acutely via transcellular shifts[4] but there was no evidence that any patient was hyperventilated during the intubation period.

Acidosis depresses glycolytic activity,[5] raises the serum phosphate concentration, and thereby intensifies excretion in the urine. Thus severe phosphate deficiency may occur in respiratory acidosis. When the pH returns to normal hypophosphataemia may develop because of increased shifts of phosphorus from the blood compartment into the intracellular compartment secondary to increased glycolytic activity.

Phosphate depletion is associated with a decreased concentration of adenosine triphosphate in skeletal muscle and may result in muscle weakness. Phosphate deficiency and hypophosphataemia lead to mobilisation of bone mineral,[1] which might explain the spontaneous return to normal of the serum phosphate concentrations. Furthermore, probably intracellular adenosine triphosphate concentrations return to normal together with serum phosphate values, thus explaining why the patients were dependent on mechanical ventilation until the serum phosphate concentration came within the normal range.

Newman et al reported that patients with hypophosphataemia responded dramatically to intravenous repletion within hours.[3]

In this series, however, the spontaneous changes in serum phosphate concentrations seen during recovery from acute respiratory acidosis (figure) suggest that phosphate replacement is not invariably necessary as a standard treatment of respiratory failure associated with low concentrations of serum phosphate. Nevertheless, patients should be monitored by repeated serum phosphate determinations, especially when it is difficult to achieve successful weaning.

Recovery from acute respiratory acidosis should be added to the list of conditions associated with severe hypophosphataemia.

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Increasing saliva (free) oestriol to progesterone ratio in late pregnancy: a role for oestriol in initiating spontaneous labour in man?

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Abstract

Oestriol and progesterone concentrations were measured in samples of saliva obtained daily from six normal women during the final four weeks before the spontaneous onset of labour. Progesterone concentrations were found to plateau whereas oestriol concentrations continued to rise so that the mean ratio of saliva oestriol to progesterone increased from 0·80 to 1·43 between 29 days and one day before labour. Saliva oestriol concentrations were 15 times higher than saliva oestradiol concentrations.

As saliva steroid concentrations reflect the unbound unconjugated (free) plasma steroid concentrations these data suggest that a changing ratio of oestriol to progesterone may play a part in initiating spontaneous labour in man.

Introduction

The onset of labour in sheep and some other mammalian species is preceded by a rise in the maternal plasma oestrogen to progesterone ratio.[1-3] No consistent change in the plasma unconjugated oestradiol to progesterone ratio has been shown before the onset of labour in the rhesus monkey or in man.[4-7]

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Plasma unconjugated steroid concentrations, however, consist of both the protein bound steroid and the biologically available unbound or "free" steroid, and as the percentage binding of the oestrogens and of progesterone to plasma proteins differs considerably[8] (H H G McGarrigle, G C L Lachelin, paper presented at 2nd joint meeting of British endocrine societies, April 1983 (abstract 64)), real changes in the ratios of the unbound unconjugated steroids cannot be shown by measurements of the total plasma unconjugated steroid concentrations.

We and others have shown that saliva oestriol, oestradiol, and oestrone concentrations in pregnancy reflect the plasma unbound unconjugated or free oestrogen concentrations[9] (H H G McGarrigle, G C L Lachelin, paper presented at 2nd joint meeting of British endocrine societies, April 1983 (abstract 64)), and similar findings have been reported for saliva progesterone and plasma unbound progesterone concentrations in late pregnancy.[10] We therefore measured saliva oestriol and progesterone concentrations in daily samples as well as saliva oestrone and oestradiol concentrations in weekly samples collected during the four weeks preceding the spontaneous onset of human labour, and we examined the ratios of oestriol, oestrone, and oestradiol to progesterone in these samples.

Patients and methods

Saliva samples were collected at intervals during pregnancy and daily during the four weeks before the spontaneous onset of labour by six normal pregnant women who delivered a healthy infant of normal birth weight vaginally after 37 weeks' gestation. The 5 ml samples were collected in plastic universal containers at about 1000 after the mouth was rinsed with water. They were stored at home in a freezer and then in the laboratory at −40°C until they were assayed.

All the steroids were measured by radioimmunoassay. Before assay all saliva samples were thawed, mixed, and centrifuged at 2000 g for 10 minutes. For measurement of oestriol and progesterone 50 μl

duplicate aliquots of saliva were pipetted into glass tubes with 25 μl sodium bicarbonate buffer pH 10·5. The contents were then extracted using 2·5 ml freshly distilled ether, the tubes frozen, the ether decanted and evaporated, and the residue assayed using a tritiated tracer for its oestriol or progesterone content. Overall recoveries were 95% for oestriol and 94% for progesterone. The intra-assay and interassay coefficients of variation were for oestriol 5·6% and 9·4% and for progesterone 8·1% and 12·2% respectively.

