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THE INFLUENCE OF INTRA-AMNIOTIC INJECTIONS OF ACTH ON THE EXCRETION OF OESTROGENS, 17-KETOSTEROIDS, 17-KETOGENIC STEROIDS, AND PREGNANEDIOL IN MIDPREGNANCY

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SUMMARY

The excretion of oestrogens, 17-ketosteroids, 17-ketogenic steroids, and pregnanediol was estimated before and after administration of ACTH to 6 women in the second trimester of pregnancy. In four cases the ACTH was injected amniotically and in two cases intravenously. The plasma cortisol level was followed in two cases.

The intra-amniotic injections caused an increase in the excretion of oestrogens, 17-ketosteroids and 17-ketogenic steroids while there was no change in the plasma cortisol concentration. The intravenous injections were not observed to effect any essential change in the excretion of oestrogens and 17-ketosteroids, but an increase was noted in the excretion of 17-ketogenic steroids and in plasma cortisol. It was concluded that ACTH may be resorbed from the amniotic cavity by the fetus but that no part thereof, or only a very small proportion, enters the maternal circulation. The hypothesis has been put forward that the changes thus effected in the excretion of steroids are caused by a stimulation of the fetal adrenal, and the results are taken to indicate the existence of a fetal hypothalamus-pituitary-adrenal 'feedback mechanism'.

INTRODUCTION

In accordance with a review by Diczfalusy and Mancuso[1] the placenta and fetus are two specialized compartments of an integrated functional unit, with a characteristic distribution of the essential steroidogenic enzyme systems between the two compartments. The fetus is regarded as the active site of intermediary metabolism and the fetal adrenal gland in particular plays the major role in the production of precursors of the large amounts of oestrogens excreted in the urine of pregnant women.

Not much light, however, has been shed on the problem concerning the primary control of the development and functioning of the fetal adrenal. The idea would immediately suggest itself that this control is exercised by tropic hormones, and that it is of much the same character as the mechanism controlling the adrenal glands in adults. No direct parallel can, however, be drawn between the two. A comparison of the adult and fetal adrenal will show pronounced structural and functional differences, and the picture is further complicated by the probable influence of the placenta and the maternal endocrine system. Observations from experiments with animals as well as clinical results seem to indicate, though, that in the fetal organism there is a certain degree of interaction between the adrenal glands, the pituitary gland and the hypothalamus.

Jost *et al.*[2, 3] demonstrated that intrauterine decapitation of the embryos of rats and rabbits caused the fetal adrenal to atrophy, but that this could be prevented by administration of ACTH to the decapitated fetuses. Similar atrophic

changes could be produced by injection of cortisone into rat fetuses. According to Jones[4] it is the outer permanent cortex especially which in rat fetuses is stimulated by ACTH whereas the activity of the fetal zone is mainly dependent on LH.

Clinical experiences likewise indicate that ACTH affects the cortex of the fetal adrenal. Frandsen and Stakemann[5] observed atrophy of the adrenal glands in a number of anencephalic children whose mothers during pregnancy had displayed a persistently low urinary oestrogen output, and Lanman[6] found it possible by treatment of an anencephalic child with ACTH over a period of 18 days to produce adrenal glands of a size normal for mature infants. In this connection it should be mentioned that Taylor *et al.*[7] identified ACTH in an extract from human fetal pituitary glands and that Stark *et al.*[8] found that cortisol was being produced in a culture fluid of human fetal pituitary and adrenal tissue, and that this production was intensified through addition of ACTH.

In recent years important results have, moreover, been achieved in the attempts made to interfere with the functioning of the fetal pituitary-adrenal 'feedback mechanism' by inhibition and stimulation, respectively. Treatment of patients in the last trimester of pregnancy with corticosteroids has occasionally effected a considerable depression of the maternal excretion of oestriol[9–11]. The authors have concluded that the steroids thus administered pass through the placental membrane, thereby inhibiting the functioning of the fetal pituitary gland.

In agreement with these results are those published by Dickey and Thompson [12]. Intravenous injections of ACTH to patients in the last trimester of pregnancy have effected a significant decrease in the excretion of oestriol accompanied by a significant rise in the excretion of 17-ketogenic steroids, while the excretion of 17-ketosteroids and pregnanediol remained unchanged, thus indicating that increased amounts of cortisol produced because of the stimulation of the maternal gland may enter the fetal circulation. Subsequent treatment of the same pregnant cases with metyrapone caused a slight increase in the excretion of oestriol and a pronounced increase in the excretion of both 17-ketogenic steroids and 17-ketosteroids. The authors interpret this to mean that the metyrapone passes through the placenta, and the resulting blocking of the 11-beta-hydrolysis in the fetal adrenal causes an increase in the fetal pituitary production of ACTH.

