endosperm is devoid of this enzyme activity and the embryo axis contains much higher enzyme level than the scutellum, it can be concluded that GAD of dry seeds is located in dry embryos. Consistent results were reported for GAD of barley seeds[9]. The GAD activities of embryos were then determined at different times of seed germination, both in the absence and in the presence of PLP. As shown in the Figure, during the first 3 h of germination, the enzyme activity maintains values close to those of the dry embryos. A significant increase is observed after 6 h of germination, when the GAD specific activity attains its maximum value. Then the enzyme levels progressively decrease during the following germination time. PLP enhances significantly the GAD activity throughout all the germination time, with higher effect in the late period. Although this activation cannot directly account for the variations of GAD activity during germination, as observed in the Figure, the possibility could be raised of a control of GAD level through the regulation of synthesis and breakdown of the apoenzyme, that was shown in mammalian organs to undergo preferential attack by group specific proteases[10]. An alternative or additional regulation mechanism, explaining the variations of GAD activity during the seed life-span, could be provided by the concentration of nucleotides and Pi. In fact, partially purified preparations of GAD from wheat embryos were found to be sensitive to 'physiologic' concentrations of these metabolites[11]. A deeper insight into these problems seems to be conditioned by further purification and characterization of the plant GAD, as well as by further investigations on amino acid and nucleotide metabolism in plants[12].

Riassunto. La glutammico decarbossilasi è localizzata nell'embrione di semi secchi di *Triticum durum*, mentre l'endosperma ne risulta praticamente privo. Durante la germinazione si ha un aumento dell'attività specifica GAD alla sesta ora di imbibizione, seguita da una lenta e progressiva diminuzione per le seguenti 24 ore. L'enzima è attivato dal piridossal-5'-fosfato, ma le variazioni di attività GAD durante la germinazione non sembrano dipendere direttamente dalla concentrazione del coenzima.

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Location of GAD activity in the isolated dry constituents of the caryopsis of *Triticum durum*

Material	GAD specific activity (μl CO_2/min/mg protein)	Relative percentages
Embryo	9.8	100.0
Embryo axis	7.3	74.4
Scutellum	2.6	26.7
Endosperm	Not detectable	—

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Effects of DHEA or its Sulfoconjugates upon c-AMP Phosphodiesterase

Only recently the interrelationship between DHEA (dehydroepiandrosterone, 3β-hydroxy-5-androsten-17-one), G-6-PDH (glucose-6-phosphate dehydrogenase, EC 1.1.1.49) and c-AMP (cyclic adenosine-3',5'-monophosphate) under physiological conditions has been reported[1]. According to these investigations, decreased plasma levels of sulfoconjugated DHEA may be at least in part responsible for the elevated activity of red blood cell G-6-PDH in psoriasis, hyperlipoproteinemia, or the menopausal syndrome, as well as for lowered concentrations of c-AMP in plasma or erythrocytes. In order to substantiate the influence of DHEA or its sulfoconjugates upon the concentrations of c-AMP, the erythrocyte-rich corpuscular fraction from blood of normal subjects was incubated with DHEA, its sulfate or sulfatide (dipalmitoyl glycerosulfate) and the intracellular content of c-AMP determined. Furthermore, the hydrolysis of 8-^{14}C-c-AMP in protein fractions from hemolysates in the presence of DHEA as well as the components of the G-6-PDH system was studied.

Material and methods. The corpuscular, erythrocyte-rich fraction from normal subjects – hence-forth called 'erythrocytes' – was prepared as previously described[2]. Duplicate samples, consisting of 3.0 ml of the 'erythrocyte' suspension in 0.9% sodium chloride/0.025% EDTA and 0.02 ml dioxane with 7α-^{3}H-DHEA, its sulfate, or synthetic sulfatide were incubated for 30 min at 37 °C, the final concentration of steroid substrate corresponding to a 10^{-6} to 10^{-4} M solution. The 'erythrocytes' were washed, hemolyzed, centrifuged, and the amount of intracellular substrate measured by its ^{3}H-activity. In the second series of experiments, the labelled substrates were replaced by corresponding concentrations of non-labelled compounds and the concentration of intracellular c-AMP determined by the protein-binding assay of Gilman[3].

