Biology Division Research Conference
Gatlinburg, April 5-8, 1965

S+P

Hydrocortisone Stimulation of RNA Synthesis in Induction of Hepatic Enzymes*

FRANCIS T. KENNEY, WESLEY D. WICKS† AND DAVID L. GREENMAN‡

From the Biology Division

Oak Ridge National Laboratory

Oak Ridge, Tennessee

---

*Research sponsored jointly by the National Institutes of Health and the Atomic Energy Commission under contract with the Union Carbide Corporation.

†American Cancer Society postdoctoral fellow.

‡Postdoctoral fellow of the National Cancer Institute, 1963-64. Present address: McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland

Five Tables; Three Figures

DISCLAIMER

Running head: RNA synthesis and enzyme induction

Send proof to: Dr. Francis T. Kenney
Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

ABSTRACT— Increased synthesis of hepatic enzymes due to hydrocortisone is preceded by an increase in the rate of synthesis of nuclear RNA. Pulse-labeled RNA from liver nuclei was fractionated by a differential thermal phenol procedure, and the labeled RNA of each fraction was characterized by sucrose gradient centrifugation and base composition analysis. Hormone treatment increases the rate of synthesis of three types of RNA: (1) the nuclear precursor to ribosomal RNA, (2) a rapid turnover component with base composition similar to the tissue DNA, and (3) transfer RNA. Much of the total isotope incorporation into transfer RNA can be traced to turnover of the terminal adenylate residue, but this type of labeling is insensitive to the hormone. The steroid also stimulates isotope incorporation into tissue precursor pools. The effect is abolished by actinomycin and thus is secondary to the hormonal stimulation of RNA synthesis. Growth hormone stimulates RNA synthesis in both intact and adrenalectomized rats, but induces the rapid turnover enzymes (tyrosine transaminase and tryptophan pyrrolase) only in the presence of functional adrenals. It therefore seems that glucocorticoids initiate both a generalized increase in synthesis of RNA and a selective induction of specific enzymes.

Control of metabolism through induction or repression of the synthesis of specific enzyme proteins is now well established in microbial systems, where metabolites of the affected enzyme are generally the inducing or repressing agents. Intensive study of this mode of control of protein synthesis has led to the widely accepted view that regulation of the activity of specific genetic loci, resulting in differential rates of synthesis of specific mRNAs,[1] is responsible for the differential rate of enzyme synthesis (Jacob and Monod, '61). As yet there is no unambiguous evidence for this type of metabolite-initiated control mechanism in mammalian systems. But the synthesis of enzyme proteins is controlled in mammalian systems, and it is now becoming apparent that this control is mediated by hormones. The hormonal mode of control of protein synthesis has only recently been recognized, and study of its mechanism is only beginning. We will summarize here our studies on the role of RNA synthesis in the induction of hepatic enzymes by adrenal steroid hormones. The data do not yet provide a definitive description of the mechanism of hormonal enzyme induction. They do indicate clearly that a mechanism more complex than selective activation of the structural genes carrying information for the enzymes induced is involved in this mode of enzyme induction.

RNA synthesis during enzyme induction

In these experiments we have employed the technique of pulse-labeling as an approximation to a measure of the rate of in vivo RNA synthesis. By severely limiting the time of exposure to the isotope we can exclude the possibility that changes in the rates of reactions involved in the metabolic utilization of RNA may lead to an artifactual change in the rate of isotope incorporation

into RNA. In the earlier experiments measurements of RNA radioactivity were routinely corrected for variations in the radioactivity of tissue phosphate pools. As will be shown, this procedure is essentially correct, but somewhat imprecise. Details of the experimental procedures can be found in the original publications (Kenney and Kull, '64; Greenman, Kenney and Wicks, '64; Greenman, Wicks and Kenney, '65; Wicks, Greenman and Kenney, '65).

When hydrocortisone is administered to adrenalectomized rats accumulation of the induced tyrosine transaminase begins after a lag of about an hour. Increased enzyme synthesis is preceded by an increase in the rate of nuclear RNA synthesis, but labeling of cytoplasmic RNA is unchanged (Figure 1). These (F-1) results are kinetically consistent with the interpretation that the initial response to hormone is an increased synthesis of one or more nuclear RNA components, which in turn results in increased enzyme synthesis. Inhibition of enzyme induction by actinomycin, a result implicating DNA-directed synthesis of RNA (Greengard, Smith and Aes, '63), is also consistent with this conclusion.

