counting being at infinite thickness).

	Deoxycholate pellet	Deoxycholate supernatant
Control rats { Intact	2.4	2.2
Intact	0.55	0.12
Sham-operated	0.53	0.17
Adrenalectomized rats	2.4	0.34
	2.2	0.66
	0.57	0.13
	2.3	1.4

Fig. 6. Changes after adrenalectomy, related to time after operation: □, amount of RNA (/100 g. body wt.) in the supernatant fraction; △, incorporation of orotic acid into the RNA of the supernatant fraction; ○, incorporation of orotic acid into the RNA of the microsomal fraction; ■, amount of protein (/100 g. body wt.) in the microsomal fraction; ●, incorporation of leucine into protein of microsomal fraction; ▲, incorporation of leucine into protein of whole cytoplasmic fraction. The total nos. of rats were respectively 55, 22, 22, 30, 24 and 23.

the incorporation into the microsomal fraction is undiminished after 3 days, but subsequently decreases.

Amount and labelling of protein. Re-examination of the data of Reid *et al.* (1956) showed that adrenalectomy had consistently decreased the amount of microsomal protein as judged by the yield of the residue remaining after removal of acid-soluble material and lipids. As is now shown (Fig. 6), both this decrease and the decrease in the incorporation of leucine into the protein of the microsomes or the whole cytoplasm become manifest gradually, no decrease occurring only 3 days after operation.

Catabolism of ribonucleic acid

The possible effect of adrenalectomy on the catabolism of RNA cannot readily be assessed from the data of Fig. 1, the amount of RNA and its apparent rate of synthesis being themselves affected by adrenalectomy. The following approach was adopted. Adrenalectomy was performed 8 hr. after injection of isotope, presumably without effect on the time or extent of maximal labelling since, as was shown in Fig. 6, the amount of RNA in the supernatant fraction is not increased 1 day after operation. During the following 2 days the amount of this RNA rises to a maximum. If this rise were the consequence of a decrease in the catabolism of RNA, supernatant fractions prepared from the adrenalectomized rats after a few days' interval should have retained their labelling more fully than those from controls.

Table 6. *Catabolism of ribonucleic acid shortly after adrenalectomy*

Experimental rats were adrenalectomized, and the control rats sham-operated, 8 hr. after injection of 2.3 μ C of orotic acid; the rats were killed after 5 days. Values represent percentage recovery of injected isotope.

	Microsomal fraction	Supernatant fraction
Control rats	6.9 4.2 5.0	3.75 3.45 3.2
Adrenalectomized rats	4.95 4.1 4.9	2.7 2.35 3.95

As is shown in Table 6, the labelling of the supernatant fraction after 5 days was no greater for the adrenalectomized rats than for the controls. The labelling of the microsomal fraction was also little affected, as would be expected in view of the observations that adrenalectomy does not affect either the amount of microsomal RNA (Reid, 1955), or its labelling shortly after operation (Fig. 6).

Effect of massive doses of orotic acid on liver ribonucleic acid levels

It is possible that orotic acid, or a uridine compound derived therefrom, is a limiting factor in RNA synthesis. To test this possibility, analyses for RNA were performed on livers from intact rats given massive doses of unlabelled orotic acid. Table 7 shows that this treatment appeared to increase markedly the amount of acid-soluble RNA precursors, as judged by the ultraviolet-light extinction at 260 m μ . The amount of RNA in the supernatant fraction was slightly increased, but that in the liver as a whole tended to decrease. Preliminary chromatographic studies with the acid-soluble fractions indicated that the increase in extinction was largely attributable to an increased amount of non-acidic material, possibly nucleosides.

A possible interpretation of these data is that the increase in supernatant-fraction RNA occurred only by virtue of enhanced breakdown or depressed formation of RNA elsewhere in the cell. Although the amount of RNA in the supernatant fraction did increase in response to administration of a uridine precursor, it is nevertheless possible that the incorporation of uridine nucleotides into RNA in normal rats is to some extent limited by the amount of the enzyme or enzymes concerned.