When injecting ACTH into the bloodstream of women in late pregnancy with a live fetus Dässler[13] observed an increase in the maternal excretion of oestriol whereas no such change was noted from similar treatment of patients 10–11 weeks pregnant or of patients carrying a dead fetus. Evaluation of these results is difficult, since experience derived from anencephalic fetuses, coupled with the normal excretion of oestriol observed in hypophysectomized pregnant women [14, 15] make it reasonable to believe that maternal ACTH plays no important part as a stimulant of the fetal adrenal.

An interesting experiment with more direct stimulation of the fetal adrenal has been made by Johannisson[16], who after intra-amniotic injections of ACTH and HCG observed that the fetal cortex had undergone certain ultrastructural changes, indicating increased functional activity of this portion of the gland. On the other hand, Frandsen *et al.*[17] failed in their attempt to effect an increase in the excretion of oestrogens, 17 ketogenic steroids and 17-ketosteroids in experiments involving intrafetal injections of ACTH or metyrapone to the

mother in pregnancies complicated with anencephalic fetuses. However, when ACTH was injected intrafetally into patients in the second trimester an increase in the excretion of 17-ketogenic steroids and 17-ketosteroids was observed.

Lauritzen and Lehman[18, 19] produced an increase in the excretion of DHA by treatment of newborn infants with ACTH and HCG. In similar experiments with ACTH Lauritzen *et al.*[20] determined an increase in the delta-5-steroids and cortisol excreted by newborns.

There is thus every reason to believe that ACTH has a controlling influence on steroidogenesis in the fetal cortex and that stimulation with ACTH will primarily result in an increased production of 17-ketosteroids, causing a rise in the excretion of oestrogens from the feto-placental unit.

Working on basis of this hypothesis we have tried to stimulate the fetal adrenal by intra-amniotic injections of large doses of ACTH since it would seem possible, judging from the material at hand, to interfere with the fetal 'feedback mechanism' through this form of administration.

EXPERIMENTAL

Complete 24-h collections of urine were continued throughout the entire investigation period. The various specimens were quick-frozen immediately, and all urine collected from the individual patient was analyzed at one time.

The methods applied were as follows:

Oestrogens: Brown *et al.*[21]

This method is based on acid hydrolysis, fractionated ether extraction, Kober and Ittrich reaction and fluorimetry.

Pregnanediol: Klopper *et al.*[22]

This method is based on enzymatic hydrolysis, chromatography on alumina columns, acetylation, a second chromatography, and sulphuric acid colour-reactions.

17-Ketogenic steroids: Wilson and Lipsett[23]

This method involves sodium borohydride reduction, sodium periodate oxidation, mild alkaline hydrolysis, ether extraction and Zimmermann reaction.

17-Ketosteroids: Vestergaard[24]

This method is based on acid hydrolysis, ethyl ether extraction, washing with alkaline solution and Zimmermann reaction.

17-Ketosteroids (fractionated): Johnsen[25]

This method involves acid hydrolysis, benzene extraction, washing with alkaline solution, chromatography on alumina columns and Zimmermann reaction. The following fractions are isolated: the dehydroepiandrosterone fraction (DHA), the androsterone fraction (A), the etiocholanolone fraction (E) and the remainder fraction (mainly corticosteroids). The value of the total 17-ketosteroid excretion measured by this method is about 30% higher than the values of other methods in general use.

Cortisol in plasma: De Moor *et al.* [26]

This procedure consists of an extraction of the plasma with dichloramethane, washing of the extract with 0·1 N NaOH and re-extraction with the fluorescence reagent ethanol–H_2SO_4.

The oestrogens and plasma cortisol were measured by the Hormone Laboratory, Frederiksberg Hospital, the pregnanediol by Medicinsk Laboratorium Copenhagen, and the other steroids by Statens Seruminstitut, Hormone Department, Copenhagen.

CLINICAL MATERIAL

The patients were admitted to our Department for the purpose of legal termination of pregnancy, the main purpose in all cases being the mental welfare of the patient.