Fractionation of pulse-labeled RNA

In order to determine what kind of RNA is synthesized in response to hormonal stimulation, we used a fractionation procedure originally described by Georgiev, Samarina, Lerman, Smirnov and Severtzov ('63), separating nuclear RNA into four fractions by repeated phenol extractions at successively higher temperatures. Essentially all of the RNA that is labeled in a brief exposure to isotope was recovered, and the RNA present in each fraction was characterized by centrifugation in sucrose gradients and by determinations of base

composition after alkaline hydrolysis. These procedures enabled us to identify each of the three major RNA species, and to determine whether hormone treatment stimulates the synthesis of one or the other of these species.

Ribosomal RNA — Approximately 85% of the total RNA of rat liver is found as the 18 S and 28 S structural RNA components of ribosomes. This RNA is characterized by a high content of G and C (ratio ATU/G + C = 0.63). In the brief labeling periods that we usually employed no isotope can be detected in the ribosomal RNA per se. However about 45% of the total ^{32}P-RNA formed (excluding labeling due to turnover of the terminal nucleotides of tRNA) can be identified as a precursor form of ribosomal RNA. The precursor RNA is synthesized in nucleoli (Perry, '64; Perry, Svinivasan and Kelly, '64) as a high molecular weight fraction that is subsequently transformed into the 18 S and 28 S ribosomal RNAs (Tamaoki and Mueller, '62; Schewer, Latham and Darnell, '63; Perry, '64; Perry et al, '64). Extraction of liver nuclei with phenol at 45 C releases much of the pRNA into the aqueous phase. Treatment with hydrocortisone for two hours causes a doubling of the rate of pRNA synthesis (Table 1). If an isotopic precursor (T-1) is administered for a period of time sufficiently long to permit conversion of pRNA to yRNA, the hormone effect is apparent on the latter component as well (Figure 2). Thus we concluded that synthesis of the major RNA component of (F-2) the liver, ribosomal RNA, is stimulated by the hormone.

DNA-like RNA — This component is not readily identifiable in functional terms, and we have used the operational designation "DNA-like" (dRNA) to identify the rapidly-labeled RNA that is relatively rich in A and U and thus resembles the liver DNA (ratio A + U/G + C = 1.34). About 50% of the total pulse-labeled

RNA is identified as dRNA by base composition and by its extensive heterogeneity of size distribution. Phenol extraction at 85 C in the presence of detergent yields a fraction that is largely dRNA, and hormone treatment doubles the rate of labeling of this component (Table 1). By comparison of the ratios of percent of labeled RNA formed to percent of total RNA we can arrive at an approximation of the relative turnover rates of the various RNA species in vivo. This calculation shows that the turnover of dRNA is 50 to 100 times faster than that of rRNA or tRNA, while turnover of the latter two RNAs is of the same order of magnitude (Table 2). Rapid turnover is a property associated with the concept (T-2) of mRNA, as are DNA-like composition and size heterogeneity, and it is thus probable that mRNA is the proper functional designation for what we term dRNA-final identification of the dRNA component in functional terms must, however, await the development of meaningful functional assays. Whatever the proper designation of dRNA, it is apparent that hormone treatment stimulates its labeling to the same extent as that of pRNA.

Transfer RNA – In our studies on labeling of tRNA we have usually extracted the entire liver homogenate, rather than the nuclear fraction as in the studies described above. Phenol extraction of liver homogenates at 3 C yields a fraction which contains most of the unlabeled RNA and about 30% of the ^{32}P-RNA formed in a brief labeling period. The labeled RNA sediments with a peak at 4 S. Much of this 4 S RNA is not transfer RNA (Greenman, Kenney and Wicks,'64), but appears to be partially degraded chains of dRNA and pRNA. Transfer RNA was isolated by salt fractionation and DEAE-chromatography and identified by its ability to act as acceptor for amino acids in aminoacyl-RNA formation. After alkaline hydrolysis much of the ^{32}P of tRNA is found as CMP, reflecting turnover

of the -pCpCpA terminus of the polynucleotide chain. The terminal nucleotides can be selectively removed by partial phosphodiesterase digestion (Anthony, Starr, Kerr and Goldthwait, '63). When this is done it becomes apparent that turnover is limited to the terminal adenylate, and that this labeling is essentially insensitive to hormone treatment. Alkaline hydrolysis of the remaining tRNA yields a pattern of ^{32}P-labeling that is indicative of de novo synthesis, and (T-3) hormone treatment stimulates this synthesis to the same extent as that of pRNA and dRNA (Table 3).