For the measurement of saliva oestrone and oestradiol concentrations 0·5 ml aliquots of saliva in duplicate were mixed with 0·25 ml bicarbonate buffer and extracted with ether. The ether was decanted and evaporated and the residue chromatographed on Sephadex LH20 micro columns using a benzene and methanol mixture. The appropriate eluate fractions were evaporated and assayed for their oestrone and oestradiol contents. The intra-assay coefficients of variation for oestrone and oestradiol were 9·2% and 10·6% respectively.

Results

The figure shows the mean saliva concentrations in the six patients. The values shown for oestriol and progesterone more than four weeks from delivery are means of single estimations in each patient, but as the timing of the sampling differed for each patient the values given are means ranging over a two week period. The values given from 29 days to one day before labour are means of daily samples. The oestrone and oestradiol concentrations shown in the figure are mean values in the saliva samples obtained on days 28, 21, 14, 7, and 1 before labour.
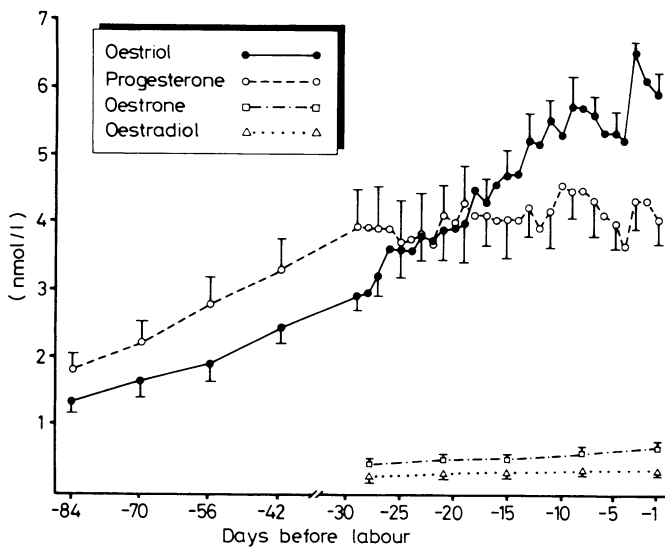
Mean (SEM) saliva concentrations of oestriol, progesterone, oestrone, and oestradiol in six patients before spontaneous labour.

Conversion: SI to traditional units—Oestriol: 1 nmol/l ≈ 0·29 ng/ml. Progesterone: 1 nmol/l ≈ 0·32 ng/ml. Oestrone: 1 nmol/l ≈ 0·27 ng/ml. Oestradiol: 1 nmol/l ≈ 0·27 ng/ml.

In five of the six patients the days on which the molar concentration of oestriol exceeded the molar concentration of progesterone in the daily saliva samples, and continued to remain above the progesterone concentration thereafter, ranged from 16 days to 28 days before labour. In the sixth patient the oestriol concentration became roughly equimolar to the progesterone concentration in the final five daily saliva samples before delivery. The mean crossover point for the six patients was on day 18 before the onset of labour (figure).

The concentrations of oestriol and progesterone increased by 100% and 6% respectively between day 29 and day 1 before labour. The means of the mean daily values for oestriol and progesterone for each of the final four weeks were calculated. The increases in the mean oestriol concentrations between weeks 4 and 3, 3 and 2, and 2 and 1 before labour were significant (p < 0·001, p < 0·001, and p < 0·01 respectively). There was no significant difference between mean progesterone concentrations during the final four weeks before labour.

The mean concentrations of oestrone and oestradiol showed increases of 64% and 46% respectively between day 28 and day 1

Ratios of oestriol, oestrone, and oestradiol to progesterone at weekly intervals before spontaneous onset of labour in six normal pregnant women. Ratios of oestriol to progesterone earlier in pregnancy are also shown

Ratio	Weeks before labour				Days before labour				
	12	10	8	6	28	21	14	7	1
Oestriol: progesterone	0·71	0·70	0·67	0·73	0·80	0·95	1·17	1·32	1·43
Oestrone: progesterone					0·104	0·131	0·124	0·143	0·156
Oestradiol: progesterone					0·070	0·076	0·077	0·079	0·092

before labour; these increases were significant ($t = 2·77$, p < 0·05 for oestrone, and $t = 4·11$, p < 0·01 for oestradiol, paired t test).

The table shows the mean ratios of oestrone, oestradiol, and oestriol to progesterone.

Discussion

Progesterone blocks the spontaneous contractile activity of the myometrium, and it has been suggested that the myometrium remains relatively inactive throughout pregnancy because it is inhibited by rising progesterone concentrations.[11] Rising progesterone concentrations are thought to inhibit and rising oestrogen concentrations to enhance the synthesis of prostaglandins in uterine tissue,[11] the formation of gap functions in the myometrium,[12] and the formation of oxytocin receptors and α adrenoceptors in the myometrium.[13][14] A rapid rise in oestrogen and a rapid fall in progesterone concentrations (in effect an increase in the ratio of oestrogen to progesterone and a withdrawal of the "progesterone block"[11]) is the initial signal for the onset of parturition in several mammalian species.[2][3][15]

Earlier reports of a rise in the ratio of oestrogen to progesterone in human plasma before labour[5] were not confirmed by subsequent studies.[6][7][16] In all of these studies, however, the frequency of sampling was at best twice weekly or weekly and only the plasma total (bound and unbound) unconjugated oestrogen and progesterone concentrations were measured.

