limited to P/S ratio determination, we feel that the importance of centrifugation is the same also for all other methods of prediction of fetal lung maturity by means of amniotic fluid studies.

SUMMARY

The results of the determination of palmitic acid/stearic acid ratio in 20 paired samples of amniotic fluid and vernix caseosa at term are presented. Vernix caseosa has a high content of fatty acids and its palmitic acid/stearic acid ratio is constantly higher than the ratio of the corresponding amniotic fluid. Centrifugation in standard conditions of amniotic fluid samples before its analysis for palmitic acid/stearic acid ratio is an essential step in order to avoid the serious consequences of an erroneous prediction of fetal lung maturity.

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Effect of tryptophan-free diet on prolactin and cortisol plasma levels in normal males

by

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INTRODUCTION

It is well known that the administration of a tryptophan-deficient diet to the rat gives rise to a diminution of the plasma concentrations of this amino acid, with consequent inhibition of cerebral serotonin synthesis ([1,6,8]).

In man too, the intensive administration by the oral route of a mixture of

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amino acids containing no tryptophan results in a diminution of the plasma levels of this amino acid ([2]) similar to that encountered in rats ([8]). The role of tryptophan in the blood is however recognized ([10]) it is probably transformed into serotonin in the central nervous system ([7]) and helps to regulate the secretion of prolactin. The secretion of ACTH and thus of cortisol seems also to be dependent upon serotoninergic control ([9]) even though the mechanism of action is still far from clear.

Our object, therefore, was to discover whether the tryptophan plasma changes brought about by the intensive administration of amino acid mixtures not containing tryptophan might bring about changes in the plasma levels of prolactin and cortisol in normal males.

MATERIAL AND METHODS

Subjects. Mixtures of amino acids were administered orally to 12 healthy volunteers between 18 and 23 years of age, between 8 and 9 a.m., after 12 hours of complete fasting. A venous catheter was inserted into each subject, and physiological solution (7 - 10 drops per minute) was slowly infused before the amino acid mixture was administered. Two blood samples were obtained at 15 minute intervals.

After the administration of amino acids, two samples were obtained 230 and 250 minutes after the start of the experiment.

Mixtures of amino acids

The mixture of amino acids were prepared as suggested by Rose *et al.* ([12]). In aggregate, they represented the mean daily protein requirement in man.

Mixture composed of the following amino acids were administered to six subjects (controls): tryptophan 0.5 g; phenylalanine 2.2 g; lysine 1.6 g; tyrosine 1.0 g; valine 1.6 g; methionine 2.2 g; leucine 2.2 g; isoleucine 1.4 g; glycine 6.0 g.

A further six (treated) subjects received a mixture of the same composition with the exception of tryptophan, which was omitted.

Analises

All the analyses were performed on samples of plasma kept at –20°C until needed for the analysis.

The plasma levels of tryptophan were evalued by the fluorimetric method, after Tagliamonte *et al.* ([13]).

The plasma levels of cortisol were measured by the method of protein competition (CIS Kit).

The method used for prolactin was the radio-immunological method, using the double antibody technique (Biodata Kit).

RESULTS

In the subjects to whom the complete mixtures of amino acids were given, the plasma levels for tryptophan in the samples obtained before administration were found to be 13.4 ± 1.9 and 13.9 ± 2.1 µg/ml. Slightly higher concentrations were found at 230 and 250 minutes; between 14.7 ± 3.3 and 14.9 ± 4.2 µg/ml.

This difference was not found to be significant on statistical analysis using Student's 't' test.

In the treated subjects, the plasma concentrations of this amino acid were diminished from 13.2±1.5 and 14.2±2.3 to 8.0±1.6 and 7.8±1.5 μg/ml respectively at times −15, 0, 230 and 250 minutes. This difference was found to be statistically significant ($p < 0.001$). In the controls, before administration, the plasma cortisol was found to be 20.0±5.3 and 19.9±4.5 μg/100 ml; after 230 minutes it was 18.9±6.8 and after 250 minutes it was 18.5±6.3 μg/100 ml, without any significant difference. In the treated subjects, the level fell from 21.9±5.8 and 20.9±3.9 to 19.3±6.1 and 18.9±5.4 μg/100 ml. Prolactin was found in the controls to be 11.7±5.5 and 12.8±6.1 ng/ml before administration, and 8.9±3.2 ng/ml after 250 minutes. In the treated subjects, prolactin gave basic values of 13.7±6.5 and 14.2±6.7 ng/ml; this fell to 9.2±2.8 ng/ml at 230 minutes. At 250 minutes, concentrations of 8.2±3.7 ng/ml were read.

