like antigens also do not represent structural components of the galactan polysaccharide, inspite of alkali resistance, as shown in Figures b and c. In this context it is interesting to note, that Concanavalin A reactive material (protease-inhibitor?) does occur in nearly all snail albumin glands and eggs, which we have investigated (*Helix, Pomacea, Achatina*, etc.).

The above mentioned observation supports our concept[9] of a fine classification based on paraimmunological findings; the eggs of very closely related snails, even of the same species, show remarkable differences in their agglutinins or precipitins, a phenomenon which may be used to distinguish or to classify them more precisely than has hitherto been possible[5,9,10]. Thus, in the eggs of *Achatina granulata* we found anti-neuraminyl specificity, whereas no agglutination of red cells by an egg extract from *Achatina fulica* was observed, even after proteinase treatment or when neuraminic acid was split off[11]. The ecological and taxonomic implications of such relationships have still to be investigated.

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## Effect of Estradiol on Aminoacid Incorporation into Proteins of Different Hypothalamic Areas in Prepuberal Rats[1]

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*Summary.* Estradiol in vitro produces a significant increase in the incorporation of $^3$H-leucine into proteins of the anterior hypothalamic area in prepuberal female rats, 15 and 20 days old, but not in younger animals. The ovarian hormone induced no changes in the protein synthetic activity of middle and posterior hypothalamus and cerebral cortex in prepuberal female rats of different ages. Estradiol did not modify the protein synthesis of the hypothalamus and cerebral cortex in prepuberal male rats.

We have previously demonstrated that changes in the secretion of gonadotrophins and /or sexual hormones are accompanied by modifications in the protein synthesis of the hypothalamus, and we proposed that such metabolic changes are related to variations in the hypothalamic synthesis of the gonadotrophin releasing factors[2,3]. Estrogen injected into prepuberal rats produces changes in the secretion of gonadotrophins, and this effect depends on the activation of hypothalamic mechanisms that mature during the prepuberal state[4,5].

Taking into account that the regulatory influence of estradiol on gonadotrophin secretion in prepuberal rats can be connected with modifications in the hypothalamic synthesis of peptides that control gonadotrophin secretion, and that changes in the protein synthesis of the hypothalamus could reflect variations in the synthesis of such regulatory peptides[6] we have studied the direct effect of estradiol on the incorporation in vitro of labelled leucine into proteins of different hypothalamic areas in male and female rats at different prepuberal ages.

*Material and methods.* Albino male and female rats were used. The litters were reduced to 7 at birth and weaned at 21 days of age. They were housed under conditions of constant temperature (23 ± 2 °C) and lighting (12 h light; 12 h darkness).

Animals were killed by decapitation at 10, 15 and 20 days of age, and the whole hypothalamus removed. The sample was placed on its dorsal surface and cut under a dissecting microscope into 3 portions by 2 frontal sections, the first section being made through the optic chiasma, and the second immediately behind the infundibulum. These sections divided the hypothalamus into the following 3 areas: a prechiasmatic portion, namely the anterior hypothalamus (including the preoptic and anterior hypothalamic areas, the paraventricular and suprachiasmatic nuclei); a retroinfundibular portion, namely the posterior hypothalamus (including the mammillary and the posterior hypothalamic nuclei); and a region between the two sections, namely the middle hypothalamus (including the median eminence, and the arcuate, ventromedial and dorsomedial nuclei).

Each hypothalamic area was divided into 2 symmetrical portions, along the anterior-posterior axis. One hypothalamic half was added to the incubation glass containing 0.01 µg/ml of estradiol benzoate dissolved in 0.09 m*M* ethanol[7]; the other hypothalamic half was incubated in a medium contained 0.09 m*M* ethanol. 2 rats were used in each single experiment.

The samples were gently blotted on filter paper, weighed on a torsion balance and incubated in 1 ml isotonic medium containing 1 µCi of L-4-5 [$^3$H] leucine (20 Ci/µmol) obtained from New England Nuclear. Incubation was for 90 min at 37 °C with gentle shaking in a Dubnoff metabolic shaker. The gas phase was $O_2$: $CO_2$ (95:5, v/v). After incubation, the tubes were rapidly removed and chilled in crushed ice, washed twice with medium, centrifuged in a refrigerated centrifuge and homogenized (Potter-Elvehjem homogenizer) in 2 ml 10% trichloroacetic acid (TCA) containing 0.2% unlabelled L-leucine (Sigma). After homogenization the suspension was centrifuged at 6,500 *g* for 15 min. The TCA-insoluble residue was washed twice with 5% TCA, twice with chloroform:methanol (1:1, v/v), and one with 2 ml ether;

