and 'Tetracanthatch' the protein composition of the particular genotype of *A. squarrosa* involved in the evolution of 'Canthatch' since some bands due to the D-genome may be obscured by those contributed by the A- and B-genomes and since the 3 genomes may carry genes in common for protein synthesis[7].

Zusammenfassung. Die Entfernung des D-Genoms aus der Handelsweizensorte «Canthatch» führte bei der Stärkegelelektrophorese zum Verlust von 4 Komponenten des Klebermusters. Aus «Tetracanthatch» und verschiedenen Genotypen von *A. squarrosa* nachgezüchtete Hexaploide zeigten bei der Stärkegelelektrophorese eine ähnliche Zusammensetzung wie die Mischung der aus den entsprechenden Elterntypen extrahierten Proteine.

W. J. R. BOYD, J. W. LEE and C. W. WRIGLEY

Institute of Agriculture, University of Western Australia, and C.S.I.R.O. Wheat Research Unit, North Ryde (N.S.W., Australia), 14 October 1968.

[7] Helpful discussion with Dr. K. W. SHEPHERD and the technical assistance of Mrs. C. TRENOUTH are gratefully acknowledged.

The Influence of Oestradiol-17β on the Rat Uterine Na^+, K^+-Mg^{++} Activated Adenosine Triphosphatase Activity

The administration of oestrogens to ovariectomized rats can alter the electrolyte composition of the uterus[1,2]. The following experiments were undertaken to investigate the possibility that these changes are secondary to alterations in the Mg^{++}-activated ATPase activity brought about by oestrogens.

Materials and methods. The method used was that of W. W. KIELLY. Spayed adult rats (250 g $\pm$ 25) were injected s.c. with oestradiol-17β in arachis oil in doses ranging from 1–50 µg/rat. Animals were killed by cervical dislocation and their uteri were removed quickly and washed in ice-cold extracting solution (0.1 *M* KCl; 0.04 *M* $NaHCO_3$; 0.01 *M* Na_2CO_3) After weighing on a torsion balance, a portion of the tissue was removed for a dry weight estimation and the remainder was ground with sand in cold extracting solution. The final volume was adjusted to 20 ml/g tissue The suspension was centrifuged for 15 min at 900 *g* and an equal volume of de-ionized water was added to the supernatant After $^1/_2$ h this was centrifuged at 1000 *g* for 30 min to sediment the actomyosin precipitate. The supernatant was assayed for ATPase activity. This was carried out by incubating the following at 37 °C: 0.1 ml 0 05 *M* $MgCl_2$, 0.3 ml 0.2 *M* *Tris*-HCl buffer pH 6.9 (in 0.15 *M* KCl), 0.1 ml 0.02 *M* adenosine 3′,5′-triphosphate (disodium salt), 0.4 ml de-ionized water, 0.1 ml supernatant. The reaction was stopped after 5 min by adding 1 ml 5% perchloric acid. Phosphate assays were performed by a modification of the method of FISKE and SUBBAROW[3].

The concentration of protein of the supernatant was measured by a biuret method.

Results and discussion. There was no significant change in Mg^{++}-activated ATPase activity 30–40 min after administration of 5 µg oestradiol compared with ovariectomized controls. ATPase activity was decreased by 35, 44 and 39% 7, 19–25 and 45–50 h respectively following 50 µg oestradiol-17β administration. With 1 µg oestradiol-17β the change in ATPase activity was similar to that produced by 50 µg oestradiol (Table).

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Na^+, K^+, Mg^{++} activated adenosine triphosphatase activity of uterine tissue homogenate after removal of actomyosin expressed as µmoles phosphorus/g protein · 5 min

Treatment	Interval after administration	Mean	S.E. of mean	No. of animals	% difference from control
0.1 ml oil	7 h	1618.7	185.1	8	−39.6
1 µg oestradiol-17β	7 h	977.1	149.4	6	$0.01 < P < 0.001$
0.5 ml oil	7–50 h	1352.3	99.3	17	
50 µg oestradiol-17β	7 h	875.5	131.35	6	−35 $0.01 < P < 0.001$
50 µg oestradiol-17β	19–25 h	751.6	69.88	8	−44 $P > 0.001$
50 µg oestradiol-17β	45–50 h	824.2	49.4	7	−39 $0.01 < P < 0.001$
0.1 ml oil	30–40 min	1662.7	195.1	7	
5 µg oestradiol-17β	30–40 min	1867.5	66.76	6	+12.33 $0.2 < P < 0.1$

The increase in uterine water content following oestradiol administration[1,2,4] and the concomitant rise in Na^+ and fall in K^+ concentrations between 6 and 8 h after the administration of oestradiol[1,2] could be a consequence of the fall in ATPase activity observed in these experiments. There is some evidence that the Mg^{++} activated ATPase is a component of the Na^+ pump mechanism[5].