In addition, hemolysates from washed 'erythrocytes' were submitted to fractionate ammonium sulfate precipitation and the protein fractions, obtained at 25%, 50%, and 75% saturation, incubated with 8-^{14}C-c-AMP in the absence or presence of DHEA and the components of the G-6-PDH system as indicated in the Table. The incubates were extracted with chloroform-methanol, the aqueous methanolic extracts lyophilized, and the residues submitted to thin layer chromatography on PEI-cellulose in 0.25 M lithium chloride for separation of

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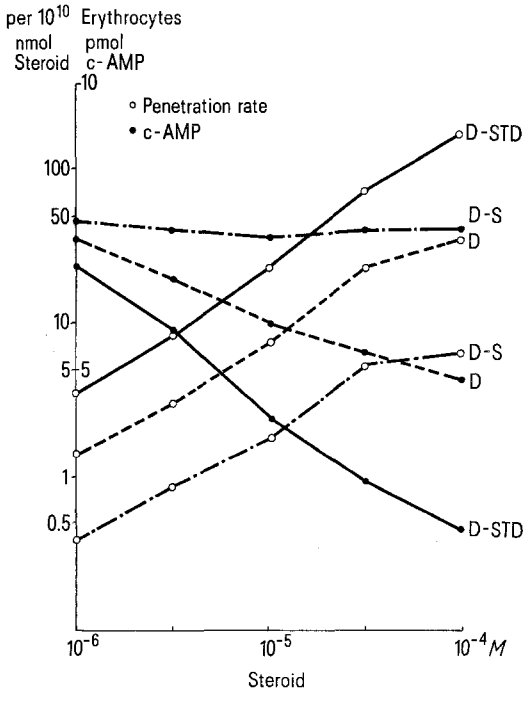
Hydrolysis of 8-^{14}C-c-AMP in different protein fractions from 'erythrocytes' of human blood in absence or presence of DHEA and components of the G-6-PDH system

Fraction	Protein/sample (mg)[a]	c-AMP (M)	Concentration of added compounds (M) DHEA	NADP	G-6-P	Hydrolysis[b] (%)
'Hemolysate'	25.2	0.165×10^{-3}	—	—	—	1.43
		0.165×10^{-3}	5×10^{-5}	—	—	4.45
25% saturation	2.01	0.165×10^{-3}	—	—	—	4.61
		0.165×10^{-3}	5×10^{-5}	—	—	8.27
50% saturation	1.96	0.165×10^{-3}	—	—	—	4.43
		0.165×10^{-5}	5×10^{-5}	—	—	7.45
75% saturation	18.9	0.165×10^{-3}	—	—	—	2.38
		0.165×10^{-3}	5×10^{-5}	—	—	4.57
50% saturation	1.96	0.165×10^{-3}	—	—	10^{-3}	2.57
		0.165×10^{-3}	—	—	10^{-2}	2.31
		0.165×10^{-3}	—	10^{-2}	10^{-3}	4.45
		0.165×10^{-3}	5×10^{-5}	10^{-2}	10^{-3}	3.15

[a] Equivalent to 1 ml whole blood. [b] Mean of duplicate analyses.

c-AMP and 5'-AMP as product of the hydrolysis. Both fractions were eluted with methanol and their ^{14}C-activity measured.

Results and discussion. From the Figure it becomes apparent that, in contrast to the low penetration rate of the hydrophile DHEA sulfate, appreciable amounts of the lipophile DHEA sulfatide had entered the 'erythrocytes'. The intracellular concentration of c-AMP, in turn, was found to be decreased in the presence of penetrated DHEA or DHEA sulfatide, while DHEA sulfate failed to influence the intracellular nucleotide. After incubation of 10^{10} 'erythrocytes' in a 10^{-5} M solution of DHEA sulfatide only 4.4 pMol of c-AMP were detected as compared to 8.1 pMol in appropriate controls. Although the corpuscular fraction used contained also leukocytes and thromobocytes, known to be rich in phosphodiesterase activity[4], it represents the fraction, usually designated as 'erythrocytes' and employed for G-6-PDH assays. The assumed stimulation of intracellular phosphodiesterase activity by DHEA or DHEA sulfatide could be verified by an increased formation of 5'-AMP from ^{14}C-labelled c-AMP during incubation of protein fractions in the presence of DHEA. Since no further ^{14}C-labelled compounds could be detected on the chromatograms, the recovered ^{14}C-activity of 5'-AMP and c-AMP representing 89–91% of total radioactivity applied, and the assay for nucleotidase[5] proved negative the rates of hydrolysis, given in the Table, may be considered valid. The phosphodiesterase activity was found to be highest in protein fractions, sedimented at 25% and 50% saturation with ammonium sulfate. When G-6-P was added to such incubates, the rate of hydrolysis of c-AMP was reduced by non-competitive inhibition of phosphodiesterase[4]. The introduction of NADP together with G-6-P, e.g. the components of the G-6-PDH system, apparently led to a removal of G-6-P by a functioning G-6-PDH, and hence to a reduced inhibition of phosphodiesterase. On the other hand, in presence of DHEA the inhibition of G-6-PDH by this steroid[6] caused a simultaneous inhibition of phosphodiesterase by unused G-6-P. It seems that the direct activation of phosphodiesterase by DHEA and especially by DHEA sulfatide is counteracted by an indirect inhibition of this enzyme via inhibition of G-6-PDH, the latter effect apparently prevailing under physiological conditions[1].



