TABLE 1. Number and Cell Content of Colonies in Spleens of Intermediate Recipients

Source of CFU_s	Number of colonies per spleen	Number of nucleated cells per colony
Embryonic blood	1,4±0,5	$12,6 \cdot 10^6$
Embryonic liver	0,8±0,3	$14,5 \cdot 10^6$

TABLE 2. Self Support of Embryonic CFU_s from Peripheral Blood and Liver

Source of CFU_s	No. of cells injected (in colony equivalents)	Number of colonies per spleen	Number of CFU_s per colony	
			in each group	$M \pm m$
Embryonic blood	1/5	23,5±3,5	118	169±26
	1/50	4,4±0,7	220	
				$P>0,05$
Embryonic liver	1/5	19,3±5,6	96	
	1/50	3,2±0,5	160	128±26

CFU_s with reduced ability for self support into the blood stream preferentially, thus seems more likely to be correct.

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IMMUNOREACTIVE ACTH LEVEL IN THE HUMAN PITUITARY AND BLOOD SERUM DURING PRENATAL DEVELOPMENT

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From the published evidence there is no doubt that the hypophyseo-adrenocortical system in man becomes functionally active before birth [6, 8, 9, 11]. The first evidence of the presence of ACTH in the pituitary of human fetuses at 21 and 27 weeks was published in 1953 by Taylor et al. [12]. Skebel'skaya [4] showed that adrenocorticotrophic activity is present in acetone extracts of pituitary glands of human fetuses after the 9th-10th week of intrauterine life. The indicator of ACTH activity was a fall in the ascorbic acid concentration in the adrenal cortex of male rats previously treated with DOCA.

So far, however, no direct systematic study has been made of the adrenocorticotrophic function of the human fetal adenohypophysis. This is largely explained by the small size of

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TABLE 1. ACTH Level in Pituitary of Human Fetuses at 7-11 Weeks of Intrauterine Development

Age of fetuses, weeks	No. of fetuses	ACTH concn. in pituitary, μg/mg	ACTH content in pituitary, μg
7	5	0,58±0,07	0,22±0,02
8	4	0,73±0,08	0,34±0,03
9	9	0,73±0,09	0,50±0,08
10	3	1,05±0,23	1,08±0,17
11	3	1,13±0,25	1,73±0,33

the pituitary, especially in the early stages of development, by the low blood level of the hormone and, most important of all, by the absence of a sufficiently sensitive method of ACTH assay until very recently. In 1963 the first radioimmunologic study of adrenocorticotrophic activity was published [14]. Since that time this method has been considerably improved and it is now highly sensitive and accurate.

The object of this investigation was a radioimmunologic study of the development of adrenocorticotrophic function during human prenatal development.

EXPERIMENTAL METHOD

Altogether 109 human fetuses of both sexes from the 7th to the 34th week of intrauterine life inclusive, and 7 newborn infants were used. The fetuses were obtained from gynecological hospitals and maternity homes in Moscow from mothers free from endocrine diseases. The age of the fetus was determined from the assumed date of ovulation, the gynecologist's opinion, and the length of the fetus.

Pituitary glands of fetuses aged 7-11 weeks were removed under the MBS-2 microscope 3-4 h after death, weighed on torsion scales with an accuracy of 0.05 mg, transferred into 0.5 ml of 0.1 N HCl and kept until investigation at −15°C.

On the day of assay the glands were frozen and homogenized with the addition of 0.1 N HCl and the following dilutions were prepared: for fetuses aged 7-20 weeks 0.25, 1 and 5 μg in 0.1 ml; for fetuses older than 20 weeks of intrauterine life 0.1, 0.25, and 1 μg in 0.1 ml.

Fetal blood was collected from the heart and chest, centrifuged for 25-30 min at 4000 rpm, and then one drop of 4 mM EDTA was added to the serum thus obtained, which was kept until investigation at −15°C.

Kits from CEA-IRE-Sorin (France) were used for radioimmunologic assay of ACTH in the pituitary and serum. The lower limit of sensitivity of the method was 10 ± 4 pg/ml. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Radioimmunologic assay of ACTH shows that by the 7th week of intrauterine life adrenocorticotrophic activity appears in the human pituitary (ACTH concentration 0.58 ± 0.07 μg/mg, ACTH content in the pituitary 0.22 ± 0.02 μg; Table 1). With an increase in age of the fetus the ACTH level rises progressively, so that by 12-13 weeks its concentration has increased by 2.5 times and its content by 15 times (Table 2).