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Table I. Incorporation of L-[$^3$H] leucine into proteins of the hypothalamus and cerebral cortex of prepuberal female rats

| | Age | | | | | |
|---|---|---|---|---|---|---|
| | 10 days | | 15 days | | 20 days | |
| Tissue | Control | Estradiol | Control | Estradiol | Control | Estradiol |
| Hypothalamus | | | | | | |
| Anterior | 17,378 ± 1,837[a] (6) | 19,237 ± 2,634 (6) | 13,569 ± 1,832 (14) | 19,343 ± 2,439[b] (14) | 8,109 ± 1,028 (10) | 12,520 ± 1,677[b] (10) |
| | | | $p < 0{,}025$ | | $p < 0{,}025$ | |
| Middle | 15,756 ± 2,000 (6) | 15,266 ± 1,959 (6) | 14,300 ± 1,661 (13) | 12,163 ± 1,611 (13) | 6,524 ± 862 (8) | 8,404 ± 1,751 (8) |
| Posterior | 18,948 ± 2,163 (6) | 19,977 ± 3,796 (6) | 17,134 ± 2,131 (10) | 21,904 ± 2,756 (10) | 8,115 ± 1,506 (10) | 9,169 ± 1,728 (10) |
| Cerebral cortex | 12,508 ± 2,510 (6) | 11,124 ± 2,551 (6) | 10,801 ± 1,300 (13) | 9,972 ± 1,522 (13) | 2,952 ± 557 (10) | 3,762 ± 620 (10) |

[a] DPM/mg of protein (means ± SEM). Number of determinations in parenthesis. [b] Statistically significant as compared with the control.

the precipitate was separated by centrifugation in the cold. The residue was then resuspended in 2 ml TCA and heated at 90 °C for 15 min after cooling and centrifuging, the protein residue was dissolved in 0.5 ml 1 *M* NaOH, and aliquots were taken to determine protein by the method of LOWRY et al.[8] using crystalline serum albumin as a standard, and to measure radioactivity.

Radioactivity was measured in a Packard Tri-Carb liquid scintillation counter and each sample was counted long enough to give a standard error of less than 3%. Counts were corrected to 100% efficiency by the channels ratio method. The radioactivity incorporation into proteins was expressed as specific activity, i.e. dpm/mg protein. All results are presented as the means ± SEM and analyzed using the Student's *t*-test.

*Results.* The effects of estradiol in vitro on the protein synthesis of different hypothalamic areas and cerebral cortex of prepuberal female rats are shown on Table I.

As can be seen, 0.01 µg/ml of the hormone (minimal effective dose) produces a significative increase in the in vitro incorporation of [$^3$H] leucine into proteins of the anterior hypothalamic area in rats of 15 and 20 days of age, but does not modify the synthetic activity of this area in 10-day-old rats. On the other hand, the ovarian hormone induced no changes in the protein synthetic activity of the middle and posterior hypothalamus and cerebral cortex in prepuberal female rats of different ages (Table I). Higher concentrations of estradiol also did not induce any changes in these structures.

The results obtained in prepuberal male rats have shown that neither 0.01 µg/ml of estradiol (Table II) nor higher concentrations modify the in vitro incorporation of [$^3$H] leucine into proteins of different hypothalamic areas and cerebral cortex in 10–, 15– and 20-day-old rats.

*Discussion.* The results of the present paper show that estradiol only modifies the incorporation of labelled leucine into proteins of the anterior hypothalamus in female rats of 15 and 20 days of age, producing a significative stimlulatory effect. In this respect, it is interesting to note that, around this age, in the hypothalamus of female rat, the mechanisms mature which are involved in the hypothalamic estrogen binding activity[9].

A positive feed-back effect of estrogens on gonadotrophins secretion has been described in prepuberal female rats[4,10]. Apparently this feed-back mechanisms depends on the activation of the cyclic centers placed in the anterior hypothalamic areas of female rats, since neither female rats, in which the development of these hypothalam-

Table II. Incorporation of L-[$^3$H] leucine into proteins of the hypothalamus and cerebral cortex of prepuberal male rats

| | Age | | | | | |
|---|---|---|---|---|---|---|
| | 10 days | | 15 days | | 20 days | |
| Tissue | Control | Estradiol | Control | Estradiol | Control | Estradiol |
| Hypothalamus | | | | | | |
| Anterior | 11,017 ± 2,824[a] (6) | 11,169 ± 2,872 (6) | 10,338 ± 2,131 (6) | 10,827 ± 2,053 (6) | 9,742 ± 1,877 (7) | 8,178 ± 1,478 (7) |
| Middle | 9,281 ± 2,140 (6) | 11,905 ± 2,854 (6) | 8,890 ± 1,734 (6) | 9,798 ± 1,964 (6) | 7,920 ± 1,709 (8) | 6,229 ± 834 (8) |
| Posterior | 12,229 ± 1,763 (7) | 11,230 ± 2,140 (7) | 11,462 ± 2,332 (6) | 12,208 ± 1,638 (6) | 11,347 ± 2,650 (7) | 10,050 ± 2,202 (7) |
| Cerebral cortex | 4,947 ± 677 (7) | 6,288 ± 819 (7) | 4,300 ± 1,022 (6) | 4,805 ± 921 (6) | 3,008 ± 677 (6) | 2,358 ± 238 (6) |

[a] DPM/mg of protein (Means ± SEM). Number of determinations in parenthesis.

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 |