Dopa, the precursor for catecholamine synthesis, like catecholamines themselves, has an inhibitory action on cell proliferation, but it is evidently not connected with adrenoreceptor activation. This hypothesis is confirmed by data in the literature indicating that dopa does not interact with binding sites of plasma membranes of turkey erythrocytes, with which either β-agonists or β-antagonists can interact [4]. Our own data for the effect of dopa on cell proliferation show that the precursor of catecholamine synthesis can exert an independent action on tissue metabolism, and not only through catecholamine formation; this is a problem which requires further study in connection with the use of dopa for the correction of trophic disorders [2].

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TESTICULAR INHIBIN-LIKE FACTOR OF FETAL AND NEWBORN RATS

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The problem of the existence of a factor limiting growth of an organ or maintaining the state of equilibrium in constantly renewed populations has engaged the attention of embryologists, endocrinologists, and clinicians for a long time. We know that during the prenatal period of mammalian ontogeny active processes of proliferation of the genocytes take place with preparation for meiosis. However, in some species at the end of pregnancy, and in others during the first days after parturition, proliferation of the primary sex cells is inhibited, as also is their entry into meiosis, until the period of puberty. The discussion on which factors affect this process still continues and opinions of investigators are extremely contradictory. An inhibin-like substance, concerned in the regulation of spermatogenesis and also in maturation of follicles in adult humans and mammals has been isolated [2, 5, 6, 8, 9, 11]. The authors cited state that there is more inhibin in the testes and ovaries of sterile men and women than in subjects with normal fertility. However, the question of the existence of an inhibin-like factor in prenatal and early postnatal periods of human and mammalian ontogeny, which could exert its action on the gonocytes, remains debatable.

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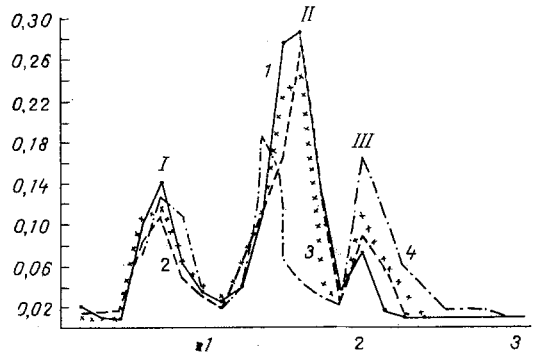

Fig. 1. Fractionation of testicular extracts from fetal and neonatal (first 2 weeks of life) Wistar rats on Sephadex G-100. Curves: 1) fetuses at 19th day of development, 2) fetuses at 20th day of development, 3) newborn rats aged 7 days, 4) newborn rats aged 14 days. I, II, and III) Principal protein peaks. Abscissa, time (in h); ordinate, optical density at 280 nm (in relative units).

TABLE 1. Dynamics of Weight of Utero–Ovarian Complex of Immature Rats after Injections of Inhibitory Fractions of Testicular Extracts from Fetal and Neonatal Rats (M ± m)

Age, days	Weight of utero–ovarian complex	
	experiment	control
Fetuses		
19	35,3±0,4*	49,8±0,5
20	33,3±0,7*	46,0±0,7
Newborn rats		
7	31,7±0,6*	48,3±0,9
14	30,7±0,8*	50,0±1,0

Legend. *P < 0.01.

This paper describes an attempt to isolate an inhibin-like factor and to determine the time and source of its appearance in the testes of fetal and neonatal rats.

EXPERIMENTAL METHOD

Testes from fetuses of Wistar albino rats from the 17th day of intrauterine life and of newborn rats during the first 2 weeks of development were investigated. Extracts were prepared from a homogenate of pools of testes by fractionation in the cold using isotonic NaCl solution, butanol, and ether. Protein in the extracts was determined by Lowry's method. Bioassay of the inhibin-like effect [2, 10] consisted of determining the degree of decrease in weight of the utero–ovarian complex of immature female rats aged 21-22 days and weighing 37-40 g. After fractionation of the testicular extracts on columns with Sephadex G-100, the inhibitory action of the resulting fractions was studied by Chari's method [10] after intraperitoneal injection directly into rat fetuses through the wall of the uterine tube. The fetuses were removed 6-12 h after injection of the test fractions, weighed, fixed in Carnoy's fluid or 8-10% formalin solution, and embedded in paraffin blocks, from which series of histological sections were cut. These were stained with Mayer's hematoxylin and eosin, for RNA by Brachet's method, and by the PAS reaction. The results were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

