TABLE I. Urinary Excretion of C^{14}-Activity from Normal and Riboflavin-Deficient Rats During 24 Hours Following an Intraperitoneal Injection of D-Riboflavin-2-C^{14} With and Without Additional Riboflavin or 5'-Hydroxypentylflavin.

Riboflavin added to diet* (μg/15 g diet)	Avg wt gain during 2 wk on diet† (g)	Compound injected with 10 μg of D-riboflavin-2-C^{14}‡ (40 μg/ml/rat)	Urinary excretion of C^{14}-activity/rat (c/m-background)	Increase in urinary excretion of C^{14}-activity due to compound injected (%)
0	12.5	none	14,652	0
		riboflavin	18,972	30
		5'-hydroxypentylflavin	17,680	21
20	54.2	none	18,500	0
		riboflavin	19,861	7
		5'-hydroxypentylflavin	18,752	2

* Rats were divided into 2 groups of 9 each. † Initial average weight was 69 g. ‡ Each injection group was comprised of 3 rats.

stant rate of urinary excretion(6). The ω-hydroxyalkyl analogs may decrease destruction of riboflavin as evidenced by its increased excretion when either an analog or excess vitamin is injected, especially in a deficient animal. The inability of ω-hydroxyalkylflavins to be converted to physiologically active coenzyme forms(1,2), but marked sparing efficiency for riboflavin in the rat, is somewhat similar to the behavior of L-lyxoflavin. This latter riboflavin analog was found not to serve as a partial substitute for riboflavin in *L. casei* or chicks, but enhances the efficiency with which limited supplies of riboflavin are utilized(7). Related findings have been reported for certain ring-substituted analogs of riboflavin in microbiological and rat assays (8-10).

Summary. A series of ω-hydroxyalkylflavins with 2 to 6 carbon chain lengths are shown to markedly enhance the utilization of normally suboptimal amounts of riboflavin given to growing rats. These analogs cannot function in place of the vitamin, but may decrease its metabolic destruction.

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Effect of Cortisol on Nuclear RNA-Synthesis *in vitro*.*(31267)

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Although it is now well accepted that steroid hormones cause a stimulation of RNA synthesis followed by formation of specific proteins in target organs(1,2), the primary reaction of the steroid molecule with a speci-

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fic cellular receptor which induces the release of specific information from the DNA and an increase in RNA synthesis remains an enigma. As yet, only very little experimental evidence supports the view that hormones act directly at the genetic level, as postulated by Karlson(3) and Zalokar(4). Dukes and Sekeris(5) reported the *in vitro* stimulation by cortisol of 2-C^{14}-uracil uptake into the RNA of isolated rat liver nuclei.

In this communication we present evidence that isolated liver cell nuclei can indeed synthesize RNA *in vitro*. There is, however, a concomitant breakdown of RNA, even after very short periods of incubation. Cortisol, although bound by isolated nuclei in small amounts, does not increase nuclear RNA synthesis when added *in vitro*. Such an increase, however, can be obtained if the hormone is injected into the animals as short a time as 20 minutes before the nuclei are isolated and incubated in the presence of a labelled precursor.

Materials and methods. Liver nuclei were isolated from 200-300 g adrenalectomized Wistar rats by the method of Chaveau *et al* (6), suspended in ice cold sucrose-Tris buffer (0.25 M sucrose, .003 M $CaCl_2$, 0.01 M Tris · HCl pH 7.4) and sedimented again at 450 *g*. The nuclear fraction thus obtained was practically free of cytoplasmic components and whole cells and contained less than 5% of damaged nuclei (DNA/RNA ratio: 5.87). The nuclei were gently resuspended with a Teflon-Glass homogenizer, filtered through 3 layers of cheesecloth and counted in a Coulter counter. (Final concentration 4×10^7 nuclei/ml.)

