standards. Maltose, poorly resolved slower-moving saccharides and traces of glucose were found in the incubation mixture of peak II, while almost exclusively glucose and some slower-moving oligosaccharides were detected in peak I (Figure 2). Non-migrating dextrins were present in both cases.

In further experiments the peak I enzyme was incubated with starch, and the glucose split off was measured, at intervals, by means of both the TGO and the phenylhydrazine reagents. When the results were calculated as μmoles of glucose split off, the same amount of sugar was found with the 2 methods, this fact being taken to indicate that the only reducing sugar liberated by enzymic hydrolysis was glucose.

Preincubation with EDTA (0.025 M) caused 95% inhibition of peak II enzyme, but had no appreciable effect on peak I enzyme.

The data reported here show that the 2 enzymes are different for both molecular and catalytic properties. While peak II exhibits some catalytic properties of an α-amylase (α-1,4-Glucan 4-glucanohydrolase, E.C. 3.2.1.1), the peak I enzyme is able to split maltose and hydrolyzes starch by splitting off glucose molecules: therefore it may be regarded as an α-glucosidase (α-D-Glucoside glucohydrolase, E.C. 3.2.1.20) or as a glucoamylase (α-1,4-Glucan glucohydrolase, E.C. 3.2.1.3.). In this respect it bears some relationship with the corresponding enzymes of human urine[10].

Hence, on the basis of the data herein reported, the 2 amylolytic enzymes cannot be regarded as amylase isoenzymes.

Further studies are now in progress in order better to characterize the molecular and catalytic properties of the peak I enzyme.

Riassunto. Mediante gel filtrazione si sono separati due enzimi amilolitici dal siero di cane; mentre il primo mostra alcune proprietà della alfa-amilasi, il secondo attacca l'amido con liberazione di glucosio ed è inoltre capace di idrolizzare il maltosio. I due enzimi pertanto non possono essere considerati come isoenzimi, ma come enzimi differenti.

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[10] C. FRANZINI and P. A. BONINI, to be published.

Early-Estrogen Syndrome in the Rat by the Non-Steroidal Estrogen Diethylstilbestrol

A complex of characteristic disorders of the regulatory mechanisms of the estrous cycle, with resulting anovulation and sterility following the application of a sufficient amount of estrogen during the early post-natal development in the female rat, was characterized in this communication as 'early-estrogen' syndrome in the sense of the term 'early-androgen' syndrome of SWANSON and VAN DER WERFF TEN BOSCH[1]. It seems to be possible to induce analogical disorders in addition to androgens and estrogens also by the suitable application of other steroids, such as progesterone and desoxycorticosterone and lastly cholesterol alone (reviewed by TAKEWAKI[2]), although our attempts to confirm this influence of progesterone and cholesterol were unsuccessful[3]. It seemed of interest, therefore, to attempt to induce the early-estrogen syndrome by a non-steroidal estrogen diethylstilbestrol.

19 females and 21 males of our laboratory strain of rats received at the age of 5 days a single s.c. injection of 1.25 mg diethylstilbestrol in 0.1 ml peanut oil. The vaginae opened at the age of 29.3 $\pm$ 0.8 days (S.D. 2.9; 95% confidence interval 27.5–31.1), i.e. significantly ($P < 0.01$) earlier than in the controls (47.8 $\pm$ 1.3 – S.D. 4.3; 44.7–50.9). At 100 days of age, treated females were placed in a cage with normal fertile males and treated males with normal fertile females, in the proportion of 2 males to 3 females, for 3 weeks. All treated animals were entirely sterile. At the age of 150 days the animals were killed and their gonads examined histologically.

Immediately after death the gonads were fixed in Bouin solution and paraffin embedding was employed. Sections were stained by hematoxylin-eosin. In the ovaries numerous primordial, growing, and Graafian follicles were present. Corpora lutea were absent. In some Graafian follicles the oocytes with corona radiata were free in antrum. A considerable finding were papillae of connective tissue penetrating into the granulosa cell layer (Figure 1). The interstitial cells among the follicles were hyperplastic and hypertrophied. In the testicles

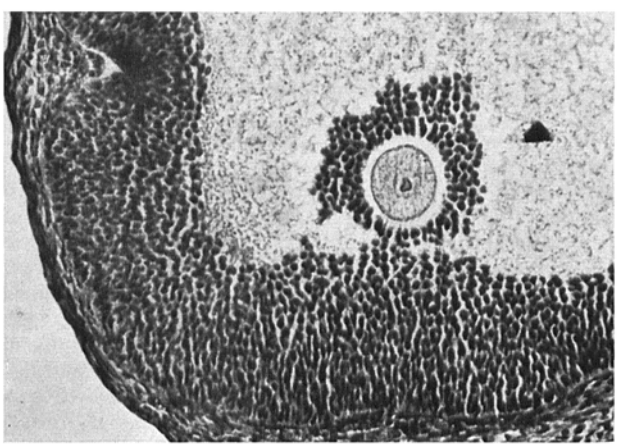


Fig. 1. Ovary of a rat with early-estrogen syndrome by diethylstilbestrol. Detail of a large Graafian follicle. On the left a characteristic fold in the granulosa layer due to a papilla of the theca interna. × 200.

