acceptor activities that are clearly in the sensitive group are for amino acids with two, and apparently only two, known codons. We can speculate that pyrimidines that are sensitive to osmium tetroxide are involved in allowing two forms of base-pairing in the third position of the codon. This explanation has the advantage of accounting for the sensitivity of phenylalanine-acceptor activity. On either type of hypothesis, the results provide strong support for other arguments that the aminoacyltransferases interact specifically with the anticodon segment of the t-RNA (Ebel, Weil, Rether & Heinrich, 1965; Hayashi & Miura, 1966).

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The 'Permissive' Effect of Hydrocortisone on the Induction of δ-Aminolaevulate Synthetase

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The concept of permissibility of hormone action has been extensively reviewed by Ingle (1954). A hormone plays a permissive role in a process when a stimulus produces a specific effect in the presence of the hormone but not in its absence (Ingle, 1954). Although hydrocortisone has been shown to exert such an effect on a number of tissue responses (Ingle, 1954; Ingle, Ward & Kuizenga, 1947), a permissive role of this hormone in enzyme synthesis has not been previously demonstrated.

The present findings demonstrate the permissive effect of hydrocortisone on the induction of hepatic δ-aminolaevulate synthetase, the rate-controlling enzyme of haem biosynthesis in the liver (Granick, 1963; Granick & Urata, 1963; Tschudy, Welland, Collins & Hunter, 1964). This enzyme catalyses the reaction between pyridoxal phosphate-activated glycine and succinyl-CoA to form δ-aminolaevulate (Wriston, Lack & Shemin, 1955; Gibson, 1958; Kikuchi, Kumar, Talmage & Shemin, 1958). Administration of certain compounds, including allylisopropylacetamide, causes a marked increase in the RNA-dependent synthesis of δ-aminolaevulate synthetase in the livers of rodents or in cultured chick-embryo liver cells (Granick & Urata, 1963; Granick, 1963; Tschudy *et al.* 1964; Marver, Tschudy, Perlroth & Collins, 1965; Tschudy, Marver & Collins, 1965*a*). A single subcutaneous injection of allylisopropylacetamide to starved rats causes a three- to five-fold increase of hepatic δ-aminolaevulate synthetase after 1hr. and a maximum 20–25-fold increase in 12–24hr. This rise is prevented by puromycin, actinomycin or glucose. The half-life of the induced enzyme after puromycin administration is 67–72min. (Marver *et al.* 1965; Tschudy *et al.* 1965*a*). The excessive excretion of porphyrin precursors in the genetic disease acute intermittent porphyria results from a marked increase in the activity of hepatic δ-aminolaevulate synthetase (Tschudy *et al.* 1965*b*).

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δ-Aminolaevulate synthetase was measured in liver homogenates (Tschudy *et al.* 1965*a*; Marver, Tschudy, Perlroth, Collins & Hunter 1966*b*) prepared in 3 vol. of 0·9% NaCl in 0·5 mM-EDTA–10 mM-tris–HCl buffer, pH 7·4. The incubation mixture contained 0·5 ml. of homogenate, 200 μmoles of glycine, 20 μmoles of EDTA, 150 μmoles of tris-HCl buffer at a final pH of 7·2 and in a total volume of 2·0 ml. The incubations were performed in air at 37° for up to 1 hr. and then terminated by adding 0·5 ml. of 25% (w/v) trichloroacetic acid. The amino ketones present were converted into pyrroles by reaction with acetylacetone at pH 4·6 and separated on Dowex 1 (acetate form) (Marver, Tschudy, Perlroth, Collins & Hunter, 1966*b*). The δ-aminolaevulate pyrrole (3-acetyl-2-methyl-4-propionic acid pyrrole) thus separated was determined by reaction with modified Ehrlich's reagent (Mauzerall & Granick, 1956).

The rate of δ-aminolaevulic acid production was constant in porphyric-liver homogenates for at least 60 min. and for 30–40 min. in liver homogenates from non-porphyric rats. In previous studies, the generation of succinyl-CoA from endogenous substrates was close to the optimum amount required for δ-aminolaevulate production in liver homogenates (Marver *et al.* 1966*a*). This also appeared to be true of the liver homogenates prepared from the adrenalectomized and hydrocortisone-treated rats used in this study. Exogenous succinyl-CoA (20 μmoles to 1·8–2·0 ml. of incubation mixture every 10 min.) did not significantly augment δ-aminolaevulate synthesis in liver homogenates prepared from the livers of adrenalectomized rats (8% increase) or adrenalectomized rats treated 5 hr. previously with 5·0 mg. of hydrocortisone/100 g. (3% increase).

