were calculated from slopes of lines obtained by plotting incubation time against NAD cleaved. For calculating the amount of NAD split, 5.9 was used as the millimolar extinction coefficient of NAD-CN complex at 327 nm. (In preliminary experiments for plasma NADase assay, minor negligible differences were found using the pH's of 7.5, 7.0, 6.5, 5.8 and 5.0. The pH of 5.8 was chosen for the assay as a matter of convenience.)

Results and discussion. As seen in Table II the plasma NADase activities of all tuberculous Ethiopians were similar to those of normal controls, whereas in the tuberculous guinea-pig an almost 3-fold elevation of plasma NADase activity was noted over controls. Previous results reported an increase in NADase activity of tuberculous guinea-pig plasma of up to 7-fold[3]. In general, the rates for normal human plasma NADase were much lower than those of the normal guinea-pigs, which might be helpful for a detection of any possible increase.

Table II. NADase in plasma of tuberculous Ethiopians and guinea-pigs

	Ethiopians		Guinea-pigs	
	Tuberculous[a]	Control	Tuberculous	Control
1	0.10[b]	0.12	0.41	0.14
2	0.07	0.03	0.27	0.10
3	0.05	0.02	0.58	0.20
4	0.08	0.00	0.41	0.17
5	0.02	0.03		
6	0.09	0.15		
7	0.07	0.12		
8	0.05[b]	0.12		
9	0.13[b]	0.08		
10	0.10	0.09		
Mean ± S.D.	0.08 ± 0.03	0.08 ± 0.04	0.42 ± 0.13	0.15 ± 0.04

NADase unit = micromole NAD cleaved at pH 5.8/1 h at 37 °C/1 ml heparinized plasma. [a] Tuberculous patients sequential numbers as shown in Table I. [b] Positive staining for acid fast bacilli in sputum.

The increase of soluble plasma NADase is known to vary with the degree and duration of the experimental tuberculous infection[18]. It is possible, therefore, that a longer duration of the disease in man may show an elevation in the plasma NADase; but one would not expect to follow a case of tuberculosis for a long duration without treatment. On the other hand, at least 3 of the patients had tubercle bacilli in their sputum (Table I) which indicates a highly progressive disease, and the remainder may also have been tuberculous long before the present diagnosis. At any rate, it seems probable that the disease in man differs from that of the guinea-pig.

The very low activity of normal plasma NADase in man, and the absence of an increased activity during tuberculosis, in contrast to what is found in the tuberculous guinea-pig, rule out any clinical application of plasma NADase for the diagnosis of tuberculosis in man.

Résumé. Des cobayes tuberculeux montrent dans leur plasma une augmentation de l'activité de l'enzyme NADase d'environ 3 fois supérieure à celle du plasma d'animaux normaux. Chez des sujets tuberculeux noirs (Ethiopiens), l'activité de la NADase plasmatique est trés faible et semblable à celle de sujets normaux. Pour le diagnostic de la tuberculose humaine, une application clinique éventuelle du «test NADase» plasmatique est donc exclue.

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Partricin Methyl Ester, a Semisynthetic Polyene Antibiotic

Only a few of the many polyene antifungal antibiotics produced by different strains of *Streptomyces* have found clinical applications. The antifungal antibiotics are usually toxic, almost insoluble in water and unstable, and the efforts made to increase their manageability have been as yet hardly successful[1,2].

A new polyene (partricin, SPA-S-132) produced by a strain of *Streptomyces aureofaciens* (NRRL 3878) has recently been isolated[3], and found to have biological properties similar to those of other known antibiotics. Its structure has not yet been elucidated but it is presumably macrolidic with amphoteric properties. Partricin is very active against fungi and protozoa: the minimum inhibitory concentrations (MIC) on *Candida albicans* were about 0.2 µg/ml and the MIC on *Trichomonas vaginalis* were about 0.25 µg/ml. It is tolerated by oral route (LD_{50} 300 mg/kg), but is very toxic by i.p. administration in mice (LD_{50} 0.5 mg/kg) and shows a high hemolytic activity.

In an attempt to improve the biological properties of partricin, its methyl ester was prepared. Partricin methyl ester (SPA-S-160) was obtained by treating a solution of partricin in dimethylsulfoxide with diazomethane and the product isolated following precipitation with ether was purified by suitable organic solvents.

