Effect of Indoleacetic Acid on Protein and Ribonucleic Acid Synthesis

Though the mechanism of the action of indoleacetic acid (IAA) on growth has not yet been fully elucidated, it has been shown unequivocally that IAA application results in an alteration of various metabolic pathways[1]. In this connection, particular mention should be made of metabolism of nucleic acids[2–4] and also of protein synthesis[5]. From the work of NOODEN and THIMANN[5], it is apparent that the locus of action of auxin in cell enlargement is on a nucleic acid system controlling synthesis of an essential protein. We wish to report our data on RNA and protein synthesis to pinpoint the exact stage at which these are affected in presence of IAA.

Peas obtained from the market were germinated at 25°C. 250–300 sections (10 mm) from the internode were suspended in 0.05*M* Tris buffer pH 7.0 containing $10^{-5}M$ IAA for 20 h. Another set was similarly kept without IAA, as a control. The sections were then washed thoroughly with water and dried with filter paper. Ribosomes and pH 5 fractions were isolated from those sections according to the method of Tso' et al.[6]. Protein and RNA content was estimated by biuret[7] and Orcinol test[8] respectively. Radioactive pyrophosphate was prepared according to the method described earlier[9]. Aminoacyl-sRNA was isolated by the method of HOAGLAND et al.[10].

When pyrophosphate exchange was determined with pH 5 fractions from the control and treated tissues in the presence of 20 amino acids, it was observed that there was no stimulation. The synthesis of aminoacyl-sRNA was studied with pH 5 fractions in the presence of C^{14}-amino acids (obtained from algal protein hydrolysate, Radiochemical Centre, Amersham, UK). Incorporation of amino acid into RNA was not found to be stimulated in the experiment. But when incubated in the presence of RNP particles from the experiment, a 1.5–2 fold increase in the acid-precipitable fraction was observed. The data are given in Table I. Then a hybrid experiment was designed. The pH 5 fraction from the control, and RNP from the treated, and vice versa, when incubated with C^{14}-amino acid mixture, only a stimulation of about twofold was observed in the former case. Ribosomes isolated from the treated tissue and amino acid loaded sRNA from the control tissue, when allowed to synthesize protein, showed higher incorporation of amino acids into the acid-insoluble fraction than that of the control. The data are recorded in Table II.

From the above data it is apparent that under the influence of IAA no change occurs in the capacity of aminoacyl sRNA synthesis, but a consistent change is discernible with RNP particles so far as amino acid incorporation is concerned. When analysed for the RNA content of the ribosomal pellet, it was observed that there is a 12–13% increase in the case of treated ones over the control. If 3–4% of the total RNA is mRNA, and 40% is the ribosomal RNA, then in terms of rRNA, mRNA will be approximately 9–10%, which may partly be correlated with the increase of RNA from treated ribosomes, provided all the mRNA synthesized in the IAA-treated tissue is in association with the ribosomes. According to the observation of IWAMOTO et al.[11], TALWAR et al.[12] and KORNER[13], administration of growth hormone to rats increases the rates of incorporation of labelled precursor into nuclear RNA of rat liver. With the application of IAA, metabolism of nuclear RNA has also been found to be mostly affected in the present case[14]. In terms of the chemical

Table I. Pyrophosphate exchange and amino acid incorporation by pH 5 and RNP fractions

Experiment No.	Fraction	Control cpm/mgP	Treated cpm/mgP	Labelled compound
1	pH 5	180	209	P^{32} P^{32}
2	pH 5	6,500	6,300	C^{14}-algal hydrolysate
3	RNP + pH 5	7,400	10,300	

Complete system in the case of experiment no. 1 contains the following expressed in μmoles in a final volume of 1 ml: Tris pH 7.4, 100; $MgCl_2$, 5; ATP, 3; KF, 5; mixture containing 20 1-amino acids, 0.5 each; P^{32} P^{32}, 0.1 ml ($2.9 \cdot 10^6$ cpm/ml) and pH 5 enzyme, 1 mg. After 20 min incubation at 37°C, reaction was stopped with $HClO_4$, washed, and plated for counting after adsorption on activated charcoal. For experiment no. 2, besides Tris, $MgCl_2$ and ATP, C^{14}-algal protein hydrolysate ($6.5 \cdot 10^6$ cpm/mg/ml), 0.1 ml was added, and for experiment no. 3, KCl 20, GTP 2, creatin kinase 0.5 mg, creatin phosphate 1, and RNP 0.95 mg were added. Incubated for 60 min. *Abbreviations.* IAA, indole acetic acid; mRNA, messenger ribonucleic acid; rRNA, ribosomal ribonucleic acid; sRNA, soluble ribonucleic acid; RNP, ribonucleoprotein particle; ATP and GTP, adenosine and guanosine nucleoside-5'-triphosphate respectively.

