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ideal means of deducing cancer of the portio, to be placed alongside those currently used, which are based on entirely different principles.

SUMMARY

The authors report on the preliminary results obtained in a telethermographic study of the uterine cervix. The findings proved encouraging for further studies of this technique in the diagnosis of cancer of the uterine cervix.

Translated by Samil Pabyrn Foundation

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Gas chromatographic studies on certain urinary steroids in obese women

by
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The lack of agreement in the literature, and above all, the absolute lack of analytical data relating to the individual urinary steroid hormones in obesity led us to investigate this aspect of this complex metabolic problem. It must first be noted that most of the results reported by various authors in regard to 17-hydroxycorticosteroid and 17-ketosteroid levels were obtained by the traditional colorometric method. Further, division of the urinary steroid metabolites merely into the two groups as 17-hydro and 17-keto derivatives is now considered too crude.

Until recently the identification of the individual hormones in each group was not possible because of technical difficulties, but this can now be done by gas chromatography. Consequently, titration of the individual urinary steroids which intervene at some stage in glycolytic metabolism may help to clarify their possible role in the pathogenesis of obesity.

On the basis of this theory we undertook an investigation of the adrenocortical function in a group of obese women.

MATERIALS AND METHODS

The study involved 21 obese women and 11 normal control subjects; the age range of the group was 18-40 years. The obese patients had the clinical characteristics of diffuse gynecoid obesity. No other type of endocrine metabolic pathology was present; hepatic, cardiac and renal functions were normal. During the

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study period none of the subject received pharmacological treatment or observed any dietary restrictions. In all cases a 12-hour urine collection was made (from 6 p.m. to 6 a.m.); during this period the urinary steroid production is less affected by the action of the corticotropic hormones.

Urine samples were collected on three successive days in order to obtain a more valid average.

For determination of the steroid hormones we used gas chromatography, with the Fractovap G. I. Carlo Erba gas chromatograph, and a « urinary profile » method proposed by Horning and Gardiner ([1]), modified by Ros and Sommerville by the use of glass capillary colums and trimethylsilil ethers-enoltrimethylsilil ethers ([2]); this method allowed us to fraction qualitatively and to titrate quantitatively 24 of the urinary metabolites listed in Table I. To obtain standard samples we carried out the same process with a mixture of the 24 steroids of known quantity.

Comparison with these and with the internal standard peaks allowed us to measure the steroid levels in the samples under study.

As shown in Figure 1 the various steroid fractions were divided into three groups; group one was made up of androgens, group two of pregnan and pregnen derivatives, and group three of the 17-hydroxycorticoid tetra-hydro derivatives and their metabolites. In addition a peak referable to cholesterol was always present.

The statistical analysis of the results was based on the Student « t » test.

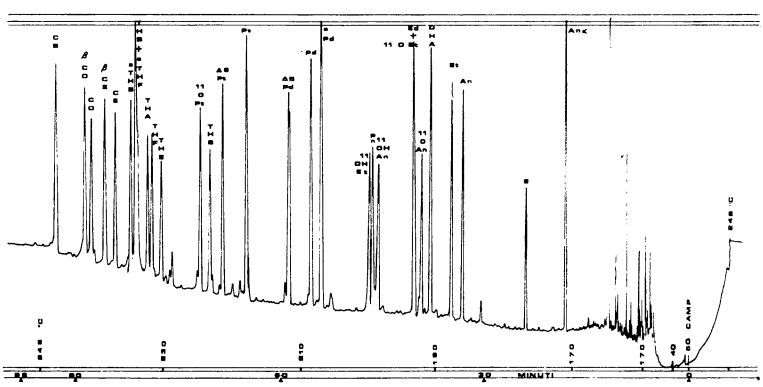

FIG. 1 - Gas chromatography of the mixture of the pure steroids TMSi, enol-TMSi. Each peak corresponds to 40 ng. Glass capillary column ∅ 0.30 mm, length 25 m, coated with OV 101. Temperature of the flash heater and of the detector: +250 °C. Automatic analysis programme; at the moment of sampling the temperature of the column is 50 °C; in the next two minutes it falls to 40 °C, then rises to 170 °C in 5 minutes, remains isothermic for 10 minutes and encreases thus 1 °C/min, until the CB has been eluted. Hydrogen for the flame: 0.20 Kg/cm² as entry pressure, corresponding to a 9 ml/min flow. Air for the flame: 0.60 Kg/cm² = 190 ml/min. Carrier gas: hydrogen with an entry pressure of 1 Kg/cm² and a flow, at 170 °C, of 2.8 ml/min. Attenuation, with the recorder turned on to 1 mv f.s.d. is 1 × 32, which corresponds to 1.8 f.s.d. for 10^{-12} Amper.