DISCUSSION

It is probable, although not proven for liver, that nucleic acids are formed at least in part from nucleoside 5'-diphosphates (pyrophosphates) or 5'-triphosphates (Grunberg-Manago, Oritz & Ochoa, 1955; Kornberg, Lehman, Bessman & Simms, 1956). Potter, Hecht & Herbert (1956) have achieved synthesis of liver RNA *in vitro* with 14 C-labelled orotic acid or UMP as precursor, having shown in

Table 7. *Effect of massive doses of orotic acid on liver-ribonucleic acid levels in intact rats*

The last column gives the mean differences (treated rats minus controls) $\pm$ s.e. of differences (with no. of degrees of freedom in parentheses).

	Control rats: mean value	Rats treated with orotic acid: mean difference from controls
Extinction at 260 m μ of acid-soluble fraction, diluted so that 1 l. is equivalent to 100 g. body wt.	0.264	+0.099 $\pm$ 0.0196 (18)*
RNA, mg./100 g. body wt. { Whole liver	34.0	-3.1 $\pm$ 2.52 (17)
Supernatant fraction	7.0	+1.0 $\pm$ 0.46 (18)†
* $P \leq 0.1\%$.		† $P < 5\%$.

earlier experiments that liver contains a 'pool' of uridine nucleotides which rapidly become uniformly labelled after injection of orotic acid (Hurlbert & Potter, 1954; Schmitz, Hurlbert & Potter, 1954). The view that orotic acid, despite its apparent absence from normal liver (Hurlbert & Potter, 1952, 1954), is a physiological precursor of uridine nucleotides is supported by evidence from other laboratories (Lieberman, Kornberg & Simms, 1955; Hurlbert & Reichard, 1955; Wu & Wilson, 1956). Orotic acid is also a precursor of cytidine nucleotides, although the study of this conversion is handicapped by the difficulty of analysing the relatively small amounts of these nucleotides in liver (Hurlbert & Potter, 1954).

It has now been shown that at least some of the uridine nucleotides are markedly decreased in amount 2-3 weeks after adrenalectomy. That this observation does not hold for adenosine or guanosine nucleotides is suggested by the data for AMP and GMP, the amount of the former being actually greater after adrenalectomy. The changes in uridine nucleotide levels may have wide implications with respect to processes such as glucuronide synthesis. Here it need only be pointed out that if uridine nucleotides are in fact immediate precursors of supernatant-fraction RNA, as is discussed below, an increased incorporation of injected orotic acid into this RNA does not signify an increased rate of synthesis of RNA unless the increase in incorporation outweighs the decrease in the uridine nucleotide pool. Consideration of Table 2, together with Fig. 6, suggests that the rate of synthesis of supernatant-fraction RNA 2-3 weeks after adrenalectomy is increased not by about 100%, as Fig. 6 would suggest, but by less than 30%. That some increase does in fact occur is indicated by the data obtained 3 days after adrenalectomy, as is discussed below. (If supernatant-fraction RNA is formed, not from nucleotides but from nuclear RNA, the increased labelling of supernatant-fraction RNA may truly reflect increased synthesis, since adrenalectomy tends to increase, rather than diminish, the amount of RNA in the nuclear fraction [Reid, 1956].)

Obviously there may be other variables which could affect the interpretation of incorporation values in terms of rates of RNA synthesis. Adrenalectomy might conceivably have altered the rate or extent of the conversion of injected orotic acid into uridine nucleotides in liver. However, the possibility that adrenalectomy increases the amount of isotope entering the liver is ruled out by the results for the acid-soluble fraction (Fig. 5), the radioactivity of which is largely attributable to uridine nucleotides (Hurlbert & Potter, 1954). The results for radioactive uridine nucleotides in this fraction (Table 4), 40 min. after injection of orotic acid in a high dosage such that free orotic acid could be detected in the

liver, suggest that the formation of uridine nucleotides is slowed rather than accelerated by adrenalectomy. Evidently, increased recovery of labelled material in supernatant-fraction RNA after adrenalectomy is not a consequence of a rise in the number of radioactive molecules in the acid-soluble fraction.