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Intracellular c-AMP after incubation of 'erythrocytes' with DHEA or its sulfoconjugates.

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Zusammenfassung. Nach Bebrütung von «Erythrocyten» mit DHEA oder DHEA Sulfatid konnte eine Abnahme des intracellulären c-AMP's festgestellt werden. Eine angenommene Aktivierung der Phosphodiesterase liess sich durch die verstärkte Hydrolyse von c-AMP zu 5'-AMP in Gegenwart von DHEA bestätigen. Auf der anderen Seite bewirkte DHEA über die Hemmung der G-6-PDH auch eine indirekte Hemmung der Phosphodiesterase, die auf einem geringeren Verbrauch inhibierenden G-6-P's beruht. Unter physiologischen Bedingungen scheint letztere Wirkung vorzuherrschen.

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Presence of Adenosine Triphosphate in Tobacco (*Nicotiana tobacum*) Tissue Grown in Nutrient Medium Containing Various Concentrations of Kinetin

Kinetin has been known to affect cell division, differentiation, and organ development[1]. Kinetin (cytokinin) has also been reported to be an inhibiting agent for the production of glycolytic kinases, hexokinase, and pyruvic kinase[2]. Furthermore, it is known that cytokinins induce RNA synthesis and consequently affect protein synthesis[1]. While logic would make one assume that ATP contents of tissues would vary according to the kinetin concentration present, this paper sets down the computed ATP contents of tissues grown in vitro with various concentrations of kinetin.

Materials and methods. Cylinders of fresh tissue were cut through the stem of *Nicotiana tobacum* var. Wisconsin 38 with a cork borer (6 mm ⌀). 5-mm segments were then cut from the cylinder. Each piece of tissue weighed 0.31 ± 0.02 g. 3 pieces of such tissue were grown in a 125 ml flask containing 50 ml nutrient agar medium. The medium contained the basic substances previously used[3]. Plant hormones in the medium included 2 mg/l of indole acetic acid and varied concentrations of kinetin. All of the flasks were placed in a growth chamber with a temperature of 27 °C and approximately 100 Lux of light.

The liquid culture was identical to the solid medium except for the agar. Flasks containing pith tissue in nutrient solution were placed on a shaker in a room kept at a constant temperature of 24 °C and approx. 350 Lux of light.

Adenosine triphosphate was extracted from the pith tissue (2 g) with 5 ml of boiling water for a period of 10 min. The extract was cooled and the ATP content was analyzed by the luciferin-luciferase method[4] and an Aminco Chem-Glow Photometer. The luciferin-luciferase was prepared from the commercial firefly lantern extract (Sigma Chemical Co., St. Louis, Mo.). The ATP standard and the N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid/magnesium buffer were also from the same source.

Percentage of increases of ATP contents or fresh weights shown by Tables I and II was calculated as:

$$\text{Percent increase} = \frac{\text{Tissue grown in vitro} - \text{Fresh pith tissue}}{\text{Fresh pith tissue}} \times 100$$

Table I. Changes of ATP content and fresh weight of tobacco pith tissue grown in vitro comparing to fresh pith

Kinetin in medium (mg/l)	Increase of fresh weight (%)	Increase of ATP content in tissue (%)
0	174	300
0.02	266	150
0.20	550	720
2.00	145	658

Each number (for either fresh weight or ATP content of tissue) was calculated from 3 pieces of pith tissue in 1 flask comparing same number of fresh pith of tobacco stem.

Table II. Changes of ATP content in tobacco pith tissue grown in liquid nutrient medium containing various concentration of kinetin

Kinetin in medium (mg/l)	Increase of ATP in pith tissue (%)	
	8 days[a]	14 days[a]
0.02	33	120
0.20	337	820
1.00	718	929

ATP content was calculated based on 1 g of tissue comparing to the fresh pith (0 day growth in medium). [a] Growth period in medium.

Results and discussion. The Figure shows the changes of ATP contents of tobacco pith tissue grown in nutrient agar. Different experiments utilized different concentrations of kinetin in the nutrient. Tissue grown in the medium containing 0.2 or 2 mg/l of kinetin produced more ATP than tissue grown on the medium without kinetin (control). Tissue grown for 6 days in medium containing 0.02 mg/l of kinetin contained much less ATP per g of fresh weight of tissue than the control. The longer growth period (14 days) made the difference even more dramatic.

Table I shows a comparison of the fresh weight and ATP contents of the tissue grown in vitro with that of pith tissue freshly cut from tobacco stem. After 14 days of growth, it can be seen that kinetin in the medium enhanced the growth, both by weight and overall size. High concentrations of kinetin (2 mg/l) retarded the growth when compared to tissue grown in the absence of kinetin. The greatest ATP content was found in tissue grown in such medium (2 mg/l).

Similar experiments were performed using liquid media. The overall growth rates of tissue in any of these media were faster than those grown on agar, probably due to

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