MEANS and HAMILTON have reported an increase in protein synthesis which is preceded by stimulation of both the synthesis of nuclear RNA and uptake of RNA precursors in the uterus within 2 min following administration of oestradiol-17β to ovariectomized rats[6]. Thus although oestrogens are capable of stimulating net protein synthesis in the uterus by increasing the rate of formation of template RNA, the present work suggests this is not a universal effect in uterine proteins and that a loss of ATPase accompanies these changes. Although this could represent a direct interaction of oestrogen and enzyme, the delay in onset of the effect suggests a selective inhibition of synthesis of enzyme protein[7].

Zusammenfassung. 17-β-Östradiol wirkt am Rattenuterus hemmend auf die ATPase, von der bekannt ist, dass sie auf die sogenannte Natriumpumpe in der Zelle wirkt.

P. K. KARMAKAR

Pharmacology Department,
Guy's Hospital Medical School,
London, S.E 1 (England), 7 October 1968

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Persistence of a Physiological Circadian Rhythm of Plasma-Free 11-Hydroxycorticosteroid Levels in Totally Fasting Obese Subjects

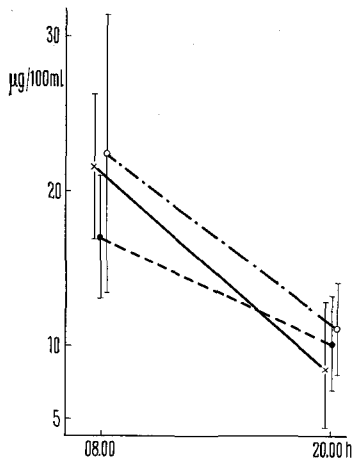
Rhythmic variations of pituitary ACTH output are the likely explanation for the high early morning and the low late evening levels of blood cortisol concentrations[1]. The physiologic pattern of circadian variations of plasma cortisol levels may be disturbed by numerous conditions such as psychic stress, central nervous disease and a wide variety of acute and chronic illnesses (for a review see SAWIN[2]).

Thus, it was tempting to speculate that long-lasting severe fast in obese subjects, a rather well-defined procedure associating considerable psychological stress with tremendous metabolic upset, would also interfere with normal pituitary-adrenal function.

In order to put this hypothesis to the test the normal circadian rhythm of free plasma 11-hydroxycorticosteroid levels (further referred to as 'cortisol') was first determined by fluorimetrically[3] measuring blood steroids at 08.00 and 20.00 in a group of 21 healthy young volunteers instructed to adhere to their normal activity schedule. The mean cortisol levels were: 21.8 ± 4.8 µg/100 ml at 08.00 and 8.7 ± 4.2 µg/100 ml at 20.00 (mean decline $60.6 \pm 15.9\%$). All individuals had definitely lower values in the evening than in the morning.

The same procedure was then applied in 8 hospitalized obese patients whose characteristics are given in the Table. The patients were subjected to a program of physiotherapy and active physical training. Again a rather uniform pattern of steroid rhythm was found. After total starvation (6 subjects) or 600 Cal. intake (2 subjects) for 16–33 days with a mean weight loss of 8.8 kg the patients again had their morning and evening blood steroid levels measured. In the Figure the mean decline of the evening values before and after starvation is indicated: The pattern remains rather identical, except for patient B.T. (not included in the Figure). In this case the diurnal rhythm became reversed. However, this patient's fast led to complications such as severe E.C. volume depletion, hypotonia and hypokaliemia which prompted precocious termination of total starvation.

Furthermore, it can be seen in the Table that the mean values for absolute 'cortisol' concentrations in



Circadian rhythm of free 11-hydroxycorticosteroid concentration (mean values ± 1 S.D.) in the blood of normal non-obese controls (×), obese patients before fasting (●) and at the end of a therapeutic fast (○). The evening cortisol levels are significantly lower than the morning levels in all 3 groups of subjects ($p < 0.005$). There is no significant difference between the 3 groups.

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