Specificity of enzyme induction

The experiments summarized above clearly indicate that synthesis of all three of the major RNA species of liver is stimulated by hydrocortisone. The hormonal mode of enzyme induction is thus apparently not explained by models wherein enzyme synthesis is controlled by mRNA levels alone. However, we are left with the difficult problem of reconciling a general increase in the synthesis of RNA with what appears to be specific induction of selected enzymes. One solution to the problem is to assume that one of the other of these results is incorrect, e.g., perhaps enzyme induction is not really specific at all. Support for this suggestion can be found in the fact that two rapidly induced enzymes, tyrosine transaminase and tryptophan pyrrolase, and induced at identical rates and to the same extent by hydrocortisone. Each of these enzymes has a turnover time (half-life) in vivo of 2 to 3 hr, and thus both rate and extent of enzyme induction appear to be strictly a function of turnover time. This is entirely consistent with a mechanism wherein synthesis of all the liver proteins is increased, since specific proteins would then be expected to increase in amount at rates determined by their half-life.

Growth hormone and enzyme induction – If the interpretation given above is correct, we would expect that any means by which hepatic protein synthesis is increased would result in apparent inductions of the same enzyme that are induced by hydrocortisone. Growth hormone is known to effect increases in hepatic protein synthesis (cf. Korner, this volume), and in a recent study Talwar, Gupta and Gros ('64) demonstrated increased synthesis of both pRNA and dRNA following growth hormone treatment. Synthesis of tRNA was not studied, but in all other respects the hepatic response in RNA and protein syntheses seem to be nearly the same, whether stimulated by growth hormone or by glucocorticoids. However growth hormone does not result in induction of the enzymes induced by hydrocortisone, if the test animals are adrenalectomized (Figure 3). We established that growth (F-3) hormone does stimulate RNA synthesis in the absence of adrenals (Table 4) and (T-4) hence it is clear that, not withstanding the similarity in response of RNA synthesis, a specific action of glucocorticoids is necessary for induction of these enzymes. This is substantiated by the slight induction which does occur in the animals with functional adrenals. It would thus appear that enzyme induction is selective and not a consequence of a general increase in protein synthesis.

Nucleotide pools; effect of actinomycin

The obvious alternative solution to the reconciliation problem discussed above is the possibility that the general response in RNA synthesis is incorrect, and represents an experimental artifact. Such an error could come about if the primary action of the hormone was to increase the rate at which isotope is incorporated into nucleotide precursors of RNA, and subsequent incorporation of the nucleotides into the various RNA species would yield

an apparent increase in overall RNA synthesis. This possibility was investigated with a technique described by Ycas and Vincent ('60), in which the entire tissue homogenate is subjected to the conditions of alkaline hydrolysis required to digest RNA to constituent 2' - 3' nucleotides. Alkali also causes hydrolysis of nucleoside triphosphates to the monophosphate level, and hence after incubation the mixture contains the precursor 5'-nucleotides and the product 2' - 3'-nucleotides. The adenine nucleotides of such digests were separated by chromatography on Dower - 1 columns and DEAE papers. The results of such an experiment, where effects of actinomycin on the response to hormone were also studied, are presented in Table 5. The limited enzyme induction seen after 2 hr of hormone treatment was completely abolished by actinomycin (cf. Greengard et al., '63). An unusually large increase in P_i specific activity was appreciably reduced by the antibiotic, which inhibited RNA synthesis in control animals by about 50%. Synthesis of RNA (measured as 3' - AMP) was doubled by the hormone, but not in animals given both hormone and actinomycin. Labeling of the RNA precursor, 5' - AMP, was increased by hormone treatment to the extent of about 50%, but this increase, too, was abolished by actinomycin. This stimulation of the labeling of precursor nucleotides is secondary to the hormonal stimulation of RNA synthesis, and probably reflects release of feedback inhibitions of the type studied by Wyngaarden and Ashton ('59). (T-5)