During the entire investigation period complete 24-h collections of urine were continued, to be analyzed for oestrogens, 17-ketosteroids, 17-ketogenic steroids, and pregnanediol. In some cases blood tests were also made for cortisol.

At the end of two or three 24-h control periods the patients were given corticotrophin (Acton) (see Table 1). On four patients a transabdominal amniocentese was performed in which ACTH in 20 ml isotonic sodium chloride was injected into the amniotic cavity following the aspiration of an equivalent volume of amniotic fluid. These operations were all without complications and resulted in every instance in a clear amniotic fluid without traces of blood. In a further two cases ACTH in a 5% concentration of glucose was introduced into the maternal bloodstream over a period of four hours.

Immediately before the abortive operation on the first mentioned four patients they were again subjected to amniocentese for the purpose of collecting specimens of amniotic fluid for analysis. All these specimens were tested for oestrogens.

In none of the cases were there complications or side effects ascribable to the procedures followed in these experiments.

Table 1 shows case history and information on the nature of the operation,

Table 1. Clinical features of the 6 pregnant patients receiving ACTH

Case	Age	Parity	Gestational age	Present pregnancy	Type of operation	Fetal weight	Crown-heel	Placenta	Dose ACTH
'AC'	20	I	16 weeks	Uncompl.	Lap. c. sectio parva	180 g	31 cm	Nat.	150 I.U.
'LA'	26	II	15 weeks	Uncompl.	NaCl 20% intraov.	?	26 cm	Nat.	225 I.U.
'SR'	24	I	17 weeks	Uncompl.	Lap. c. sectio parva	290 g	32 cm	Nat.	225 I.U.
'TL'	28	II	19 weeks	Uncompl.	Lap. c. sectio parva	480 g	37 cm	Nat.	225 I.U.
'LT'	22	I	15 weeks	Uncompl.	NaCl 20% intraov.	?	?	Nat.	30 I.U.
'VN'	24	III	15 weeks	Uncompl.	NaCl 20% intraov.	?	?	Nat.	30 I.U.

Table 2. The excretion of oestrogens, total 17-ketosteroids (17-KS), dehydroepiandrosterone (DHA), androsterone (A), etiocholanolone (E), 17-ketogenic steroids (17-KGS) and pregnanediol (P-DIOL), the concentration of oestrogen in amniotic fluid and of cortisol in plasma before and after intra-amniotic injection of ACTH (patients: 'AC', 'LA', 'SR', 'TL') and intravenous infusion of ACTH (patients: 'LT', 'VN'). a = before injection, b: after injection, c: after abortion

		Urine							Amniotic fluid	Plasma
		Oestrogen (mg/24 h)	17-KS (mg/24 h)	DHA (mg/24 h)	A (mg/24 h)	E (mg/24 h)	17-KGS (mg/24 h)	P-DIOL (mg/24 h)	Oestrogen (μg/100 ml)	Cortisol (μg/100 ml)
		4·3	8·3				12·8			
	a	3·6	6·6				9·8			
'AC'		3·2	4·9				8·6		19·4	
		4·6	12·9				20·1			
	b	6·6	7·5				11·3		25·7	
		6·0	5·8	1·7	0·8	1·2	2·6	3·2		
	a	7·1	10·3	4·1	0·9	1·6	4·5	7·4	55·0	
		4·8	17·8	7·7	1·9	2·1	7·2	10·5		
	b	7·1	18·5	8·0	2·4	2·3	6·8	8·9		
'LA'		7·8	18·1	8·4	1·5	1·7	6·7	12·3	42·7	
		3·7	12·4	5·5	2·3	1·4		10·9		
	c	1·4	9·7	4·0	1·6	1·5	5·9	8·9		
		0·7	7·8	3·2	0·7	1·4	3·6	5·3		
		6·7	10·4	3·6	1·4	1·8	9·7	10·0		÷20 h: 17·8
	a	7·6	16·1	5·6	1·3	2·9	9·6	4·5	21·0	÷ 4 h: 22·6
'SR'		9·1	—	—	—	—	—	12·2		Injection
	b	10·4	30·8	11·3	2·7	6·4	19·5	11·7		+ 4 h: 25·9
		7·0	16·1	7·6		3·3	9·4	15·4	17·0	+ 20 h: 24·0
		4·2	10·7	2·0	1·2	3·2	6·1	12·2		
	a	3·9	10·0	1·6	1·3	3·3	7·1	13·2	25·2	
'TL'		5·6	18·1	2·5	2·8	4·5	11·0	5·1		
	b	9·4	16·2	2·1	2·1	4·3	9·7	15·7		
		6·4	17·6	2·8	2·5	5·2	10·4	13·1	35·8	
		3·1	10·7	1·5	3·6	2·5	7·8	11·7		
	a	3·3	10·0	1·2	2·9	1·9	7·3	9·9		÷20 hr: 28·8
'LT'		2·2	11·7	1·6	3·6	2·7	16·0	10·5		÷ 4: 30·0
	b	1·5	6·5	1·1	2·1	1·2	6·1	6·4		Infusion
	c	0·9	5·0	0·8	1·6	0·9	5·3	5·1		+ 4 h: 75·8
		0·9	4·6	0·7	1·5	0·6	5·8	4·8		+ 20 h: 23·9
		1·0	7·9				10·4	11·2		
	a	2·0	7·8				8·6	10·7		
'VN'	b	2·0	8·6				16·9	9·4	16·1	
	c	2·0	5·4				4·7	3·5		
		2·0	5·7				3·9	2·1		