As shown in Table 1, administration of allyl-

Table 1. *Permissive effect of hydrocortisone on the induction of hepatic δ-aminolaevulate synthetase*

Both intact and adrenalectomized Sprague–Dawley female rats were starved for 36 hr. before the administration of allylisopropylacetamide subcutaneously or hydrocortisone, intraperitoneally, or both, and starvation of the rats was continued until they were killed. After adrenalectomy, rats were maintained on 1% NaCl for 1 week before they were used and during the course of the experiment. Groups of four rats were studied under each of the conditions outlined below. δ-Aminolaevulate-synthetase assays were performed on the pooled liver homogenates of each group of animals.

Compounds administered (mg./100 g.)		Time of administration (hr. before killing of rats)	δ-Aminolaevulate-synthetase activity (mμmoles of δ-aminolaevulate/g. of liver/hr.)	
Allylisopropyl-acetamide	Hydrocortisone		Expt. 1	Expt. 2
Intact rats				
—	—		17·9*	
—	1·0	5	18·0	
—	5·0	5	23·8	
40	—	5	172·0	
40	1·0	5	169·4	
40	5·0	5	177·3	
Adrenalectomized rats				
—	—		18·8	15·8
—	0·2	5	22·9	
—	0·5	5	24·2	
—	1·0	3		18·0
—	1·0	5	22·1	16·2
—	1·0	12		20·1
—	5·0	3		20·2
—	5·0	5	32·2	26·5
—	5·0	12		19·3
—	5·0	16, 11, 5		24·9
40	—	5	41·7	47·0
40	0·2	5	153·0	
40	0·5	5	161·0	
40	1·0	5	154·2	181·0
40	5·0	5	167·6	

* The range of values of δ-aminolaevulate-synthetase activity in 89 starved Sprague–Dawley female rats as determined by the present method was 8–24 mμmoles/g. of liver/hr. (Marver *et al.* 1966*a*).

isopropylacetamide and various doses of hydrocortisone (0·2–5·0mg./100g.) to adrenalectomized rats produced a marked increase of hepatic δ-aminolaevulate synthetase comparable with that produced in intact animals given only allylisopropylacetamide. This response did not appear to depend on the amount of hydrocortisone within the given range of dosages. However, when allylisopropylacetamide alone was given to adrenalectomized rats, only a small rise in δ-aminolaevulate-synthetase activity was noted. Adrenalectomy did not affect the normal hepatic activities of δ-aminolaevulate synthetase. Administration of hydrocortisone slightly and variably increased hepatic δ-aminolaevulate synthetase only when given in amounts (5·0mg./100g.) far in excess of those required by adrenalectomized rats for maximal induction of δ-aminolaevulate synthetase by allylisopropylacetamide. The small increase in δ-aminolaevulate synthetase resulting from 5·0mg. of hydrocortisone/100g. was not enhanced by repeated injections of that dosage of the hormone.

Since the stimulus of injections plus 1·0mg. of hydrocortisone/100g. did not increase hepatic δ-aminolaevulate-synthetase activity in intact rats, induction of this enzyme, unlike several other mammalian enzymes, can be studied in intact rather than in adrenalectomized rats. A dosage of 1·0mg. of hydrocortisone/100g. is adequate for the induction of a number of liver enzymes in adrenalectomized rats. At 4–5hr. after the administration of 1·0mg. of cortisone/100g., or hydrocortisone to adrenalectomized rats, there is a fourfold increase of tryptophan pyrrolase (Feigelson & Greengard, 1962) and a tenfold increase of tyrosine–α-oxoglutarate transaminase (Kenney & Flora, 1961).

The effect of adrenalectomy on depressing the allylisopropylacetamide-mediated increase of hepatic δ-aminolaevulate synthetase is not related to the production of inhibitors of enzyme activity. The addition of equal quantities of liver homogenates of adrenalectomized rats to porphyric-rat-liver homogenates did not decrease δ-aminolaevulate production.

Thus, though hydrocortisone does not appreciably induce hepatic δ-aminolaevulate synthetase, it is necessary for the allylisopropylacetamide-mediated induction to occur to a significant extent. The effect of hydrocortisone on the induction of δ-aminolaevulate synthetase would appear to fulfil the criteria of permissibility of hormone action. Among previous studies of the effect of hydrocortisone on hepatic enzyme activities (Knox, Auerbach & Lin, 1956; Civen & Knox, 1959; Feigelson, Feigelson & Greengard, 1962; Weber, 1963) there is no example of an enzyme that is not induced significantly by hydrocortisone but whose induction requires hydrocortisone.

The mechanism of this permissive effect of hydrocortisone on hepatic δ-aminolaevulate induction is unknown, although it may be related to the stimulatory effect of the hormone on the synthesis of RNA (Garren, Howell & Tomkins, 1964).

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