While the enhancement of nucleotide synthesis is secondary to increased RNA synthesis, it does lead to increased labeling of RNA that is greater than the actual increase in RNA synthesis. Measurements of the rate of RNA synthesis must then be corrected for this effect, and this can be done by measuring

radioactivity of 5' - nucleotides and correcting RNA measurements appropriately. Transfer RNA was isolated from the same livers used in the analyses of Table 5. When corrected for 5' - AMP, internal labeling of tRNA was increased by the hormone to about 50%, and labeling due to terminal turnover was completely unaffected. Thus it is clear that earlier experiments, wherein RNA measurements were corrected only for P_i radioactivity, have indicated a hormone effect somewhat larger than the actual increase in rate of RNA synthesis. Nevertheless the results indicating increased synthesis of all three species of RNA are essentially correct.

The dilemma then remains, for we observe hormonal stimulation of the synthesis of all major species of RNA and specificity in the response of enzyme synthesis. It is possible that there are specific effects on RNAs within the three categories examined, and that our analyses thus far have been too gross to detect these changes. This can be tested by further fractionation of each type of rapidly-labeled RNA. A second possibility is that specificity in enzyme synthesis reflects an unknown mechanism of control at the level of translation, i.e., in the final assembly of amino acids. The existence of control mechanisms of this type, and their significance in the hormonal mode of induction, are suggested by the recent repression studies of Garren, Howell, Tomkins and Crocco ('64).

Literature cited

Anthony, D. D., J. L. Starr, D. S. Kerr, and D. E. Goldthwait 1963. The incorporation of nucleotides into amino acid transfer ribonucleic acid, J. Biol. Chem. 238: 690-696.

Garren, L. D., R. R. Howell, G. M. Tomkins and R. M. Crocco. A paradoxical effect of actinomycin D: the mechanism of regulation of enzyme synthesis by hydrocortisone. Proc. Natl. Acad. Sci. U.S. 52: 1121-1129.

Georgiev, G. P., O. P. Samarina, M. I. Lerman, M. N. Smirnov and A. N. Severtzov 1963, Biosynthesis of messenger and ribosomal ribonucleic acids in the nucleolochromosomal apparatus of animal cells, Nature 200: 1291-1294.

Greengard, O., M. A. Smith and G. Aes. 1963, Relation of cortisone and synthesis of ribonucleic acid to induced and developmental enzyme formation, J. Biol. Chem. 238: 1548-1551.

Greenman, D. L., F. T. Kenney and W. D. Wicks 1964, On equating low molecular weight RNA with transfer RNA, Biochem. Biophys. Res. Communs. 17: 449-453.

Greenman, D. L., W. D. Wicks and F. T. Kenney 1965, Stimulation of RNA synthesis by steroid hormones. II High molecular weight components, J. Biol. Chem., in press.

Jacob, F. and J. Monod 1961, Genetic regulatory mechanisms in the synthesis of proteins, J. Mol. Biol. 3: 318-356.

Kenney, F. T. and F. J. Kull 1963, Hydrocortisone-stimulated synthesis of nuclear RNA in enzyme induction, Proc. Natl. Acad. Sci. U.S. 50: 493-499.

Perry, R. P. 1964, The role of the nucleolus in RNA metabolism and other cellular processes, Natl. Cancer Inst. Monograph 14: 73-81.

Perry, R. P., P. R. Srinivasan and D. E. Kelley 1964, Hybridization of rapidly labeled nuclear ribonucleic acids, Science 145: 504-506.

Scherrer, K., H. Latham and J. E. Darnell 1963, Demonstration of an unstable RNA and of a precursor to ribosomal RNA in Hela cells, Proc. Natl. Acad. Sci. U.S. 49: 240-245.

Talwar, G. P., Gupta, S.H., and F. Gros 1964, Effect of growth hormone on ribonucleic acid metabolism. 3. Nature and characteristics of nuclear subfractions stimulated by hormone treatment, Biochem. J. 91: 565-572.

Tamaoki, T. and G. C. Mueller 1962, Synthesis of nuclear and cytoplasmic RNA of Hela Cells and the effect of actinomycin D. Biochem. Biophys. Res. Communs. 9: 451-454.

Wicks, W. D., D. L. Greenman and P. T. Kenney 1965, Stimulation of RNA synthesis by steroid hormones. I Transfer RNA, J. Biol. Chem., in press.