*According to the students t-test the differences in hormone excretion between 'a' and 'b' were: for oestrogens in urine: $0{\cdot}02 < p < 0{\cdot}05$; for 17-ketosteroids in urine: $0{\cdot}02 < p < 0{\cdot}05$; for 17-ketogenic steroids in urine: $0{\cdot}02 < p < 0{\cdot}05$; for pregnanediol in urine: $0{\cdot}05 < p < 0{\cdot}1$.

the product of the conception and the amount of ACTH administered. The first four patients ('AC', 'LA', 'SB' and 'TL') were given intra-amniotic injections while in the last two patients ('LT' and 'VN') the ACTH was administered intravenously.

RESULTS

Table 2 illustrates the excretion of hormones before and after administration of corticotrophin.

Excretion of oestrogens

When comparing the maximum increase in excretion with the average output

for the preceding two 24-h control periods it was found that *intra-amniotic injections* produced an increase in oestrogen excretion by 76%, 20%, 46% and 130%, respectively. In three cases ('AC', 'SR' and 'TL') the maximum increase occurred during the second 24-h period. In the fourth case ('LA') the reaction was rather uncertain with an initial fall in oestrogen excretion being followed by a comparatively slight increase.

In the two patients ('LT' and 'VN') *intravenous administration* of ACTH caused a decrease in oestrogen excretion by 53% and 0%, respectively, of the average initial value.

Following the induced abortion the excretion dropped rapidly ('LA').

Excretion of 17-ketosteroids

After *intra-amniotic injection* an increase was seen in all cases in the maternal excretion of 17-ketosteroids. The rate of increase was 95%, 128%, 132%, and 74%, respectively, of the average initial value. When studying the individual cases, no significant change was, however, ascertained in the ratio between the fractions: dehydroepiandrosterone, androsterone and etiocholanolone.

The induced abortion resulted in a rather rapid fall in the excretion of 17-ketosteroids ('LA').

The *intravenous administration* of ACTH had no appreciable effect on the excretion of 17-ketosteroids.

Excretion of 17-ketogenic steroids

Intra-amniotic injections resulted in an increase in the excretion of 17-ketogenic steroids by 93%, 100%, 101%, and 67%, respectively of the average initial value. Here also a rather rapid fall was seen following the termination of pregnancy.

During the 24-hour period immediately following *intravenous administration* the excretion of 17-ketogenic steroids rose by 111% and 78%, respectively.

Excretion of pregnanediol

An *intra-amniotic injection* of ACTH effected an increase in pregnanediol excretion in two patients ('LA' and 'SR'). The maximum increase occurred during the 3rd 24-h period following the injection, being at the rate of 132% and 111%, respectively, of the average initial value. In a third patient ('TL') there was an initial decrease in the excretion of pregnanediol, followed by a slight increase.

The excretion of pregnanediol was not effected by *intravenous administration* of ACTH.

The oestrogen content of the amniotic fluid

In two patients ('AC' and 'TL') *intra-amniotic injection* of ACTH was followed by an increase in oestrogen concentration of 33% and 42%, respectively, over the initial value. In two other patients, however, the oestrogen concentration decreased by 22% ('LA') and 11% ('SR').