The experiments showed that testicular extracts from Wistar albino rat fetuses at the 17th-18th day of intrauterine life had no inhibin-like effect. This was shown by the equal weight of the utero–ovarian complex of immature female rats in the experimental and control series. Testicular extract from rat fetuses at the 19th-20th day of development had a weak inhibin-like action. During fractionation of testicular extract at this age on columns with Sephadex G-100 the protein fraction corresponding to peak III (Fig. 1) gave an inhibin-like effect (Table 1). Testicular extracts from young rats aged 2, 7, and 14 days likewise had an inhibin-like effect. The fraction corresponding to peak III obtained from testicular extracts from young rats of these age groups gave an inhibin-like effect which was stronger than that given by injection of whole extract. The strongest effect was found when testicular extract from 14-day-old rats was used.

After injection of protein fractions giving an inhibin-like effect on the utero–ovarian complex of immature rats, and obtained from extracts of pools of testes from rat fetuses at the 19th-20th days of intrauterine development and newborn rats aged 2, 7, and 14 days (intraperitoneally, through the wall of the uterine tube into rat fetuses at the 15th-18th day of intrauterine development) the proliferative activity of the gonocytes in the testes of these fetuses was found to be inhibited. Whereas in testes of control fetuses, 280-300 mitoses

could be counted in the primary sex cells in 100 fields of vision of the microscope (objective 40, ocular 10), in the experimental fetuses the number was 50-70. In the latter, moreover, death of gonocytes could be observed, especially those in the center of the seminiferous tubules. Epithelial cells, like fibroblasts, participating in the formation of the wall of the seminiferous tubules in the experimental fetuses were more compactly arranged than in the controls.

At the end of pregnancy, an inhibin-like factor which inhibits proliferation of the gonocytes and also, probably, their entry into the phase of meiosis, appears in the testes of Wistar albino rat fetuses. This factor is protein in nature. It is evidently secreted by the epithelial cells of the rete testis. Evidence in support of this view is given, first, by the fact that in fetuses into which a protein fraction with inhibitory action was injected, the epithelium of the rete testis was observed to be inhibited. Whereas in control fetuses the epithelium of the rete was high and cubical, and its cells had a branching, pyroninophilic cytoplasm, containing many glycogen granules, in the testes of the experimental fetuses the epithelium was flattened, and the cytoplasm of its cells contained less glycogen. Epithelial cells of the rete testis of fetuses injected with the fraction possessing inhibitory action also contained less mucopolysaccharides than the controls. Second, in this part of the anlage of the testis the tubular epithelium reaches its highest level of differentiation in normally developing fetuses and newborn rats. The important role of the rete testis of fetuses and newborn animals in the secretion of androgens and other biologically active substances is well known [1, 4, 12, 13]. Secretion of the inhibin-like factor is increased during the first weeks of postnatal life, as shown by the results of our experiments with testicular extracts and their fractions taken from young rats aged 2, 7, and 14 days. Although secretion of the inhibin-like factor by the fetal testes of Wistar rats begins only at the end of pregnancy, the gonocytes and fibroblasts and epithelium forming the wall of the seminiferous tubules are sensitive to it even on the 15th-16th day of pregnancy, further confirmation of the view [3, 7] that receptors are formed before the factor which binds with them begins to be produced. The appearance of an inhibin-like factor in the fetal testes of Wistar rats simultaneously with or a little earlier than secretion of FSH, LH, prolactin, and other adenohypophyseal hormones concerned in the regulation of formation of the generative and endocrine part of the testes is essential for the conduct and maintenance of normal homeostasis in the developing testes.

It can be concluded from the facts described above that a factor of protein nature, inhibiting proliferation of the gonocytes and also, probably, their entry into meiosis, is produced in the testes of fetal and neonatal rats. It evidently also participates in the formation of certain components of the blood—testis barrier (the wall of the seminiferous tubules), the density of which it increases. The inhibin-like factor is produced in all probability in the rete testis. It can be postulated that it differs somewhat in its structure and mechanism of action from the testicular inhibin of adult men and animals. The term "inhibin" is perhaps a collective term, but verification of this hypothesis requires further investigation.

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