Remarkable Seasonal Variations of Urinary Gonadotrophin Excretion in Young Girls

Pursuing our studies on gonadotrophin excretion during infancy and puberty[1], we have collected a large quantity of urine from young girls (11 to 14 years of age) in order to measure once again the gonadotrophin content.

We observed that the urinary extracts (prepared according to ALBERT[2]) had remarkable differences in their gonadotrophin activity depending upon the season at which they were obtained, spring or autumn.

We repeated the collection of urine twice in the spring and twice in autumn, between the 29th and 30th of April and between the 29th and 30th of October, both in 1961 and 1962.

The gonadotrophic activity of the extracts was assayed according to KLINEFELTER[3], the potency thus being determined in mouse units. The procedure of ALBERT[4] was also employed. Several doses of the extracts were injected into normal intact weanling rats to obtain the characteristic dose response curve for ovaries and uterus growth.

It was demonstrated that the gonadotrophic activity of the extracts of urine collected in spring was about 10 times higher than that of similar extracts collected in autumn.

Similar results were obtained by the determination of LH content in the same urinary extracts, using the method of PARLOW[5], as modified by SCHMIDT-ELMENDORFF and LORAINE[6]. The ovarian ascorbic acid depletion was measured according to ROE and KUETHER[7]. Statistical analysis of our results was done by the method of BLISS[8].

The results of both determinations are presented in the Table, in which the official meteorological data for the days selected are also reported. Our results indicate that there is a marked seasonal variation of urinary excretion of gonadotrophins, which are approximately 9–10 times higher in the spring than in the autumn. The values for the LH content of the samples examined are in good agreement with those of the total gonadotrophic activity of the same extract.

Riassunto. Si è dosato il contenuto gonadotropinico di urine di soggetti di sesso femminile, tra gli 11 ed i 14 anni di età raccolte in due anni successivi (1961–1962) a mezza primavera ed a mezzo autunno.

Si è osservato che il contenuto gonadotropinico era sia nell'una sia nell'altra serie di determinazioni molte volte maggiore (circa dieci volte) nel periodo primaverile in confronto che nel periodo autunnale.

B. CARLETTI, E. KEHYAYAN, and F. FRASCHINI

Istituto di Farmacologia e di Terapia, Università degli Studi, Milano (Italy), March 14, 1964.

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Days of urine collection	Hours of sunshine[a]	Mean temperature °C[a]	Atmospheric pressure[a] mm Hg	Total quantity of extracted urine (l)	Total gonadotrophic activity mU/l	LH activity µg/l
29–30 April 1961	5.30	16.6	74.1	97	48.0	339.8
29–30 October 1961	1.35	13.3	74.8	85	5.5	38.2
29–30 April 1962	7.35	14.3	74.3	125	44.0	359.0
29–30 October 1962	3.35	10.2	74.3	75	4.8	42.2

[a] *Official records of the Milan University Observatory.*

Keto Acids in the Haemolymph of *Dysdercus koenigii* (Fabr.)

The composition of haemolymph or blood of insects differs a great deal from that of other animal groups[1]. Insects in general are characterized by high concentrations of free amino acids and organic acids in the haemolymph. The concentrations of various components of haemolymph, however, show a great variation and differ in detail from one insect species to another. The present work was, therefore, undertaken to determine the various keto acids in the haemolymph of *Dysdercus koenigii* (Fabr.).

The adults of *Dysdercus* were collected from the Government Agricultural Gardens, Kanpur. The haemolymph was collected by cutting the antennae of insects and allowing the haemolymph to drain into small tubes kept in freezing mixture. The keto acids in the haemolymph were estimated as dinitrophenylhydrazone derivatives by the method of FRIEDEMANN and HUGEN[2]. For the identification of various keto acids present in the

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haemolymph, one-dimensional ascending paper chromatography was used[3].

