Occurrence of a Blood Group A-like Substance in Eggs of the Prosobranch Snail *Pomacea canaliculata*

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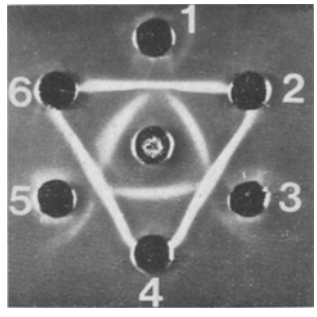
Summary. In the eggs of the prosobranch snails *Pomacea canaliculata* and *Pomacea insularum* a blood group A-like substance has been detected by anti-A from the snails *Helix pomatia, Helix aspersa* and *Cepaea nemoralis.*

Snail eggs have in recent years attracted much attention from biochemists and immunologists, since they contain substances which are not encountered elsewhere in nature, or only very rarely. These materials are of three kinds: 1. special inhibitors of proteinases, such as those from *Helix pomatia*[2] or ovorubin from *Pomacea canaliculata*[3]; 2. anti-carbohydrate or blood group-specific agglutinins and precipitins, e.g. anti-A from *Helix pomatia*[4] or anti-A + B from *Biomphalaria glabrata*[5]; 3. different forms of galactans, which can be distinguished by means of naturally occurring anti-galactans[6].

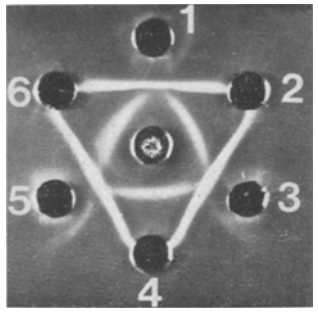
This latter galactan group is of special interest, since it shows blood group H-like activity, probably on account of its content of terminal, non-reducing bound L-fucose or L-galactose[7]. Since anti-blood group A or anti-A-like activity is found in the eggs of most snails studied up to the present, we have been very surprized to find an A-like substance in the eggs of *Pomacea canaliculata* and *Pomacea insularum*, though this does not occur in other species of the same genus, such as *P. scalaris* and *P. urceus*, the anti-B-like agglutinins of which latter species have recently been reported[8]. This powerful blood group A-like activity could be clearly demonstrated with the anti-A precipitin obtained from other snail eggs. The results are shown in Figures a and b (agar gel diffusion) and in Figure c (agar gel electrophoresis).

Blood group A-like receptors could not be found in purified ovorubin, a chromoglycoprotein, which, on the other side however, strongly reacted with Concanavalin A (red precipitate in agar), thus offering a method for purification of ovorubin by affinity chromatography. A-

[1] Bedford College, University of London, London NW1 4NS, England.
[2] G. Uhlenbruck, I. Sprenger and I. Ishiyama, Z. klin. Chem. klin. Biochem. *9*, 361 (1971).
[3] D. F. Cheesman, Proc. R. Soc. *B 149*, 571 (1958).
[4] O. Prokop, D. Schlesinger and A. Rackwitz, Z. ImmunForsch. *129*, 402 (1965).
[5] J. C. S. Filho, A. Dmytraczenko and J. H. Duarte, IRCS Int. Res. Commun. System, July 1973, reprint.
[6] G. Uhlenbruck, G. Steinhausen and B. A. Baldo, *Galactane und Anti-Galactane* (Verlag Josef Stippak, Aachen 1975).
[7] B. A. Baldo and G. Uhlenbruck, Immunology *25*, 649 (1973).
[8] G. Uhlenbruck, G. Steinhausen and D. F. Cheesman, Experientia *29*, 1139 (1973).

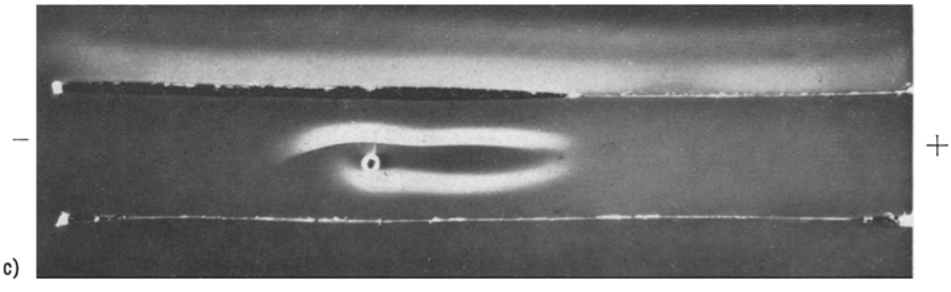


a)



b)

a) Immunodiffusion experiment with *Pomacea canaliculata* egg extract (a similar picture was obtained with *Pomacea insularum*). Note identity line with blood group A substance from peptone. In the central well: *Pomacea canaliculata* egg extract, tested against extracts from other snail eggs. 1 + 4 = *Helix pomatia*, purified anti-A; 2 = *Helix aspersa*, crude anti-A; 3 + 5 = blood group substance A; 6 = *Cepaea nemoralis*, crude anti-A. The anti-A lectin from the plant *Dolichos biflorus* did not react.
b) Non-identity of the anti-galactan reaction and the reaction with anti-A from *Helix pomatia*. In the central well: *Pomacea canaliculata* egg extract. 1 + 3 + 5 = *Tridacna maxima* precipitin (= anti-galactan); 2 + 4 + 6 = *Helix pomatia*, anti-A. The wells are described clockwise.



c)

c) Electrophoresis of *Pomacea canaliculata* snail egg extract (trough). Upper well: *Tridacna maxima* precipitin, which detects the galactan. Lower well: Anti-A precipitin, which detects the blood group A-like material. This is not destroyed by treatment with alkali.

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.

[9] G. UHLENBRUCK and A. WEIS, Z. ImmunForsch. *145*, 356 (1973).
[10] B. CLARKE, Scient. Am. *233*, 50 (1975).
[11] G. UHLENBRUCK, G. STEINHAUSEN and H. A. KAREEM, unpublished results.

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;

[1] Supported by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
[2] J. A. MOGUILEVSKY, P. SCACCHI and J. CHRISTOT, Proc. Soc. exp. Biol. Med. *137*, 653 (1971).
[3] J. A. MOGUILEVSKY and J. CHRISTOT, J. Endocr. *55*, 147 (1972).
[4] L. CALIGARIS, J. J. ASTRADA and S. TALEISNIK, J. Endocr. *55*, 97 (1972).
[5] P. SCACCHI and J. A. MOGUILEVSKY, Experientia *29*, 877 (1973).
[6] J. A. MOGUILEVSKY, M. A. ENERO and B. SZWARCFARB, J. Endocr. *64*, 155 (1975).
[7] In preliminary experiments, different concentrations of estradiol were tested.