Partricin methyl ester is a deep yellow crystalline material, almost insoluble in water and in the usual organic solvents, very soluble in dimethylsulfoxide, dimethylformamide, dimethylacetamide and methylcellosolve. In solid form and preserved from light, it is almost stable. Elemental analysis has given the follow-

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ing results: C 63.3%; H 7.8%; N 3.2%; O 24.8%. UV-spectrum shows absorption maxima in ethanolic solution at 401, 378, 359, 340 nm, a characteristic pattern for heptaenic structures[4]. Thin layer chromatography on silica gel shows a single spot in many solvent systems[5]; in butanol-ethanol-acetone-25% ammonium hydroxide (2:5:1:3), the compound has a Rf value (about 0.8) different from that of the starting material (0.5). IR-spectrum (nujol mull) shows the ester carbonyl absorption at 1715 cm^{-1} and the NMR-spectrum (dimethyl-d_6 sulfoxide solution) presents the signal due to the methyl ester protons at 3.25 ppm.

Partricin methyl ester is inactive against bacteria, but active against several saprophytic and pathogenous fungi (in particular yeasts) and against some protozoa. The MIC on *C. albicans* (Fluid Sabouraud medium, Difco) are about 0.05 µg/ml and the MIC on *T. vaginalis* (CPLM medium) are about 2 µg/ml. On the whole, the inhibitory activity of partricin methyl ester is higher against the yeasts and lower against *T. vaginalis*, compared to the starting substance.

The LD_{50} of partricin methyl ester in mice is over 2 g/kg by oral route and about 200 mg/kg by i.p. administration (carboxymethylcellulose suspension). The hemolytic activity is low. The compound administered by oral route for 6 months to dogs and rats (200 mg/kg die) has not modified body weight growth, WBC, RBC, blood urea nitrogen, serum creatinine, SGOT, SGPT, alkaline phosphatase, etc. Local applications on the normal and scarified skin of rabbits and on the conjunctival mucosa and corneal epithelium are well tolerated. In clinical trials the compound proved effective on *Candida* and *Trichomonas* vaginal infections and on fungal skin and mucosa infections following local application.

The esterification seems to have increased the activity against yeasts and to have reduced the toxicity and the hemolytic activity of partricin. The above data represent a new approach to the synthesis of semisynthetic derivatives of natural polyenes with biological properties improved over those of the parent compounds. Preliminary results show that esterification can decrease the toxicity while preserving the antifungal activity of other polyene antibiotics.

Zusammenfassung. Es wurde der Methylester von Partricin, eines polyenischen Antibiotikums, hergestellt. Das Produkt zeigte eine gesteigerte Aktivität gegen Hefen und eine verminderte Toxizität.

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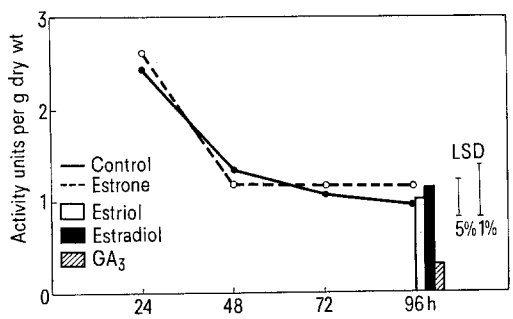
Effect of Estrogens and Gibberellic Acid on Cytokinin and Abscisic Acid-Like Compound Contents in Pea

Steroidal hormones are found in small amounts in plant tissues[1] and they may have influence on the growth[2,3], flowering[4–6] and sex-expression[7–9] in plants. It was also found that an increased biosynthesis of estrogens occurs at the time of flower bud formation and reaches a maximum at the time of flower development[10,11]. The mechanism of action of the steroid substances in plants is unknown. In previous papers the positive influence of the exogenously applied estrogens on the content of endogenous gibberellins and auxins, and lack of effect of GA_3 and IAA on the endogenous estrogens content in plants was stated[12–15]. At the same time kinetin increased and abscisic acid (AbA) decreased the amounts of estrogens[15]. The present paper deals with the effect of estrogens and gibberellic acid on endogenous cytokinin and inhibitor level.

Material and methods. Pea seeds (*Pisum sativum* var. Cud Kelwedonu) were germinated and cultivated in sterile sawdust under red light (λ_{max} 610 nm, E 0.915 µW/mm²/s) at 20–22 °C. After 6 days the seedlings were selected and treated with estrone, estriol, estradiol-17β on one hand or gibberellic acid on the other, in 0.1 and 0.001 µg doses of hormone per plant, respectively. Growth regulators determination were carried out on the



Influence of estrogens and gibberellic acid (GA_3) on the abscisic acid like compound contents in pea. LSD, significant differences at $P=1$ and $P=5$.

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