Wyngaarden, J. B., and D. M. Ashton 1959, The regulation of activity of phosphoribosylprobphosphate amido transferase by purine ribonucleotides: A potential feedback control of purine biosynthesis, J. Biol. Chem. 234: 1492-1496.

Yeas, M. and W. S. Vincent 1960, A ribonucleic acid fraction from yeast related in composition to desoxyribonucleic acid, Proc. Natl. Acad. Sci. U.S. 46: 804-811.

Footnotes

* Research sponsored jointly by the National Institutes of Health and the Atomic Energy Commission under contract with the Union Carbide Corporation.

† American Cancer Society postdoctoral fellow.

‡ Postdoctoral fellow of the National Cancer Institute, 1963-64. Present address: McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland

1. The various RNA species discussed are abbreviated as follows: mRNA, messenger RNA, dRNA, DNA-like RNA; rRNA, ribosomal RNA; pRNA, the nuclear precursor to ribosomal RNA, and tRNA, transfer RNA. The designations A, G, U and C refer to adenylic, guanylic, uridylic and cytidylic acids, respectively.

Table 1. Hydrocortisone effect on pulse-labeling of high molecular weight components of nuclear RNA

Extraction Conditions	Major ^{32}P-RNA Component	Relative Specific Activity of RNA	
		Untreated	+ Hydrocortisone
45°	pRNA	2290	5660
85° + SDS	dRNA	6070	14500

Relative specific activity is cpm per mg RNA/cpm per $\mu\mu$ mole P_i.

Adapted from Greenman et al ('65).

Table 2. Estimated relative turnover rates of liver RNAs

Component	% of total* ^{32}P-RNA Formed in Pulse (a)	% of Total Liver RNA (b)	Ratio a/b
rRNA	45	85	0.53
tRNA	4	12	0.33
dRNA	50	1.5	33

* Excluding labeling due to turnover of terminal A of tRNA

Table 3. Distribution of isotope in transfer RNA after limited phosphodiesterase treatment.

Hydrocortisone Treatment	Terminal Labeling (as 5'-AMP)	Internal Labeling, Total cpm in			
		AMP	GMP	UMP	CMP
-	5350	450	650	850	550
+	7350	1250	1800	1900	1850

Phosphodiesterase (snake venon) treatment was for 1 hr at 15°. Terminal labeling is that rendered acid-soluble by this treatment, and was essentially all in 5'-AMP. Internal labeling determined after alkaline hydrolysis of the RNA not hydrolyzed by the enzyme. The data are recorded as cpm per nucleotide/ cpm per μμ mole acid-soluble AMP, and have been corrected for different amounts of RNA recovered. Adapted from Wicks et al ('65).

Table 4. Growth hormone effects on RNA and enzyme synthesis

Treatment	Relative Specific Activity of Nuclear RNA	Tyrosine Transaminase (Units/mg Protein)
Adrenalectomized	2.50	7.0
Adrenalectomized + STH	4.06	7.2
Sham	2.91	12.8
Sham + STH	4.02	20.0

Relative specific activity is cpm per mg RNA/cpm per $\mu\mu$ mole P_i. ^{32}P was given 20 min, and growth hormone (STH, 3 IU/100 g) 4.5 hr before the animals were killed.

Table 5. Effects of actinomycin on early responses to hydrocortisone

Component	No Actinomycin D		+ Actinomycin D	
	Control	+ F	Control	+ F
Tyrosine Transaminase (Units/mg protein)	26 ± 2	63 ± 4	23 ± 1	20 ± 1
Inorganic Phosphate (cpm/μμ mole)	2.5 ± .2	6.2 ± .3	2.3 ± .1	3.2 ± .3
5'-AMP $\left[\frac{\text{cpm/m}\mu\text{ mole}}{\text{cpm/}\mu\mu\text{ mole P}_i}\right]$	70 ± 6	108 ± 4	60 ± 3	69 ± 0
RNA -3' AMP $\left[\frac{\text{cpm/m}\mu\text{ mole}}{\text{cpm/}\mu\mu\text{ mole P}_i}\right]$	1.1 ± .1	2.2 ± .2	0.52 ± .01	0.50 ± .07

Actinomycin (150 μg/100 g) was given 3 hr, hydrocortisone (F 2.5 mg/100 g) 2 hr, and ^{32}P (2 mc) 20 min before the animals were killed. The data are the mean ± S.E. of 2 or 3 animals in each group.