Incubation. Per ml, the incubation mixture contained 4×10^7 nuclei, Tris · HCl pH 7.4 15 μMol, KCl 30 μMol, NaCl 30 μMol, GTP, UTP, ATP 0.6 μMol each, 12.5 μC CTP (H^3) (sp. act. 2C/mMol), $MgCl_2$ 0.125 mMol, 3 μMol of creatine phosphate, and 0.006 ml of a 1% solution of creatine kinase. Cortisol was added to the incubation mixture in a small volume (5-10 μl per ml) of ethanol/water or DMSO/water 1:10 to final concentrations between 10^{-12} and 10^{-4} molar. 0.2 ml samples were incubated at 37°C for various time intervals, ranging from 3 to 30 minutes. Incubations were stopped by rapid chilling and dilution with ice cold sucrose-Tris solution and all following operations were carried out at 0° to 4°C. After centrifugation the nuclear pellets were dissolved in 0.4 ml of a neutralized detergent (Hemo-Sol®) and transferred to filter paper discs, which were then treated with ice cold 10% TCA (twice), ethanol/ether 1:6 and ether for 30 minutes each, dried and transferred to glass vials with 15 ml of PPO/POPOP Toluene and the radioactivity measured in a Packard liquid scintillation counter. Results were expressed as moles of CTP incorporated per nucleus. For *in vivo/in vitro* experiments cortisol phosphate‡ (1 mg/100 g b.w.) was injected intraperitoneally into male white rats at various time intervals before sacrificing the animals. The rats had been adrenalectomized at least 5 days prior to use. To determine the uptake of cortisol by isolated nuclei *in vitro*, 0.4 to 1.0 μC of C^{14}-cortisol (sp. act. 450 mC/mMol) dissolved in 0.01 ml of DMSO-water 1:1 was added to 1 ml of incubation mixture which contained an equimolar amount of CTP instead of CTP · H^3 in these experiments. Incubations were carried out in 0.2 ml samples for 5, 10 and 15 minutes. The nuclei were then spun down, washed twice with 3 ml of sucrose-$CaCl_2$-Tris buffer, which contained non-radioactive cortisol in concentrations corresponding to that of C^{14}-cortisol in the incubation mixture, transferred to filter paper discs in 0.4 ml of water, dried and assayed for radioactivity. The results were expressed as cpm/100 g dry weight of nuclei. Dry weight was determined by lyophilization.

Chromatography. 4 ml of the incubation mixture which was used to measure RNA synthesis were incubated for 30 minutes, the nuclei centrifuged, washed with TCA, ethanol/ether and ether and the remaining pellet hydrolyzed overnight in a small volume of 0.3 M KOH. After removal of insoluble materials by centrifugation the clear supernatant was neutralized with $HClO_3$, 2 μMol of 5-CMP, 2′-,3′-CMP and cytidine were added and the solution applied to a Dowex AG 1-×8 column (25 cm long, 1 cm i.d.) which was eluted with a H_2O/1 N formic acid linear gradient;

‡ Hydrocortone®

TABLE I. Typical *in vitro* Experiment from a Series of 5 Experiments, Conducted in a Similar Way. The figures represent moles of CTP incorporated per nucleus.

	Incubation time (min)			
	4	8	15	30
Control *in vitro*	1.54×10^{-19}	2.1×10^{-19}	1.93×10^{-19}	2.24×10^{-19}
Cortisol *in vitro* (10^{-5} molar)	$.86 \times 10^{-19}$	1.49×10^{-19}	1.94×10^{-19}	1.99×10^{-19}

3.8 ml fractions were collected, OD was recorded with a Guilford Spectrophotometer at 260, 280, and 245 mμ and aliquots taken for assays of radioactivity. The identity of the compounds eluted from the column was confirmed by determination of their 245/260 and 280/260 ratios and their λ max.

RNA extraction and sucrose gradients. Two RNA fractions were extracted from isolated and incubated nuclei with water-saturated phenol at 0°C and pH 7.6 and at 38°C and pH 8.3 (A. Hadjivassiliou and G. Brawerman 1966)(7). 100-150 μg samples were applied to 5 ml 5%/20% sucrose gradients and centrifuged for 4 hours at 35,000 rpm. Twelve drop fractions were collected and radioactivity and optical density measured as described above.

Results and discussion. 85% of the radioactivity incorporated into a batch of nuclei during a 15-minute incubation can be recovered as 2',3'-CMP, approximately 12% is found as 5'-CMP and a negligible amount comes out as cytidine. The radioactivity peaks coincided exactly with the peaks obtained from measurements of optical density. Thus, the radioactivity measured actually represents synthesis of RNA rather than non-specific binding of labelled precursor. The addition of cortisol to the incubation mixture does not significantly enhance the incorporation at any concentration and time interval studied (Table I). However, cortisol causes a significant stimulation *in vitro* of nuclear RNA synthesis, if it is injected into the rats as short a time as 20 minutes before sacrifice (Table II). This observation is in accordance with the results from other laboratories(8). C^{14}-labelled cortisol is taken up by isolated nuclei in amounts which are in the same order of magnitude as those found by Sekeris and Lang in similar experiments carried out *in vivo*(9). The highest uptake is measured after 15 minutes of incubation. The decrease observed after 30 minutes of incubation may be due to a leakage of protein from the nuclei or to other degenerative changes of the nuclei. The relationship between cortisol concentration in the medium and cortisol uptake by the nuclei is linear within the range of concentrations tested (Table III). These findings show that the intranuclear presence of cortisol does not necessarily produce an increase in nuclear RNA synthesis or any other specific response. This reservation must also be made with respect to all presently available data concerning interactions of steroids with nuclear components *in vivo*.