The fall in the level of prolactin in the treated subjects was significant according to Student's 't' test ($p < 0.05$), while in the controls the diminution in the levels of prolactin, although present, was not significant.

All the results are listed in Table 1.

Tab. 1. *Progress of plasma levels of trytophan, cortisol and prolactin before and after the administration of a tryptophan-free diet. Tryptophan ($p<0.001$) and prolactin ($p<0.05$) undergo significant modifications (mean±standard deviation).*

	Minutes		−15	0	+230	+250	P
	Tryptophan	(ug/ml)	13.4±1.9	13.8±2.1	14.7±3.3	14.9±4.2	N.S.
Controls (6.	Cortisol	(ug/100ml)	20.0±5.3	19.9±4.5	18.9±6.8	18.5±6.3	N.S.
	Prolactin	(ng/ml)	11.7±5.5	12.3±6.1	8.4±4.4	8.9±3.2	N.S.
	Tryptophan	(ug/ml)	13.2±1.5	14.2±2.3	8.0±1.6	7.8±1.5	0.001
Treated (6)	Cortisol	(ug/100ml)	21.9±5.8	20.9±3.9	19.3±6.1	18.9±5.4	N.S.
	Prolactin	(ng/ml)	13.7±6.5	14.2±6.7	9.2±2.8	8.2±3.7	0.05

DISCUSSION

The possibility of modifying the plasma levels of an essential amino acid such as tryptophan by our experimental approach has already been mentioned by other authors as regards both the rat and man ([2,8]).

The possibility, in man, of confirming changes in cerebral serotonin ([1]) as demonstred in the rat, is subordinated to the possibility of having a parameter that can easily be ascertained in the human species. It seems to us that this parameter may exist in two pituitary hormones, ACTH and prolactin, both of which are subject to control of a serotoninergic type ([4,9]).

The plasma levels of ACTH are assessed not directly, but via cortisol, whose secretion is notoriously dependent upon that of pituitary corticotrophin.

The plasma levels of cortisol, which underwent no change in the subjects we treated, provided a confirmation of the difficulty of altering the basal levels of this steroid by an experimental approach which could probably only modify the rate of synthesis of cerebral serotonin. Even using a drug with a powerful antiserotoninergic activity like metergoline, it is not possible to alter the basal levels of ACTH and cortisol, only their response to certain stimulus tests such as the metyrapone test ([3]) or the insulin hypoglycemia test ([4]).

We may therefore conclude that the intensive administration of a tryptophan-

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free diet cannot alter the basal levels of cortisol, nor can it alter its circadian rhythm, which was probably present unchanged in our subjects, givin a difference in the basal levels of cortisol between 8 a.m. and 12 noon of about 10% [11].

The behaviour of prolactin appear to be different. When measured in the controls it showed no significant modification, while in the treated subjects it decreased significantly.

This observation confirms that our experimental approach was capable of altering the mono-aminergic cerebral equilibrium.

In particular we may remember that in man too, the oral administration of a « pool » of tryptophan-free amino acids may probably induce cerebral depletion of serotonin. We were unable to confirm this indirectly, via changes in the basal levels of prolactin.

However, we cannot exclude interference by other factors. It is certain that serotonin acts upon the capacity of the pituitary to secrete prolactin; and this can be obtained either by the intravenous administration of tryptophan [10] or by the use of antiserotoninergic drugs, such as metergoline [5].

Therefore the results obtained further reinforce the concept of the existence of the serotoninergic regulation of the secretion of prolactin in man. These observations do constitute a first step in the study of the hormonal changes induced by this experimental approach, and they provide a stimulus for the assessment of this phenomenon either by different techniques (long-term administration, intensive parenteral administration) or by including tests of stimulation or suppression.

The utilization of stimulation may also explain more clearly the mechanism of action and the levels at which the changes in serotonin (induced by a tryptophan-free diet) act upon the secretion of prolactin.

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