ic centers is altered by the administration of testosterone soon after birth[11], nor male rats show the positive effect of estrogen on LH and FSH secretion[5]. On this experimental basis, and taking into account the peptide nature of the hypothalamic releasing factors that control the LH and FSH secretion, it could be postulated that the stimulatory effect of estrogens on the protein synthesis of the anterior hypothalamus of female rats is representive of a stimulatory action of the sexual hormone on the hypothalamic synthesis of peptides related to the LH and FSH secretion. The fact that, in prepuberal male rats, estrogen neither stimulates gonadotrophin secretion nor modifies protein synthesis of the hypothalamus further supports this point of view.

It is a well known fact that estrogen, besides its positive action on gonadotrophin secretion in female rats, also exerts a negative influence on these pituitary hormones in male and female rats. Nevertheless, we did not observe any effect of the sexual hormone on the protein synthesis of male hypothalamic areas and only the stimulatory effect on the anterior hypothalamus of female rats. On this basis, and supposing that the changes produced by estradiol on the protein synthesis of anterior hypothalamus of female rats are connected with the positive feed-back effect of the hormone, it could be postulated that different mechanisms are involved in the negative and positive feed-back effect of estrogens on gonadotrophin secretion; moreover an extrahypothalamic mechanism for negative feed-back cannot be eliminated.

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Effect of the Extracts from *Aristolochia indica* Linn. on Interception in Female Mice

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Summary. The crude petroleum ether, chloroform and alcoholic extracts of the roots of *Aristolochia indica* (Linn.) showed 100% interceptive activity in mature female mice at the single dose of 100 mg/kg body wt. The follow-up studies with the chloroform extract showed the most significant effect in the basic part and two acidic fractions at the single dose levels of 50 mg/kg body wt. No toxic effect was observed at the dose levels used.

Aristolochia indica Linn. (N.O. Aristolochiaceae), locally known as Isharmul (Bengali and Hindi) is a shrub with long twining stem growing all over the tropical region of India. Its very bitter root is reputed to have emmenagogic[2,3] and abortifacient properties[4].

Materials and methods. 5 kg air-dried roots were milled and successively extracted in a Soxhlet apparatus with petroleum ether (b.p. 60°–80°), benzene, chloroform and alcohol for 20 h each. Each extract was then evaporated to dryness. The chloroform extract was further separated into acidic, basic and neutral components. 3 g crude acid fraction, due to its low solubility, was again extracted in a Soxhlet with 2 l chloroform in which the total material gradually dissolved. On cooling a solid (Fraction I, 0.5 g m.p. $>274°$, decomp.) that separated out was filtered and crystallized from chloroform-methanol. The filtrate was then added slowly over a column of silica gel. Elution with 10% methanol in 3 l chloroform yielded a solid material (Fraction II) which crystallized out of chloroform-methanol into pale yellow needles (140 mg), m.p. 278°–79°. Further elution with 7 l of the same solvent and 15% methanol in 3.5 l chloroform afforded another solid (Fraction III, 280 mg; m.p. $>280°$, decomp.) on crystallization from D.M.F.-alcohol.

Colony-bred proven Swiss albino mice at the estrous or early estrous stage were caged with proven males in the ratio of 1 male to 2 females in a controlled room temperature (24–25 °C). Vaginal smear were recorded daily. The day of vaginal plug was marked as Day 1 of pregnancy. The test samples, pasted with gum acacia powder and suspended in water, were administered orally in a single dose of 100 mg/kg body-weight for the crude extracts (Table I) or 50 mg/kg body weight, for the purified fractions (Table II) on day 6–7 of pregnancy. In all cases, laparotomy was performed under ether anaesthesia after observing change in the vagina. Control animals were treated with a suspension of gum acacia in water only.

The results in Table I show that the crude petrol, chloroform and ethanol extracts exert highly significant interceptive activity. Laparotomy revealed the inter-

Table I. Effect of various solvent extracts of the root of *Aristolochia indica* (Linn.) on fertility of mice in post implantation stage given at a dose of 100 mg/kg body wt.

Solvent	No. of mice	Mice showing interceptive effect (%)
Vehicle only	10	0.0
Petroleum ether extract	10	100
Benzene extract	15	73.3
Chloroform extract	10	100
Alcohol extract	10	100

Table II. Effect of various fractions of the chloroform extract of root of *A. indica* (Linn.) on fertility of mice in post-implantation stage given at a dose of 50/kg body wt.