[1] H. E. SWANSON and J. J. VAN DER WERFF TEN BOSCH, Acta endocr., Copenh. *45*, 1 (1964).
[2] K. TAKEWAKI, Experientia *18*, 1 (1962).
[3] Unpublished results (1964).

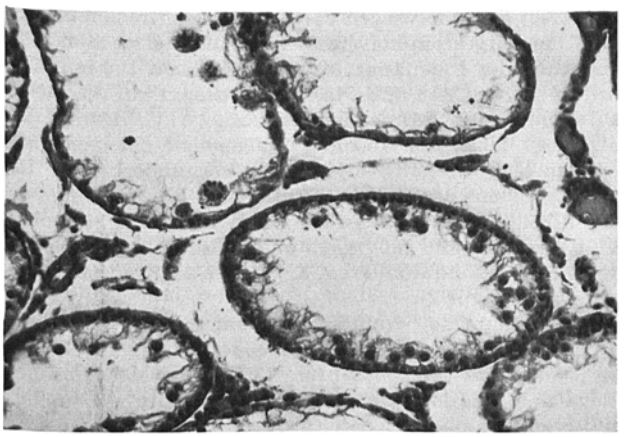
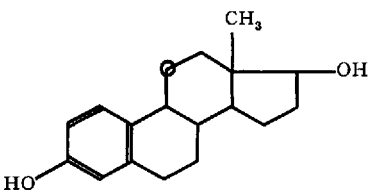
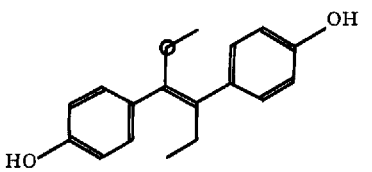


Fig. 2. Testis of a rat with early-estrogen syndrome by diethylstilbestrol. Seminiferous tubules lined only with spermatogonia and Sertoli-cells. In the lumen many multinucleated giant spermatocytes. × 100.



Estradiol



Diethylstilbestrol

○ = 11-β-H

Fig. 3

heavy degenerative changes could be seen. In some tubules spermatogonia, spermatocytes, praespermatides, spermatides and spermatozoa, while in others only spermatogonia, sporadic spermatocytes and giant polynuclear cells were present (Figure 2). The basal membranes were thin and in the interstitium the loose connective tissue with interstitial Leydig cells could be found.

CAMPBELL[4] recently assumed that, for all the steroids effective in inducing the early-steroid syndrome, the 11-β-H structure is common. This is taken into consideration with the supposed mechanism of their action at the origin of this syndrome. It has been shown in this paper that a comparable syndrome was induced also by diethylstilbestrol, thus confirming the experience of HALE[5] and KINCL et al.[6]. Although this estrogen is not related to steroids structurally, it is possible to presume a similar mechanism of its action from certain similarity in the spatial location of the carbon atoms and spatial arrangement of the molecule (Figure 3).

Zusammenfassung. Typisches, durch Östrogene (frühpostnatale Periode) bei weiblichen und männlichen Ratten induziertes Syndrom, wird auch durch nicht-steroidales Östrogen, das Diäthylstilböstrol, ausgelöst. Auffallend wellenförmige Granulosazellenschicht in den grossen Graafschen Follikeln.

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Institute for the Care of Mother and Child, Prague (Czechoslovakia), 14th November 1966.

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[5] H. B. HALE, Endocrinology *35*, 499 (1944).
[6] F. A. KINCL, A. F. PI, M. MAQUEO, L. H. LASSO, A. ORIOL and R. I. DORFMAN, Acta endocr., Copenh. *49*, 193 (1965).

Die Abhängigkeit der Ca^{++}-Aufnahme isolierter Mitochondrien des Herzmuskels von der Na^+- und K^+-Konzentration als mögliche Ursache der inotropen Digitaliswirkung

Die Kontraktilität der Muskelzelle wird nach heutigen Vorstellungen durch die wechselnde intrazelluläre Konzentration an freien Calciumionen gesteuert. Im Skelettmuskel erfolgt diese Änderung durch eine Bindung und Speicherung von Calcium in den Vesikeln des sarkoplastischen Retikulums. Dieser Mechanismus reicht jedoch für die Steuerung des Kontraktionszyklus im Herzmuskel nicht aus, so dass hier zusätzliche Bindungs- bzw. Austauschmechanismen für Ca^{++} vorhanden sein müssen (HASSELBACH und WEBER[1]; PORTZEHL[2]).

Aus Untersuchungen von SLATER und CLELAND[3] ist bekannt, dass Lebermitochondrien Calcium speichern können. VASINGTON und MURPHY[4] zeigten, dass die Ca^{++}-Bindung bei Nierenmitochondrien ein energieverbrauchender Prozess ist, und BRIERLEY et al.[5] konnten an Mitochondrien des Herzmuskels einen Substratabhängigen und einen ATP-abhängigen Ca^{++}-Transport unterscheiden.

Nachdem wir in früheren Untersuchungen festgestellt hatten, dass der K^+-Transport an Mitochondrien des Herzmuskels durch Ca^{++} gehemmt wird[6], machten wir in den vorliegenden Untersuchungen die Beobachtung, dass die Ca^{++}-Aufnahme massgeblich durch den K^+/Na^+-Quotienten des Inkubationsmediums beeinflusst wird. Da bekannt ist, dass sich der intrazelluläre K^+/Na^+-Quotient während der Muskelkontraktion ändert, könnte

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