RESULTS

Of the androgen fractions identified by our method, only four were of interest: androsterone, ethiocolanolone, 11-hydroxy-androsterone and ethiocolandiol. The remaining fractions were either at the lower limit of the sensitivity

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Table 1.

Abbreviation	Trivial name	Systematic name
An α	Androstenol	5α-androst-16-en-3α-ol
E	Oestratetrienol	Oestra-1, 3, 5, (10), 16-tetrien-3-ol
An	Androsterone	3α-hydroxy-5α-androstan-17-one
Et	Aetiocholanolone	3α-hydroxy-5β-androstan-17-one
DHA	Dehydroepiandrosterone	3β-hydroxy-5-androsten-17-one
epi-An	Epiandrosterone	3β-hydroxy-5α-androstan-17-one
11-O-An	11-Oxo-Androsterone	3α-hydroxy-5α-androstane-11,17-dione
11-O-Et	11-Oxo-aetiocholanolone	3α-hydroxy-5β-androstane-11,17-dione
Ed	Aetiocholanediol	5β-androstan-3α, 17β-diol
11-OH-An	11-Hydroxyandrosterone	3α, 11β-dihydroxy-5α androstan-17-one
Pn	Pregnanolone	3α-hydroxy-5β-pregnan-20-one
11-OH-Et	11-Hydroxyaetiocholanolone	3α, 11β-dihydroxy-5β-androstan-17-one
a-Pd	allo-Pregnanediol	5α-pregnane-3α, 20α-diol
Pd	Pregnanediol	5β-pregnane-3α, 20α-diol
Δ^5-Pd	Δ^5-pregnenediol	preg-5-en-3β, 20α-diol
Pt	Pregnanetriol	5β-pregnane-3α, 17α, 20α-triol
Δ^5-Pt	Δ^5-pregnenetriol	pregn-5-en-3β, 17α, 20α-triol
THS	Tetraidrodesossicortisol	3α, 17α, 21-trihydroxy-5β-pregnan-20-one
11-O-Pt	Pregnanetriolone	3α, 17α, 20α-trihydroxy-5β-pregnan-11-one
THE	Tetrahydrocortisone	3α, 17α, 21-trihydroxy-5β-pregnane-11, 20-dione
THF	Tetrahydrocortisol	3α, 11β, 17α, 21-tetra-hydroxy-5β-pregnan-20-one
THA	Tetrahydrodehydro-corticosterone	3α, 21-dihydroxy-5β-pregnane-11, 20-dione
THB	Tetrahydrocorticosterone	3α, 11β, 21-trihydroxy-5β-pregnan-20-one
a THF	allo-Tetrahydrocortisol	5α-pregnan-3α, 11β, 17α, 21-tetrol-20-one
a-THB	allo-Tetrahydrocorticosterone	3α, 11β, 21-trihydroxy-5α-pregnan-20-one
CE	Cortolone	3α, 17α, 20α, 21-tetra-hydroxy-5β-pregnan-11-one
βCE	β-Cortolone	3α, 17α, 20β, 21-tetra-hydroxy-5β-pregnan-11-one
CO	Cortol	5β-pregnane-3α, 11β, 17α, 20α, 21-pentol
βCO	β-Cortol	5β-pregnane-3α, 11β-17α, 20β, 21-pentol
CB	Colesteryl butyrate	

of the method, or their quantitative determination was unreliable because of the superimposition of impurities in the formation of the relevant gas chromatographic peak.

The androsterone levels were 0.47 mg $\pm$ 0.34/12 h in the normal subjects

Table 2.

URINARY STEROIDS mg./12h.	NORMAL SUBJECTS	OBESITY	
Androsterone	0,47±0,34 *	1,01±0,53	$p<0,01$
Aetiocholanolone	0,47±0,30	0,62±0,39	NS
Aetiocholanediol	0,09±0,03	0,11±0,03	NS
11-OH-Androsterone	0,32±0,20	0,44±0,17	NS
Pregnanetriol	0,20±0,15	0,49±0,40	$p<0,05$
Δ^5 Pregnenetriol	0,11±0,07	0,19±0,13	NS
Tetrahydrocortisol (THF)	0,30±0,13	0,52±0,27	$p<0,02$
Tetrahydrocortisone (THE)	0,72±0,34	1,30±0,79	$p<0,05$
Cortolone	0,49±0,21	0,73±0,24	$p<0,01$
β-Cortolone	0,18±0,14	0,26±0,18	NS
Tetrahydrodehydrocorticosterone (THA)	0,35±0,15	0,37±0,18	NS
allo-Tetrahydrocorticosterone (a-THB)	0,19±0,05	0,32±0,12	$p<0,01$