Our data show an increase in the ratio of unbound unconjugated oestriol to progesterone during the final four weeks before labour. This ratio remains fairly constant until about four weeks before labour (table). At this point progesterone concentrations plateau whereas oestriol concentrations continue to increase. The molar concentration of saliva oestriol has been equal to or greater than that of saliva progesterone by the start of labour in all the patients that we have studied—namely, the six reported on here and four others who were not included here because of infrequency of sampling.

Previous studies of changes in the ratio of oestrogen to progesterone in maternal plasma before labour in man concentrated on the relation between oestradiol and progesterone.[5-7] Oestriol appears to have been largely ignored, even though it is found in the unconjugated form in women only during pregnancy, is produced in much larger quantities during the third trimester of pregnancy than oestrone or oestradiol, and is derived mainly from steroid precursors of fetal origin, whereas oestrone and oestradiol originate in roughly equal proportions from maternal and fetal adrenal steroid precursors.[17] This disregard of oestriol is presumably because it was long considered to be a weak or impeded oestrogen.[18] It was subsequently shown, however, to be equipotent to oestradiol in its oestrogenic effects when administered to rats continuously[19] rather than as a single injection.[18] Furthermore, oestriol will displace oestradiol in vitro from human myometrial oestrogen receptors,[20] and human myometrium actively accumulates oestriol in vivo after the infusion of tritiated oestriol tracer into postmenopausal women for 12 hours before hysterectomy.[21]

The data presented here and our previous findings[22] (H H G McGarrigle, G C L Lachelin, paper presented at 2nd joint meeting of British endocrine societies, April 1983 (abstract 64)) show clearly that plasma unbound unconjugated oestriol concen-

trations are 15 times higher than plasma unbound unconjugated oestradiol concentrations. This suggests that in late pregnancy there is a dominant role for oestriol in competition with oestradiol for available oestrogen receptors in the myometrium. In our view this finding, allied to the inference that plasma unbound oestriol concentrations equal or exceed plasma unbound progesterone concentrations during the last few weeks before delivery resulting in an increasing ratio of oestriol to progesterone, strongly suggests that oestriol may play a part in initiating normal spontaneous labour in man.

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Sex steroid binding patterns in primary biliary cirrhosis complicated by hepatocellular carcinoma

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Abstract

Owing to recent findings of certain unusual sex steroid binding in liver disease—particularly an allosteric biphasic pattern (pattern A) unique to the serum of patients with hepatocellular carcinoma—the serum binding characteristics for 5α-dihydrotestosterone were examined in serum samples from six patients with primary biliary cirrhosis who had developed hepatocellular carcinoma. In all serum samples taken after the development of tumour pattern A binding only was obtained, and in four cases in which earlier samples were also examined there was a transformation from the normal, non-specific binding pattern, or an allosteric plateau pattern seen in non-malignant liver disease (designated D and C respectively), to pattern A coincident with the rise in serum α fetoprotein. In one patient chemotherapy leading to a fall in α fetoprotein abolished pattern A binding, showing further its close association with tumour growth.

The value of pattern A binding as a tumour marker in hepatocellular carcinoma warrants further study.

Introduction

Studies using the two tier column assay[1] for binding of sex steroids in serum have detected two novel patterns of binding in the Cibacron blue 3GA-Sepharose 6B (blue gel) portion of the column when sera from patients with liver disease were examined. The first—a biphasic allosteric pattern (pattern A)—was found only in sera from patients with hepatocellular carcinoma and in fetal liver cytosol,[2 3] and the second—an allosteric plateau pattern (pattern C)—was found in non-malignant liver disease, including primary biliary cirrhosis.[3] Both patterns were attributed to a single binding moiety, termed fetal steroid binding protein. These two binding patterns are distinct from the more usual form of sex steroid binding associated with high affinity, low capacity specific binding protein such as sex hormone binding globulin, androgen, and oestrogen receptors (pattern B) and the low affinity, high capacity non-specific binding to proteins such as human serum albumin (pattern D).

We have examined serum samples from six patients with primary biliary cirrhosis in whom hepatocellular carcinoma had developed. The novel sex steroid binding patterns were investigated in relation to time of diagnosis and changes in serum α fetoprotein concentrations.

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Patients and methods

Of 130 patients (18 men) with primary biliary cirrhosis seen in the liver unit during 1975-83, seven were found to have developed hepatocellular carcinoma six months to nine years after diagnosis of the cirrhosis.[4] Hepatocellular carcinoma was confirmed histologically and in six patients serum samples were available, which are the subject of