Since the amount of RNA in the supernatant fraction has already risen to a maximum 3 days after adrenalectomy (Fig. 6), an increased rate of synthesis of this RNA 2-3 weeks after adrenalectomy must be balanced by an increased rate of catabolism. This conclusion is compatible with the data of Fig. 1 and with data for ribonuclease levels (Reid & Stevens, 1956; Stevens & Reid, 1956) which will be discussed in a later paper.

It does appear that the increase in the amount of RNA occurring as an initial effect of adrenalectomy is attributable to increased synthesis rather than to decreased catabolism (Fig. 6 and Table 6), the uridine nucleotide pool being then elevated (with no increase in its labelling). This increased synthesis might be due to an increase in the amount of the enzyme(s) concerned in the formation of RNA from ribonucleotides. Daoust & Cantero (1955) suggest that 'the rate-limiting step in the synthesis of nucleic acids is not the formation of free nucleotides but rather the building up of nucleic acids from individual nucleotides'. The data of Table 7 are not incompatible with this view.

As Hurlbert & Potter (1954) have pointed out, the time course of labelling of the different fractions (cf. Figs. 1, 5) is compatible with the view that nuclear RNA is formed from acid-soluble ribonucleotides, but does not suggest a direct relationship between the latter and cytoplasmic RNA as has been assumed in the above discussion. The suggestion that nuclear RNA is the direct precursor of supernatant-fraction RNA (Jeener & Szafarz, 1950) is not supported by observations from other laboratories (Barnum, Huseby & Vermund, 1953; Smellie, McIndoe, Logan, Davidson & Dawson, 1953; Sacks & Samarth, 1956; Brachet, 1956), although Smellie *et al.* recognize the possibility that nuclear RNA contains several constituents, one of which may be such a precursor. It is, however, possible that the supernatant fraction contains at least two types of RNA, one of which is in fact formed directly from acid-soluble ribonucleotides.

It has now been found that subfractions differing in the time course of labelling can be separated from the supernatant fraction, in accordance with preliminary experiments mentioned by Barnum & Huseby (1950). Logan & Smellie (1956) have independently used ammonium sulphate to obtain subfractions which, in this instance, differed in their ability to donate ^{32}P -labelled RNA to the nucleus *in vitro*. Studies in which RNA was isolated at a fixed time after isotope injection and was deliber-

ately degraded, the products differing in specific activity values, have been reported by Sacks & Samarth (1956), whose interpretation of the results is, however, open to question, and by Moldave & Heidelberger (1954), who found 'intramolecular heterogeneity' with respect to the labelling of RNA phosphate (and guanine?) but not, in the supernatant fraction, with respect to that of adenine and pyrimidines after injection of labelled glycine and orotic acid.

The differences in labelling between the sub-fractions in the present experiments, together with the 'plateau' in the ascending portion of the curve for the whole supernatant fraction (Fig. 1), suggest the presence of two types of RNA, one of which may be the precursor of the other, the labelling of both types being enhanced by adrenalectomy. More rigorous fractionation will be required if the criteria of Zilversmit *et al.* (1943) for a precursor-product relationship are to be met. With orotic acid there is the inherent difficulty that RNA cytosine, as well as uracil, becomes labelled at long time intervals after isotope injection (Hurlbert & Potter, 1954); in the nuclear fraction from regenerating liver the RNA pyrimidines have become equally labelled at 12-24 hr. after isotope injection (Hecht & Potter, 1956).

Despite the latter complication and the probable heterogeneity of the RNA in each of the cellular elements, some evidence has been obtained in support of the postulate that microsomal RNA is formed from supernatant-fraction RNA (Jeener & Szafarz, 1950; Barnum *et al.* 1953). Whereas the specific activity values for microsomal and supernatant fractions after injection of ^{32}P showed no maxima in the experiments of Barnum *et al.* (1953) and similar maxima in the experiments of Smellie *et al.* (1953), the microsomal fractions now studied have shown a maximum, albeit broad and late, which is intersected by the descending portion of the curve for the supernatant fraction. (As has been pointed out above, the curves of Fig. 1 can be regarded as representing specific activity as well as percentage recovery.) Moreover, in the experiment of Table 7 the preferential increase in supernatant-fraction RNA suggests that the synthesis of this RNA precedes that of RNA elsewhere in the cytoplasm.