* Means values ± standard deviations; mg./12h.

and 1.01 mg ± 0.53/12 h in the obese subjects; ethiocolanolone levels were 0.47 mg ± 0.30/12 h in the normal subjects and 0.62 mg ± 0.39/12 h in the obese ones; ethiocolandiol levels were 0.09 mg ± 0.03/12 h in the controls and 0.11 mg ± 0.03 in the obese subjects; the levels of 11-hydroxy-androsterone were 0.32 mg ± 0.20/12 h in the normal subjects and 0.44 mg ± 0.17/12 h in the obese subjects (Table 2).

Statistical comparison on these findings showed that obese subjects have a significantly greater urinary excretion of androsterone than normal subjects. The results relating to the other three androgen steroids were of no statistical significance (Table 2). As to Pregnan and Pregnen derivatives, our tests were carried out with Pregnantriol and Δ^5 Pregnentriol, which always allowed optimal separation by gas chromatography.

Pregnantriol levels were 0.20 mg ± 0.15/12 h in normal subjects and 0.49 mg ± 0.40/12 h in the obese; Δ^5 Pregnentriol levels were 0.11 mg ± 0.07/12 h in normal subjects and 0.19 mg ± 0.13/12 h in obese subjects. Statistical comparison of the results showed that the increased amounts of Pregnantriol found in obese subjects in comparison with normal ones was statistically significant ($p < 0.05$); the differences found for Pregnentriol were not significant (Table 2).

As regards the 17-hydroxy steroid tetrahydro derivatives and their metabolites, we examined the results obtained with tetrahydrocortisol (THF) and tetrahydrocortisone (THE) with its direct metabolites cortolone and β-cortolone, all of which are a reliable metabolic index of the glucoactive adrenocortical steroid group; we also investigated the results obtained with tetrahydro-dehydrocorticosterone (THA) and with allo-tetrahydrocorticosterone (a-THB) which, while taking the metabolic path of active minerals, bear reference to the corticosterone group because of their glucoactive effects (Table 2).

Results were as follows: THF levels were 0.30 mg ± 0.13/12 h in normal subjects and 0.52 mg ± 0.27 in the obese subjects. The THE levels were 0.72 mg ± 0.34/12 h in normal subjects, as compared with 1.30 mg ± 0.79/12

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h in the obese; cortolone levels were 0.49 mg ± 0.21/12 h as compared to 0.73 mg ± 0.24/12 h; β-cortolone levels were 0.18 mg ± 0.14/12 h as compared to 0.26 mg ± 0.18/12 h; THA levels were 0.35 mg ± 0.15/12 h as compared with 0.37 mg ± 0.18/12 h; a-THB levels were 0.19 mg ± 0.05/12 h as compared with 0.32 mg ± 0.12/12 h (Table 2).

Statistical analysis of the 17-hydroxy derivatives showed a significant increase in cortolone and a-THB ($p < 0.01$) in the obese patients in comparison with the controls; there was also a significant increase in THE and THF, and no statistical significance in the differences obtained for THA and β-cortolone (Table 2).

DISCUSSION

Examination of our case material reveals that the values of the individual urinary hormones were on average, always higher, in obese subjects than in control subjects. The fact that statistical analysis did not yield significant results in all cases is probably due to the relatively small number of cases and the differences of the data. The results do not agree with those of other investigators who maintain that the adrenalcortical function of obese individuals is normal ([3]), or that hyperfunction only exists in sthenic android obesity and never in hyposthenic gynoid obesity ([4, 5, 6, 7, 8]). As to the behaviour of the individual urinary steroids, we examined the androgens and in the first place androsterone. Urinary elimination of this hormone appeared to be significantly increased in the obese subjects, in comparison with the controls. No significant differences were found for aethiocholanolone, although it is an isomer of androsterone; this discrepancy is not easy to explain and should be checked in a larger number of cases. In control subjects androsterone and aethiocholanolone yielded entirely identical results, as was to be expected in view of what has been said previously.

The urinary elimination of androsterone in the obese subjects was almost double that of its 5-β isomer. Since both of these androgens are direct metabolites of dehydroepiandrosterone by way of androstenedione, it must be assumed that the difference in urinary steroid elimination is due to the differences in activity of the respective enzymes, or to a difference in peripheral clearance.