Table II. Transfer of amino acids from aminoacyl sRNA in presence of treated and non-treated ribosomes

Control Aminoacyl sRNA	RNP	Experimental Aminoacyl sRNA	RNP	cpm/mg ribosomal protein
+	+	–	–	250
–	–	+	+	560
–	+	+	–	290
+	–	–	+	520

Aminoacyl sRNA was prepared by incubating pH 5 fraction from the treated and control ribosomes with C^{14}-algal protein hydrolysate as indicated in the 'Method'. Aminoacyl sRNA containing 2000 cpm, RNP 1.1 mg was used. Other conditions were the same as indicated in Table I.

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mechanism of protein synthesis, a question that might be posed is which ribosomal constituents are responsible for the variation in activity of ribosomes. It has also been observed that the nucleotide composition of the ribosomal RNA does not change significantly throughout the incubation phase, but a consistent variation in the RNA/protein ratio of the ribosomal pellet can be found. The change in the rate of synthesis of mRNA suggests that the messenger RNA content of the ribosome may differ. Such a condition could also lead to a variation of the polysome content of the cell, and these questions are now being investigated[15].

Zusammenfassung. Unter dem Einfluss der Indolessigsäure ist die Aminoacyl-SRNA-Synthese nicht verändert, dagegen zeigen die Ribosomen eine gesteigerte Fähigkeit von Aminosäure-Inkorporation.

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The Postnatal Development of Hormone Sensitive Lipase in Brown and White Adipose Tissue of the Rat

It has been shown in previous work that in suckling rats the blood level of free fatty acids (FFA) is very high and decreases during starvation (NOVÁK et al.[1]). Administration of adrenalin or noradrenalin to such animals does not lead to the rise in the blood FFA level observed in adult rats (SKÁLA et al.[2]) nor does the level of blood glucose change. Since FFA are mobilized from adipose tissue, and since hormone sensitive lipase seems to be responsible, at least in part, for this mobilization (VAUGHAN et al.[3]), the activity of this enzyme was determined in rat adipose tissue during postnatal development.

Lipase activity was determined according to VAUGHAN et al.[3] in brown and white adipose tissue after homogenization with 10 times its volume of 0.25*M* KCl, and after incubation of 0.1 ml of this homogenate in 0.9 ml Krebs-Ringer phosphate solution (pH 7.4) with 3% bovine serum albumin (fraction V) for 5–30 min at 37°C. The FFA content at the beginning and end of incubation was determined colorimetrically according to NOVÁK[1]. The rate of FFA production was linear for at least 20 min, and hence only values for the 20 min incubation period are given. Brown adipose tissue was collected from between the shoulder blades and samples from two animals were usually pooled. White adipose tissue came from the subcutaneous layer above the knee joint. In new-born rats there is hardly any white adipose tissue present and hence only brown tissue was studied.

It is apparent from Table I that lipase activity is low in both brown and white adipose tissue in the suckling period and rises between day 10 and 18, and that this is not due to changes in fat or fat free dry matter content, since these occur mostly between day 1 and 10 (Table I). Thus at a time when the level of FFA in the blood of fed infant rats decreases and is raised during starvation, lipase activity is seen to increase. In white adipose tissue, lipase activity in adult rats is much lower than in brown adipose tissue.

In view of the fact that the rate of acetoacetate production by rat liver slices has been shown to decrease between day 10 and 18 postnatally, and that the administration of corticosterone before day 10 leads to a premature fall in this rate (HAHN et al.[4]), the effect of corticosterone was also tested. 1 mg corticosterone/100 g body weight/day was injected into rats for 3 days between day 7 and 10 and lipase activity was again examined. An increase in activity was noted in both white and brown adipose tissue (Table II). It was not possible, however, to delay the rise in activity by adrenalectomy performed on day 14 or by feeding a high fat diet from day 14.

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Table I. Development of hormone sensitive lipase activity and tissue composition of white and brown adipose tissue

Age (days)	Fat content (% wet weight)[a]		Fat free dry substance (% wet weight)[a]		Lipase activity (μEq/g/20 min)	
	white	brown	white	brown	white	brown
1	0	14 ± 2.3	7 ± 1.2	18 ± 2.0	–	2.3 ± 0.70
10	65 ± 3.1	40 ± 4.0	8 ± 1.3	11 ± 2.1	3.5 ± 0.68	3.0 ± 0.10
21	62 ± 3.6	40 ± 3.5	9 ± 2.0	14 ± 2.3	8.3 ± 0.14	8.6 ± 0.20
Adult males	48 ± 4.5	55 ± 3.2	16 ± 2.1	14 ± 2.6	5.8 ± 0.25	8.0 ± 0.21

[a] Determined by differential weighing after drying to constant weight and fat extraction with petroleum ether. 6 to 10 animals for each group.