Closer consideration of the isotopic data from the kinetic aspect must await study of each fraction with respect to the amounts and specific activities of RNA cytosine and uracil. It may, however, be pointed out that at short time intervals after isotope injection the specific activity of the RNA in the supernatant fraction was somewhat increased after adrenalectomy, as in the previous experiments (Reid *et al.* 1956). The supposed translocation of RNA from the supernatant fraction to the micro-

somal fraction may therefore be retarded more than is suggested by the reduction in percentage of isotope recovered in the microsomal fraction.

The reduction in microsomal labelling, unlike the increase in the labelling of the supernatant fraction, is a late effect of adrenalectomy. From this observation and also from the data for short time intervals in Fig. 1, it can be concluded that the enhanced labelling of the supernatant-fraction RNA could not be due merely to a decrease in its supposed translocation to the microsomal fraction. Whatever the origin of microsomal RNA, it is evident that the lack of adrenal hormones affects the processes of cytoplasmic RNA synthesis at two separate points. That adrenalectomy also affects the synthesis of nuclear RNA is suggested by the results of Fig. 5, considered together with the fall in the amounts of uridine nucleotides (Tables 1 and 2); this fall in the pool of supposed precursors of nuclear RNA implies that the synthesis of this RNA is diminished more than is suggested by the slight diminution in the labelling of this RNA.

The decrease in microsomal labelling shows a striking parallelism, in its gradual onset after adrenalectomy, with the decreases in the incorporation of leucine into protein and in the amount of microsomal protein (Fig. 6). Since the rise in the labelling of supernatant-fraction RNA is much earlier, it is probable that at least some of this RNA, unlike the RNA in the microsomal fraction, is not directly concerned in protein synthesis. Admittedly it has not been proved in the present experiments that the small reduction in leucine incorporation truly signifies a fall in protein synthesis; but the absence of a significant effect of adrenalectomy on the pool of leucine in liver (Awapara, Skellenger & Manz, 1955) or on amino acid absorption by the liver (Awapara & Kit, 1954) argues in favour of this interpretation.

'Microsomal' material as prepared in some laboratories comprises both the microsomal fraction and the ultracentrifugal fraction as now prepared. The divergence between these fractions observed by Reid *et al.* (1956), with respect to the change in the incorporation of orotic acid after adrenalectomy, has now been confirmed (Figs. 1 and 2). A similar divergence has been observed by Jardtetzky & Barnum (1956) in a study of RNA formation in regenerating liver.

SUMMARY

1. Adrenalectomy leads eventually to a marked decrease (exceeding 50%) in the levels, in rat liver, of uridine 5'-phosphate, uridine 5'-diphosphate (-pyrophosphate) and its glucuronic acid derivative, and uridine triphosphate. There is also some decrease in uridine diphosphate acetylglucosamine

and possibly in uridine diphosphate glucose, but not in liver nucleotides in general.

2. Microsomal and supernatant fractions have been isolated from liver at different times after injection of labelled orotic acid. The results confirm that the labelling of the ribonucleic acid of the microsomal fraction is decreased after adrenalectomy and that of the supernatant fraction increased, and are compatible with the possibility that ribonucleic acid in the supernatant fraction is a precursor of microsomal ribonucleic acid.

3. Supernatant fractions containing labelled ribonucleic acid have been fractionated by several methods, two of which have indicated heterogeneity in this ribonucleic acid with respect to the time course of labelling. However, the labelling of the different subfractions was equally affected by adrenalectomy.

4. The labelling of nuclear and acid-soluble fractions, and of uridine nucleotides separated from the latter, shows no increase after adrenalectomy.

5. The labelling of microsomal ribonucleic acid, the labelling of cytoplasmic or microsomal protein with leucine as precursor, and the amount of microsomal protein are undiminished 3 days after adrenalectomy but decrease thereafter. The increase in the labelling of supernatant-fraction ribonucleic acid is already apparent 3 days after adrenalectomy, together with a rise in the amount of this ribonucleic acid which evidently occurs because of an increase in synthesis rather than a decrease in catabolism.

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