The recent observation that certain androgen steroids may intervene in glycolytic metabolism ([7, 9, 10]) suggests that the high urinary androsterone level may be related in some way to the state of glycolytic intolerance characteristic of obesity. It has often been found that obese women suffer from serious menstrual irregularities, often culminating in amenorrhea, and this observation also suggests the physiopathological possibility that the high androsterone levels interfere with the liberation of hypophyseal gonadotropins, in particular LH ([11]).

The findings obtained with pregnan and pregnen derivatives did not lead to any significant conclusions because their levels were too greatly affected by the menstrual cycle. However, the pregnantriol levels, which showed a statistically significant increase in the obese patients, were of some interest. Indeed, the pregnantriol level faithfully reflected that of 17-OH-progesterone, the chief substance in the intermediate metabolism of the corticosteroids, which are the direct precursors of both 11-desoxy-cortisol and of androstenedione, and thus of both the glucocorticoids and the androgens ([12]).

The increased urinary elimination of pregnantriol may be considered as a

further confirmation of hyperactivity of the adrenal cortex in obese women. As to the glucocorticoids THF, THE, cortolone and β-cortolone, we found that except for β-cortolone, the urinary values of the others were definitely increased in the obese women in comparison with the controls; the difference was statistically significant.

In this case too our results were not in agreement with those of other investigators, who obtained their findings with procedures which seem to deny the existence of glycocorticoid hyperfunction in obesity, or limit its possible presence to sthenic android obesity ([3, 6, 7, 8, 13]). In our opinion, gas chromatographic study of the glucocorticoids offers a further confirmation of the overall adrenal cortical hyperactivity, as demonstrated by tetra-hydrocortisol and tetra-hydrocortisone; the latter reached levels which were more than double those of tetrahydrocortisol. We know, however, that in Cushing's disease this relationship is reversed and the levels of the cortisol metabolites are higher than those of cortisone; it may be that under these conditions the enzyme system responsible for the reversible conversion of cortisol into cortisone functions under conditions of relative insufficiency ([14]).

Finally, the data relating to the mineral corticosteroids THA and a-THB were not in agreement with each other and did not allow interpretation for the present time.

In conclusion, gas chromatographic study of the urinary elimination of certain steroids in obese women appears to suggest a state of overall adrenal cortical hyperactivity. Of particular interest is the rise in the glucocorticoid steroids, which may be related to the increase in the total adipose mass and/or with the state of glycolytic intolerance characteristic of obesity. It is not possible as yet to determine whether the adrenal cortical hyperfunction in the obese subjects was the primary or secondary consequence of the increase in the total body mass. Another point which requires clarification and confirmation is the significance of the rise in certain androgen levels.

SUMMARY

The authors used gas chromatography to examine the behaviour of certain steroids in the urine of obese women.

The most important findings related to androsterone, which was found to be increased in the obese subjects in comparison with the controls; the difference was statistically significant. The discrepancy in the findings obtained with ethiocolanolone is discussed.

Statistically significant results were obtained with tetrahydrocortisol and tetrahydro-cortisone, which had increased levels in all the obese subjects. Possible correlations with the changes in the glycolytic and lipid metabolism in obesity were investigated.

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Assement of the uteroplacental circulation by electronic development of a scintiphotographic picture

by

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Despite the great number of studies carried out on the subject, assessment of the amount of the uteroplacental hematic flow continues to be very difficult even today. Furthermore, the various methods suggested for this type of research do not always meet the indispensable prerequisites for practical application, e.g. safety for mother and foetus, simplicity of management, the possibility of repetition during the last weeks of pregnancy and consistency of response.

We therefore decided to apply radioisotopic techniques, which are already being commonly used for the exact location of the sites for placental insertion ([4]), by extending the use of short-term half-life radionucleides (^{99m}Tc and ^{113m}In) to the functional study of uteroplacental circulatory dynamics ([5, 6]).

The isotope of choice for this kind of investigation was ^{113m}In. This nucleide has a very short physical half-life (1.7 hours), so that irradiation of the mother and foetus is much reduced; moreover, the ^{113m}In-gelatine compound used in such cases remains in the blood system for a long time (its biological semi-period is three hours), while its very low urinary elimination (approximately 1%) avoids assessment errors in cases of placenta previa which may occur when ^{99m}Tc is used ([1]).

PHOTOGRAPHIC METHOD

The pictures were taken by a Gamma Camera made by Nuclear Enterprises (Scinticamera III) connected to a continuous-image oscilloscope which allowed us to monitor directly and continuously the course of the investigation, and to check on the exact centering of the instrument.

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