TABLE II. *In vivo-in vitro* Experiment: Cortisol phosphate, 1 mg/100 g body wt was injected intraperitoneally into adrenalectomized rats. At intervals indicated the rats were killed, liver nuclei isolated and incubated as described in text for 15 min. RNA was extracted from 4 ml of incubation mixture and incorporation of radioactivity was expressed as cpm/μg RNA. Each value is the average of 2 experiments.

Control *in vivo/vitro*	50
Cortisol *in vivo* 20 min	90
Cortisol *in vivo* 60 "	116
Cortisol *in vivo* 180 "	160

TABLE III. Uptake of ^{14}C-Cortisol by Isolated Nuclei *in vitro*. Results are expressed as counts per min per 100 mg of dry weight.

	Incubation time (min)		
	5	15	30
^{14}C-cortisol			
.4 μC = 3.2 μg/ml	26,150	33,750	25,700
.5 μC = 4 μg/ml	—	35,500	—
1 μC = 8 μg/ml	—	64,990	—
1.5 μC = 12 μg/ml	—	105,000	—

Even during short incubation periods a partial degradation of nuclear RNA takes place. While RNA, isolated from non-incubated nuclei, can be separated by zone centrifugation into well characterized fractions with respect to base composition, pulse labelling and template activity, the distribution pattern of the RNA changes drastically after only 3 minutes of incubation. After 5 min-

utes, all heavy RNA is partially degraded and can be found as a large heterogenous peak in the lighter part of the gradient, between 4 and 10 s. The yields of RNA extractable from the nuclei at pH 7.6 or pH 8.3 decrease from 0.7 mg/10 g of liver to 0.4 mg/10 g after an incubation of only 15 minutes. Dukes and Sekeris used incubation periods of 2 or even 3 hours. The cortisol concentrations which stimulated the incorporation of uracil into isolated nuclei in their experiments were about 500 times as high as the normal non protein-bound plasma concentration. Compared to the effects obtainable by application of the hormone *in vivo,* the stimulation of incorporation reported by Dukes and Sekeris is still small. For these reasons it seems doubtful that their results reflect a physiologically relevant mechanism. The degree of normal structure which must be maintained by a subcellular system in order to respond to a hormonal stimulus in a specific way remains unsettled.

Summary. Nuclei isolated from rat liver cells can incorporate H^3-cytidine triphosphate into RNA *in vitro.* Cortisol added to the incubation medium is taken up by the nuclei in small amounts, but does not stimulate nuclear RNA synthesis. Administration of the hormone *in vivo,* however, results in a marked increase in incorporation of H^3-cytidine triphosphate incubated *in vitro* with nuclei isolated from 20 to 180 minutes after the injection. Synthesis of RNA *in vitro* is accompanied by a simultaneous partial degradation of RNA which affects the yields of RNA extractable from nuclei even after very short periods of incubation.

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The Nature of *Macacus rhesus* Erythrocyte Agglutinins Found in the Sera of Hepatitis Patients. (31268)

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Hoyt and Morrison(1) reported that sera of patients with infectious hepatitis agglutinated *Macacus rhesus* erythrocytes. It was suggested by Rubin *et al*(2) that the hemagglutination resulted from a specific effect of hepatitis virus, whereas Hoyt *et al*(3) suggested that the hemagglutination mechanism involved a gamma globulin of the sera of hepatitis patients. This report concerns our studies on the agglutinating component found in the sera of hepatitis patients.

Methods. Procedures for the agglutination test were essentially similar as described by Hoyt and Morrison(1). *Macacus rhesus* erythrocytes were washed 3 times with 0.9% saline and a 2% suspension prepared. Serial 2-fold dilutions of the sera, previously inactivated at 56°C for 30 minutes, were made in 0.2 ml volumes and 0.2 ml of the erythrocyte suspension was added to each. After incuba-