Since the preliminary examination of absorption spectrum and the ratio of optical density at 420 and 520 mμ indicated that there might be a number of keto acids present in the haemolymph, it was thought desirable to identify various keto acids present in the haemolymph chromatographically before any quantitative estimations were made. Data presented in the Table indicate that the dinitrophenylhydrazone derivatives of keto acids extracted from the haemolymph, resolved into two components having Rf's comparable with pyruvic and α-ketoglutaric acid, when subjected to paper chromatography. A further confirmation of the presence of pyruvic and α-ketoglutaric acid was obtained by eluting the areas corresponding to the Rf of pyruvic and α-ketoglutaric acid from the chromatograms and determining the ratio of optical density at 420 and 520 mμ. It was found to be from 1.22–1.3 for the component having Rf 0.46, and 1.98–2.10 for the slow-moving component (Rf 0.13). These ratios are characteristic of pyruvic and α-ketoglutaric acids[2].

The concentration of pyruvic acid in the haemolymph was found to be from 25–30 m*M* and that of α-ketoglutaric acid 8–10 m*M*. The presence of both pyruvic and α-ketoglutaric acid in the haemolymph of *Dysdercus* is not in accordance with the findings reported for the haemolymph of *Bombyx mori* larvae where no pyruvic acid could be detected, although α-ketoglutaric, oxalacetic and glyoxylic acids were found to be present[4]. However, MURTY and SREENIVASAYA[5] reported only pyruvic and three other unknown keto acids from the haemolymph of *Bombxy mori* larvae. Both pyruvic and α-ketoglutaric acid, nevertheless, have recently been reported from the haemolymph of *Hyalophora cecropia*.

These findings indicate that comparatively high concentrations of keto acids are present in the haemolymph of *Dysdercus* and that these keto acids must be playing a significant role in the ionic balance. The net contribution of keto acids to the total anionic pool, however, cannot be ascertained from the present study[7].

Paper chromatography of dinitrophenylhydrazone derivatives of keto acids

Substance	Rf
Pyruvic acid	0.46
α-Ketoglutaric acid	0.13
Oxalacetic acid	0.20
Haemolymph	0.46, 0.12

Solvent: *n*-butanol-ethanol-ammonia-water 70:10:5:20

Note. Dinitrophenylhydrazone derivatives of keto acids and haemolymph were prepared for chromatography as outlined by BLOCK et al.[6].

Résumé. L'hémolymphe de *Dysdercus koenigii* (Fabr.) est analysée pour la présence d'acides-kéto. La chromatographie-papier a démontré la présence d'acide pyruvique et d'acide α-kétoglutarique. La concentration de l'acide pyruvique a variée de 25 à 30 m*M* et celle de l'acide α-kétoglutarique de 8 à 10 m*M*.

K. N. MEHROTRA[8]

Department of Physiology, G.S.V.M. Medical College, Kanpur (India), December 12, 1963.

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[8] Present address: Insect Physiologist, Division of Entomology, Indian Agricultural Research Institute, New Delhi (India).

Autoradiographische Studien über die DNS-Synthese im Hypophysenvorderlappen der Ratte[1]

Über den Zellersatz im Hypophysenvorderlappen (HVL) und die DNS-Syntheserate unter funktioneller Belastung bestehen bisher noch keine sicheren Befunde. Es wurden daher autoradiographische Untersuchungen mit H^3-Thymidin an Kontrollen sowie 14 Tage nach Thyreoidektomie und Kastration durchgeführt. Männliche 185–190 g schwere Albinoratten (BD II) erhalten 500 μC H^3-Thymidin i.p. (spez. Akt. 6,0 C/Mol, Schwarz Bio Research, New York). Dekapitation der Tiere 1 h nach Applikation – gegen Ende der Verfügbarkeitszeit von H^3-Thymidin, jeweils morgens zwischen 9 und 11 h. Paraffineinbettung der Hypophyse, Waschen der entparaffinierten Schnitte in hochkonzentrierter Lösung inaktiven Vorläufers zum Auswaschen nicht inkorporierten H^3-Thymidins. Stripping film (Kodak AR 10). Exposition 8–14 d. Nachfärbungen. Bestimmung des H^3- und Mitoseindex (45000–70000 Parenchymkerne/Tier). Die Tabelle zeigt, dass der H^3-Index bei den Kontrollhypophysen zwar sehr niedrig, aber nicht gleich 0[2] ist. Auf 10000 Zellen finden sich im Mittel 12,2 markierte Kerne. Grössenordnungsmässig kann diese Proliferationsrate mit der von Leberparenchymzellen der Ratte verglichen werden[3]. Nach

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