Legends to Figures

12.187 Figure 1. Stimulation of nuclear RNA synthesis during enzyme induction. Relative specific activity is cpm per mg RNA/cpm per μ mole P_i X100. From Kenney and Kull ('63).

13,979 Figure 2. Hydrocortisone effect on synthesis of ribosomal RNA. Hormone treatment was for 4 hr, and C^{14}-oroticte (10 μc, S.A. 6.5 μc/μ mole) was given 2 hr before the animals were killed. The RNA was prepared by phenol - SDS extraction of the microsome fraction of liver - from Greenman et al ('65).

14,165 Figure 3. Growth hormone effects on rapid turnover enzymes of rat liver.

Fig. 1

12,187

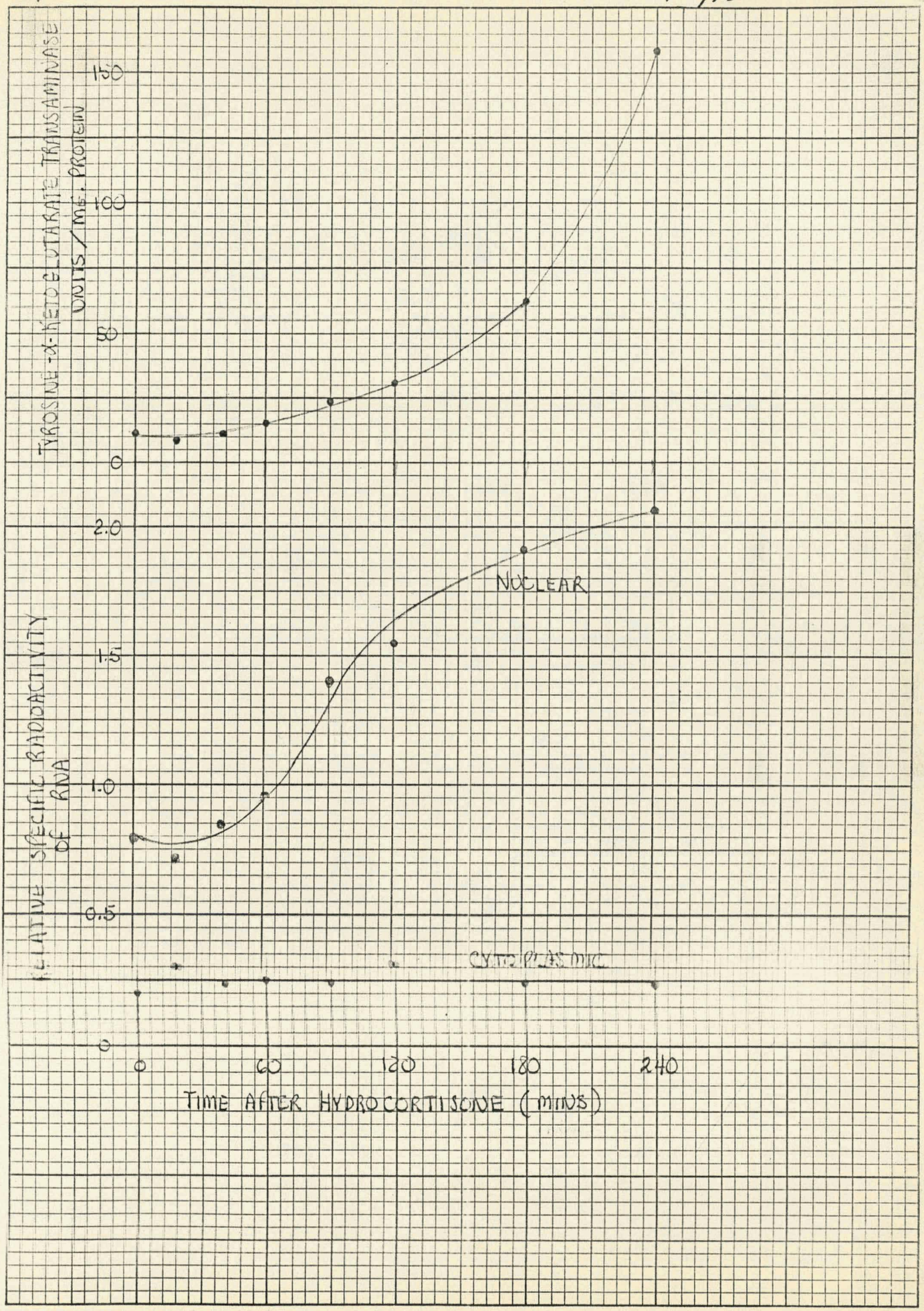

Figure 2

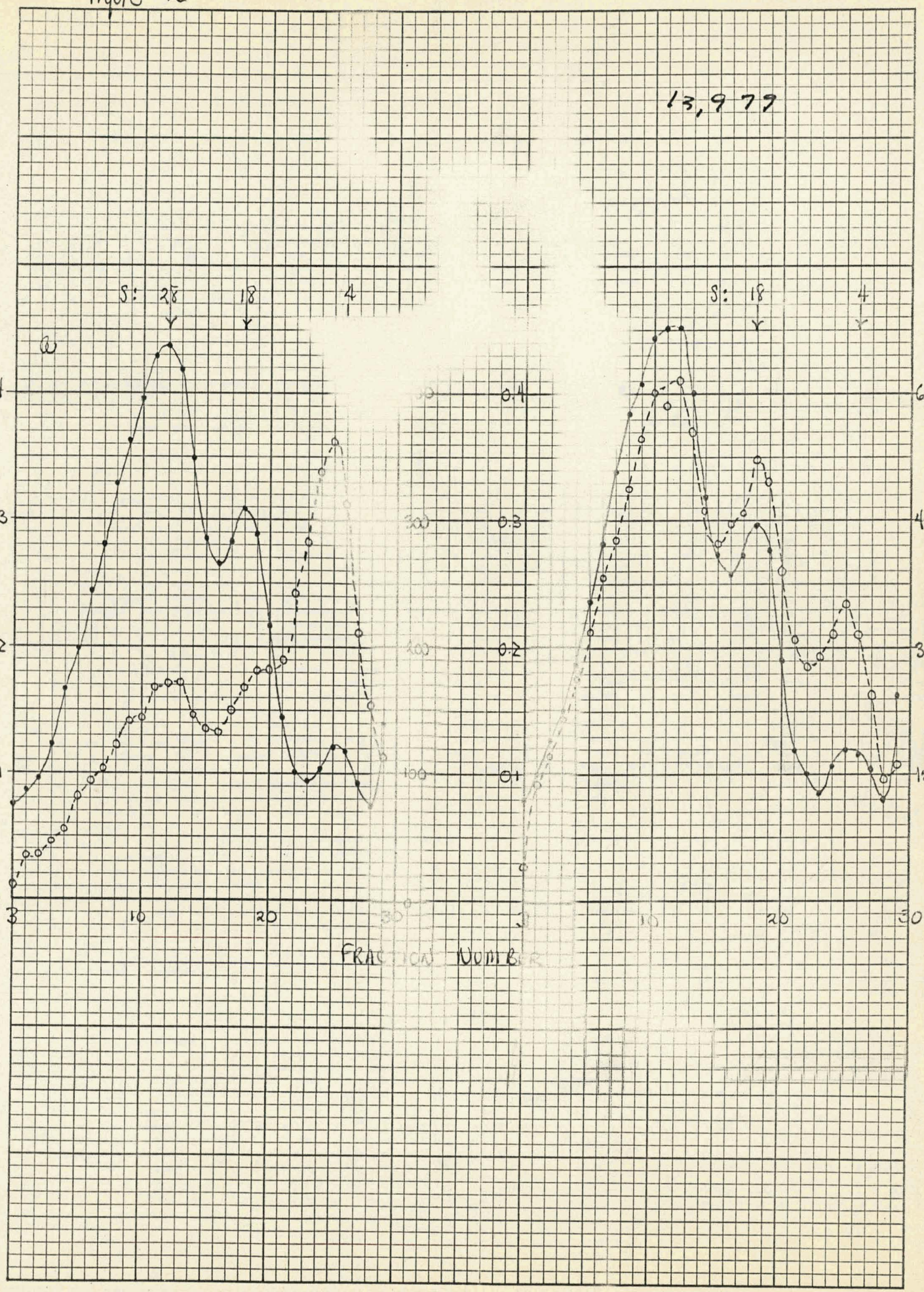

Fig 3

14,165

Kenney 3-5733

Growth Hormone Effects on Rapid Turnover Enzymes in Intact and Adrenalectomized Rats