Extract	Fraction	No. of mice	Mice showing interceptive effect (%)
Vehicle only (control)	—	10	0.0
Chloroform extract	Basic part	7	100
	Neutral part	8	87.0
	Acidic part		
	Fraction I	14	100
	Fraction II	12	75.0
	Fraction III	7	100

ception to occur from Day 8–10 and 7–9 respectively in cases of petroleum ether and chloroform extracts. Table II indicates that the basic part and the Fractions I and III of the acid part of the chloroform extract possess maximal interceptive activity which is less, though still significant, in the cases of the neutral part and the chromatographic fraction II of the acid part. The interception was complete also within the period of Day 7–9 in all these cases, except in that of the acidic fraction III which required a longer time for completion of the interceptive activity. There is no toxic effect at the particular dose level. Further work is in progress.

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Studies in Relation to Endocrine Exophthalmos: The Biochemical Composition of Human Retrobulbar Connective Tissue

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Summary. As part of studies on the pathogenesis of exophthalmos of Graves' disease, the biochemical composition of human retrobulbar tissue was investigated. The connective tissue was composed of 72.7% lipid, 23.8% water and 3.5% dried defatted tissue. Total tissue glycosaminoglycan (GAG) amounted to 0.18% of dried defatted tissue. Approximately 27% of the total hexosamine and 19% of the total tissue galactosamine were recovered in the GAG fraction. Cellulose microcolumn fractionation of GAG showed that the hyaluronic acid and dermatan sulfate were the two major GAG species.

Changes in retrobulbar connective tissue play a significant role in the production of exophthalmos of Graves' disease[2]. Major evidence of these observations is based upon studies in experimental animals. For example, the induction of exophthalmos in guinea-pig and fish was shown to result in increased metachromasia, hexosamine and hexuronic acid contents and ^{35}S-sulfate incorporation in the retrobulbar tissue[3]. Recently, we reported the use of the mouse orbital tissue as a method to study the etiology and pathogenesis of exophthalmos of Graves' disease[4]. To clarify the relevance of these observations to clinical exophthalmos, the present study was undertaken to characterize the biochemical composition of human retrobulbar connective tissue.

Materials and methods. 16 human cadavers ranging in age from 47 to 90 years (average 65 years) were studied. Within 48 h of death, retrobulbar tissue was obtained by cutting open a window in the orbital plate. Fibrofatty connective tissue was dissected free of the muscle fibres, cut into small pieces and weighed. Tissue segments were lyophilized, weighed, then passed through several changes of methanol-chloroform and dried to a constant weight in vacuo. The water and lipid contents were calculated from the wet, dried and dried defatted tissue weights. Individual eye samples were analyzed for water and lipid determinations and subsequently the tissues from both eyes were pooled for further studies.

Hydrolysis of the dried defatted tissue (approximately 10–12 mg) was performed with 4 *N* HCl, in a sealed glass tube, at 100 °C for 16 h in an oven. After dividing the hydrolyzed tissue into 2 aliquots, HCl from each aliquot was removed in vacuo over NaOH pellets. The residue was dissolved in 0.1 ml 0.1 *N* HCl for the determination of hexosamine[5] and galactosamine[6].

Glycosaminoglycans were liberated from the dried defatted tissue by proteolytic digestion with pronase[7] and recovered as potassium salt according to a previously described method[8]. Subsequently the crude glycosaminoglycans (GAG) preparation was dissolved in 0.075 *M* NaCl and divided into aliquots for chromatographic separation of GAG species and for the measurement of uronic acid[9], hexosamine[6] and galactosamine[5]. Each sample was desalted by dialysis against distilled water at 4 °C overnight prior to hexosamine and galactosamine determinations. GAG was fractioned on 0.8 × 16 cm cellulose columns according to the elution procedure of SVEJCAR and ROBERTSON[7]. In this method GAG are precipitated as cetylpyridinium chloride (CPC) complexes on cellulose

Table I. Water, lipid and protein content of retrobulbar connective tissue

	Water	Lipid	Dry weight	Protein
Mean ± SEM	23.8 ± 0.9	72.7 ± 1.0	3.5 ± 0.1	3.3 ± 0.2
Range	15.2 — 32.5	63.9 — 83.1	2.2 — 5.1	2.1 — 4.4

All values are expressed as percent of wet tissue weight. Retrobulbar connective tissue was removed within 4–48 h of death of 16 male humans and dissected free of extraorbital muscles before analysis.

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