Cortisol in plasma

ACTH injected *intra-amniotically* effected no appreciable change in the cortisol concentration in plasma.

On the other hand, ACTH administered *intravenously* effected an increase in cortisol concentration by 158% of the average initial value.

DISCUSSION

When injecting corticotrophin intra-amniotically the following alternative results must be foreseen:

(1) The drug may be resorbed to such a slight extent only or become inactivated so rapidly that the effect, if any, is not measurable.

(2) It may be resorbed into the maternal circulation, resulting in a measurable response from the maternal endocrine system.

(3) It may be resorbed into the fetal circulation and the resulting change in the fetal-placental endocrine balance may be measured.

Re 1

The results described above demonstrate that ACTH injected intra-amniotically effects a change in the endocrine balance of the pregnant organism. This is taken to mean that ACTH is being resorbed from the amniotic cavity.

The excretion of 17-ketosteroids and 17-ketogenic steroids was seen to increase even during the first 24-h period following the injection, and in three of the patients increased values were observed in the second or third such period. The maximum increase of the oestrogen output was seen in the second period. This might indicate that resorption starts rather promptly following the injection but that there may be some ACTH left in the amniotic fluid a couble of days after the injection. On the other hand, the possibility of a certain degree of local destruction of the injected ACTH cannot be ignored, the more so in view of the fact that the injected doses are rather high.

There is thus evidence that ACTH may pass from the amniotic cavity, but how this takes place has not been fully elucidated. Theoretically, there are three possibilities: through the wall of the uterus, through the fetal skin or through the fetal intestinal canal. The last alternative seems most probable for several reasons. It has thus been demonstrated[27] that Thorotrast injected intra-amniotically may be traced to the intestinal canal of 15-week-old fetuses. Migeon *et al.*[28] injected [4-^{14}C] cortisol into the amniotic cavity, and the results thus achieved seemed to indicate that cortisol is being resorbed via the intestinal canal of the fetus. The situation might very well be the same as far as corticotrophin is concerned.

Re 2

It is conceivable that the increase effected in the excretion of 17-ketosteroids and 17-ketogenic steroids may be caused by stimulation of the maternal adrenal. The estimation of plasma cortisol in one of the patients ('SR'), however, showed unchanged values before and after administration of 225 I.U. ACTH injected intra-amniotically, whereas, in another pregnant case ('LT') an intravenous infusion of only 30 I.U. ACTH effected an increase in plasma cortisol by more than 150%, while the excretion of 17-ketosteroids remained nearly unchanged.

The results thus obtained, therefore, provide no basis for the theory that after intra-amniotic injections of ACTH a considerable proportion of this drug enters the maternal circulation.

Re 3

In view of the foregoing it seems likely that the injection of ACTH into the amniotic cavity has produced a response from the feto-placental endocrine system.

This theory is supported by the increase effected in the oestrogen excretion, it having been established that during pregnancy the feto-placental unit is the main unit for production of this hormone.

Hereafter the following hypothesis seems justified:

Adrenocorticotrophin is resorbed from the amniotic cavity into the feto-placental circulation where it serves to stimulate the fetal cortex. This stimulation increases the production of 17-ketosteroids, a part of which is aromatized into oestrogens in the placenta.

By stimulation of the maternal adrenal (ACTH administered intravenously) no increase was effected in 17-ketosteroids, and in one patient ('LT') there seemed on the contrary to be a slight tendency to a depression of the oestrogen excretion. In accordance with Dickey and Thompson[12] this may be explained by the increase in the production of corticosteroids (an increase in 17-ketogenic steroids and plasma cortisol), which pass through the placental membrane and inhibit the function of the fetal adrenal.

In one patient ('LA') the change in steroid pattern was atypical. This situation is difficult to assess, but it should be pointed out that the gestational age of this patient (15 weeks) was lower than in the other cases.

The rather vague picture of the change in the excretion of pregnanediol is difficult to explain. It may be that more progesterone is being produced in the placenta to compensate for an increased consumption of progesterone in the fetal adrenal.

The results obtained must, of course, be evaluated with a grain of salt, for one thing because of the possibility of having interfered with two integrated endocrine systems. The findings, however, may be taken in further confirmation of the theory of the existence of a fetal hypothalamus-pituitary-adrenal 'feedback mechanism'. It is difficult to say whether the fact that artificial interference in this balance is possible may be of any clinical importance, but this forms a very interesting perspective from a clinical point of view.

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