

no guarantee that the phytic acid used in the experiments was free from small amounts of inositol penta-phosphate which would be difficult to detect by chemical analysis. The fact that in the early stages of the hydrolysis the experimental values for hexa- plus penta-phosphate phosphorus are less than the theoretical values may possibly indicate that some inositol penta-phosphate was present in the original phytic acid.

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## Functions of ribonucleic acid in liver cytoplasm

There is evidence that cytoplasmic ribonucleic acid (RNA) plays an important role in protein synthesis<sup>1,2</sup>. That this may not be its only role is suggested by the following observations, made in the course of a study<sup>3</sup> of hormonal influence on the incorporation of labelled precursors into the protein and RNA of liver cytoplasm.

Adrenalectomized rats (males of about 220 g body weight) maintained on saline, and control rats kept on the same food intake, were given 8  $\mu$ c of orotic acid-6-<sup>14</sup>C by intraperitoneal injection. After 2 hours, at which time the radioactivity of cytoplasmic ribonucleic acid had not reached a "plateau" (*cf* <sup>4</sup>), the livers were removed and homogenized in 0.25 *M* sucrose solution. Differential centrifugation was then performed, essentially as previously described<sup>5</sup>. The fractions thus isolated were treated with cold trichloroacetic acid solution, defatted, dried, and ground to a powder suitable for the determination of radioactivity with a thin-window counter.

Similar experiments were performed with DL-leucine-1-<sup>14</sup>C in place of orotic acid. The dose was 20  $\mu$ c, given 1½ hours before autopsy.

In the case of the mitochondrial fraction, and of the overlying "fluffy layer" (which was analysed separately), the only significant effect of adrenalectomy was a reduction in the amount of leucine incorporated into the protein of the mitochondrial fraction (the value being calculated as percentage recovery of injected isotope). The amount of orotic acid incorporated into the RNA of the crude "nuclear" fraction tended to be low after adrenalectomy.

In the case of the microsomal fraction, of the supernatant fraction, and of a "sub-microsome" fraction derived from the latter by ultracentrifugation, the effects of adrenalectomy were striking (Table I). With the microsomal fraction the incorporation of orotic acid was reduced, whereas with the ultracentrifugal fraction and particularly with the whole supernatant fraction the incorporation was markedly increased. The RNA of the supernatant fraction is known to increase in amount after adrenalectomy<sup>6</sup>, but nevertheless its actual specific activity was definitely increased in the present experiments with orotic acid.

As further shown in Table I, the incorporation of leucine was significantly lowered by adrenalectomy in the case of the microsomal fraction, and tended to be low in the case of the supernatant and ultracentrifugal fractions.

With the microsomal fraction, which is believed to be of particular importance in protein synthesis<sup>1</sup>, the data of Table I thus suggest that there is a decrease in the rate of synthesis both of protein and of RNA after adrenalectomy. Since no such correlation is evident with the supernatant fraction, the RNA of this fraction probably has, at least in part, some function unconnected with protein synthesis. The marked divergence between the microsomal fraction and the supernatant fraction, with respect to the effect of adrenalectomy on the incorporation of orotic acid, would be difficult to explain merely in terms of an effect of adrenalectomy on "pool" size, or

TABLE I

EFFECT OF ADRENALECTOMY ON THE INCORPORATION OF LABELLED PRECURSORS INTO THE RNA AND PROTEIN OF CERTAIN CYTOPLASMIC FRACTIONS

	Microsomal fraction	Supernatant fraction	Ultracentrifugal fraction (sedimented from supernatant fraction)
Incorporation of labelled orotic acid, as % recovery of injected isotope			
Mean value for control rats	0.44	1.30	0.41
Change after adrenalectomy*	$-0.12 \pm 0.034$ (7)	$+1.39 \pm 0.250$ (7)	$+0.28 \pm 0.091$ (7)
P**	< 1 %	< 0.1 %	< 2.5 %
Incorporation of labelled leucine, as % recovery of injected isotope***			
Mean value for control rats	1.57	2.09	0.76
Change after adrenalectomy*	$-0.23 \pm 0.067$ (6)	$-0.18 \pm 0.0085$ (6)	$-0.10 \pm 0.098$ (3)
P**	< 2.5 %	< 10 %	> 30 %

\* With standard error of mean change (and number of degrees of freedom).

\*\* Probability that apparent change could be due to chance.

\*\*\* Uncorrected for presence of D isomer in the injected DL-leucine.

on the supposed translocation<sup>6,7</sup> of RNA from the cell sap (supernatant fraction) to the microsomal elements. It is, moreover, unlikely that the effect of adrenalectomy on the incorporation of leucine is ascribable merely to a fall in "pool" size or in rate of absorption of injected isotope (*cf.*<sup>8</sup>).

Further study is needed, with particular attention to the time course of the incorporation of orotic acid and also to the nature of the RNA in the supernatant fraction. We have already found that the proportion of the RNA of the isolated supernatant fraction existing in "highly polymerized" form (*cf.*<sup>9</sup>) is increased after adrenalectomy (mean value 82 % for adrenalectomized rats, 73 % for controls), this effect being converse to that reported for certain cytoplasmic fractions from intact rats given massive doses of cortisone<sup>9</sup>.

In contrast with adrenalectomized rats, hypophysectomized rats have not shown an increased incorporation of orotic acid into the RNA of the supernatant fraction in comparison with that for pair-fed controls. After hypophysectomy, the incorporation of both leucine and orotic acid into the various fractions tended to be low (markedly so in the case of the microsomal fraction), even if growth hormone had been administered. With hypophysectomized rats there is evidently no indication of a divergence between changes in protein synthesis and changes in RNA synthesis, as found with adrenalectomized rats.

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