The ability of the human fetal pituitary gland to secrete ACTH *in vitro* was demonstrated by Siler-Khodr et al. [11] after the 5th week of intrauterine development. In a similar investigation Goodyer et al. [8] discovered adrenocorticotrophic activity in the pituitary of an 11-week fetus. Using antiserum against D-ACTH, Begeot et al. [6] showed that the first adrenocorticotrophic cells in the human fetal pituitary can be identified at the 8th week of development, and they increase in size and number during the first half of pregnancy. Furthermore, after the 8th week of embryonic development all enzyme systems necessary for corticosteroid synthesis are present in human fetal adrenals, and they are capable of responding to ACTH [3]. It can therefore be tentatively suggested that in the early stages of embryonic development functional interconnections is established between the pituitary and adrenal glands, and that pituitary ACTH stimulates growth of the adrenal cortex and processes of steroid production.

TABLE 2. ACTH Level in Adenohypophysis and Blood Serum of Human Fetuses at 12th-34th Weeks of Prenatal Development and in Blood Serum of Newborn Infants

Age of fetuses, weeks	Adenohypophysis			Blood serum	
	No. of fetuses	ACTH concentration, μg/mg	ACTH content, μg	No. of fetuses	ACTH concentration, ng/ml
12—13	3	1,34±0,19	3,16±0,55	1	1,1
14—16	3	0,90±0,24	8,09±2,80	4	1,57±0,48
17—18	4	0,63±0,12	6,76±1,17	6	3,23±0,88
19—20	3	0,87±0,20	16,60±4,16	5	2,73±0,77
21—22	6	1,11±0,29	23,12±6,27	7	1,86±0,22
23—24	7	1,33±0,28	33,66±5,71	17	1,78±0,22
25—26	8	1,61±0,28	45,79±5,58	14	5,86±0,88
27—28	5	1,84±0,36	69,72±13,6	8	4,22±0,79
29—31	4	1,80±0,25	77,55±11,6	11	4,80±1,11
32—34	3	2,42±0,51	144,6±81,5	5	6,82±1,29
Newborn infants	—	—	—	7	0,49±0,18

From the 12th through the 18th week of prenatal development the ACTH concentration in the gland falls sharply, whereas the circulating hormone level rises considerably. A similar fall in the ACTH level in the human fetal pituitary was discovered by Kastin et al. [10] from the 11th through the 23rd week of development. These workers judged adrenocorticotrophic activity from the increase in the blood corticosterone level of hypophysectomized rats after injection of human fetal pituitary extract.

After the 17th-18th week a gradual increase was observed in both the concentration and the absolute content of ACTH in the fetal adenohypophysis, with a maximum toward the 32nd-34th week (Table 2). After the 23rd-24th week the increase in hormone production in the gland coincided with a sharp rise in the ACTH concentration in the fetal circulation. The highest hormone concentration was found in fetuses aged 32-34 weeks (Table 2).

A marked increase in the ability of the human fetal pituitary to secrete ACTH into the culture medium also was observed by Siler-Khodr [11] in fetuses after the 15th-20th weeks of prenatal development.

The increase in hormone formation in the glands and of its secretion into the blood discovered in fetuses during the second half of intrauterine development coincided with an increase in functional activity and also with redistribution of the activity of certain enzymes in the human fetal adrenals [1]. It can therefore be tentatively suggested that growth, development, and steroid production in the embryonic adrenals are controlled by ACTH of the fetal pituitary.

The results of the present investigation do not agree with the radioimmunologic data of Winters et al. [13], who determined adrenocorticotrophic activity in the human fetal circulation and discovered the maximal ACTH level in fetuses at the 14th-20th weeks of development. In the present investigation some increase in ACTH concentration also was found in the period from the 17th through the 20th weeks of prenatal development, but the highest level of the hormone was discovered in 25-34-week fetuses. However, like Winters et al., the present writers also noted that the ACTH concentration in newborn infants falls sharply by comparison with its level in the fetus.

The present results confirmed to some degree the observations of Rassokhin [2] who assayed immunoreactive ACTH in the blood of human fetuses between the 11th and 32nd weeks of development and discovered three peaks of ACTH secretion (the first peak between the 11th and 14th weeks, the second between the 21st and 22nd weeks, and the third at the 32nd week). However, all three ACTH peaks observed in the present investigation were delayed by 2-3 weeks. This discrepancy between the data may be attributable to determination of the stage of pregnancy. Unfortunately Rassokhin did not specify the data on the basis of which he calculated the age of the fetus.

A very low ACTH concentration (0.49 ± 0.18 ng/ml) was observed in the blood of newborn infants, i.e., less than one-tenth of that found in 32-34-week fetuses (Table 2). Meanwhile, according to data in the literature [7], at birth the corticosteroid concentration in human fetal blood rises considerably. These observations suggest that the sharp fall in the circulating ACTH level in the blood of newborn infants was the result of birth stress.

The present investigation thus showed that the human fetal pituitary gland produces immunoreactive ACTH after the 7th week of development, and secretes it into the fetal blood at latest after the 12th week.

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