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Histochemical data on the skin mucous cells during the development of the trout.

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Translated by K.DICKSON.

In the skin of Salmo irideus the production of mucus is due to one type of cell specialized as a mucus cell.

Massari (1) has observed the presence of such cells in the immature prehatching stages and Porcelli (2) has seen them in the ectodermic portion of the yolk sac of the same stage. In the literature (3-5) skin mucous cells with mucus and a truly complete structure have been described only in the adult of those species of teleosts with naked skin or in those where scales appear relatively late. The histochemical research presented in this paper describes the mucous cells of Salmo irideus and demonstrates observable variation in such cells during the fish's development.

We examined the skin of successive stages of Salmo irideus using Prakash's (6) classification:

Stage 1: immature prehatching, length of the embryo 6·5-8 mm., eyes faintly pigmented.

Stage 2: late prehatching, length of the embryo 8·1-11 mm., eyes intensely pigmented.

Stage 3: immature post hatching; length of the embryo 12·5-18 mm.,

Stage 4: late post hatching; length variable, yolk sac still of moderate dimensions with respect to the fry.

Stage 5: young trout, juvenile characteristics, yolk sac completely disappeared.

Stage 6: Trout, length 15 cms.

Stage 7: Trout, length 30 cms.

Where possible, and it was possible in the first 4 stages, we examined both the somatic skin and the ectodermal portion of the wall of the yolk sac. We refer you to a paper by one of us (2) in which the development and the absorption of the yolk sac was examined in more detail and the correspondence between the two locations described.

The material was fixed in 10% formalin or in Carnoy's liquid then routinely dehydrated and embedded, or if lipids were to be looked at they were fixed in Ca - formalin and the frozen block was sectioned on a freezing microtome. It was then subjected to the following histochemical reactions besides the normal histological dyes.

a) To demonstrate the presence of mucopoly saccharides:
   1) Reaction to periodic acid - Schiff according to McManus - Hotchkiss.
b) To demonstrate the presence of glycogen:
   1) Reaction of McManus - Hotchkiss with previous digestion with α - amylase.
   2) Iodine-reaction of Langhans also according to Mancini's version.
   3) Reaction of McManus - Hotchkiss previous treatment with agitation (see Lison).
c) To demonstrate the occurrence of acid mucopoly saccharides.
   1) Reaction to colloidal iron according to Hale with Muller's modifications.
   2) Alcian blue 8 G.N. according to Stedman.
   3) Alcian blue at several pH's see Spicer.
   4) Alcian blue prior to the blocking of alcianophilia with Mg cl_2 according to Scott's method.
   5) Alcian blue with prior methylation see McManus and Mowry.
   6) Alcian blue with prior methylation followed by demethylation.
   7) Acridine-orange see Hicks & Matthaei.
   8) Acriflavine - see Takenchi.
   9) Alcian blue with prior enxymatic digestion with bacterial hyaluronidase in a concentration of 1mg./ml. in distilled water for 1 Hr. at 37 C.

10) Alcian blue with prior enzymatic digestion with sialidase see Spicer.

11) Alcian blue with prior enzymatic digestion with sialidase (see Spicer) preceeded by treatment with alcoholic KOH (see Gerard).

d) To demonstrate the presence of both a neutral and an acidic fraction in the polysaccharide material. Double reaction using Alcian blue simultaneously with Hotchkiss's reaction see Vialli.

e) To demonstrate of mucopoly saccharides at different acidities.

1) Alcian blue - alcian yellow see Ravetto.

f) To demonstrate proteinaceous material:

1) Staining with bromophenol blue see Mazia, Bravert and Alfert.

2) Morel - Sisley's reaction for demonstrating the presence of protein containing the amino acid tyrosine.

g) To demonstrate the presence of neutral lipids.

1) Sudan black B on frozen material freshly cut with a freezing microtome.

h) To demonstrate the presence of heterophasic lipids: Sudan black B on material fixed in 10% formalin and prior treatment with unmasking agents: 1% phenol 37 °C, acetone t° ambient, ethyl alcohol 90 °C, distilled water 37 °C.

The following conclusions can be drawn from an examination of the table.

In the adult stages where the epidermis is very stratified, the ventral and dorsal locations of the skin, and the superficial and deep mucous cells are shown separately.

It seems evident that the mucous cells of the trout contain mucopoly saccharide material with a complex constitution. Close to a fraction of neutral mucopoly saccharide a relatively acid fraction is present with a variable amount of sialic acid. The presence of a proteinaceous component could not be demonstrated and the reactions for lipid gave practically negative results even after the unmasking treatments. The fraction responsible for the positive response to the reactions for the sulphated mucopolysaccharides were not always present and one can observe a difference in mucous production between adjacent cells. One can therefore identify

two types of mucus producing cells on the basis of differences in content of sulphated mucopolysaccharides and the coexistence of these two types of cells occurs in all developmental stages examined as well as in the adult. All the characteristics of the mucus are demonstrable as early as the endovular stages. The principal transformation observed during the development are considered in the following by comparing the various developmental stages and the adult:

a) The relative increase of glycogen in the adult as opposed to the fry. (The digestion removes from the preparation, granular material which in general gives a positive reaction to Hotchkiss' reaction. This material is very distinctive in the undifferentiated cytoplasm which in the mucous cells gives a characteristic foamy appearance to the mucus present in the cytoplasm. The difference between the zones is not clear).

b) The increase in the reactivity of sialic acid, which is very clear in the stages following hatching increases even further in the adult. (In the pictures of the strongly stratified adult skin it is possible to identify more signs of sialic acid in the superficial mucous cells as opposed to those deeper, and in the ventral as opposed to the dorsal portions).

From the functional point of view it seems evident that, at least in the strict sense, there does not exist a correlation between the described histochemical transformations and the surrounding changes in connection with hatching. The presence of sialic acid is inseparable from the other epithelial mucous secretions with which it is described, and not alone as in other skin mucous cells of teleosts described in the literature. The presence of glycogen in the skin mucous cells is above all due to its persistence in the adult stages, this is a piece of information which hints at several interpretations. Peyrot (8) has interpreted the presence of glycogen in the duodenal epithelia of immature stages of higher vertebrates as a precursor of the glycoprotein of the adult and a function of this sort may be attributable to the glycogen of the mucus-producing cells in the adult tissues (this would allow a complete functional cycle inside a single cell.) On the other hand we can be criticized because we have not considered adult trout of 30 cms length (2 yrs. old), and it must be noted that trout retains its growth for a long time.

In some sense the glycogen demonstrated by us in the last stages can be considered a trasitional condition in the development. On the other hand it seems opportune to record that since the skin of the trout is considered deprived of specialized types of cells (eg. the clavate cells)(9) and, like the majority of teleost, lacks Keratin(10) an essential turgor due to glycogen could be called the support mechanism, (see the functional interpretations proposed for the clavate cells). Whether this information could extend to other skins deprived of clavate cells or not is debatable.

(*) Da notare che non è mai dimostrabile presenza di glicogeno nel muco secreto.

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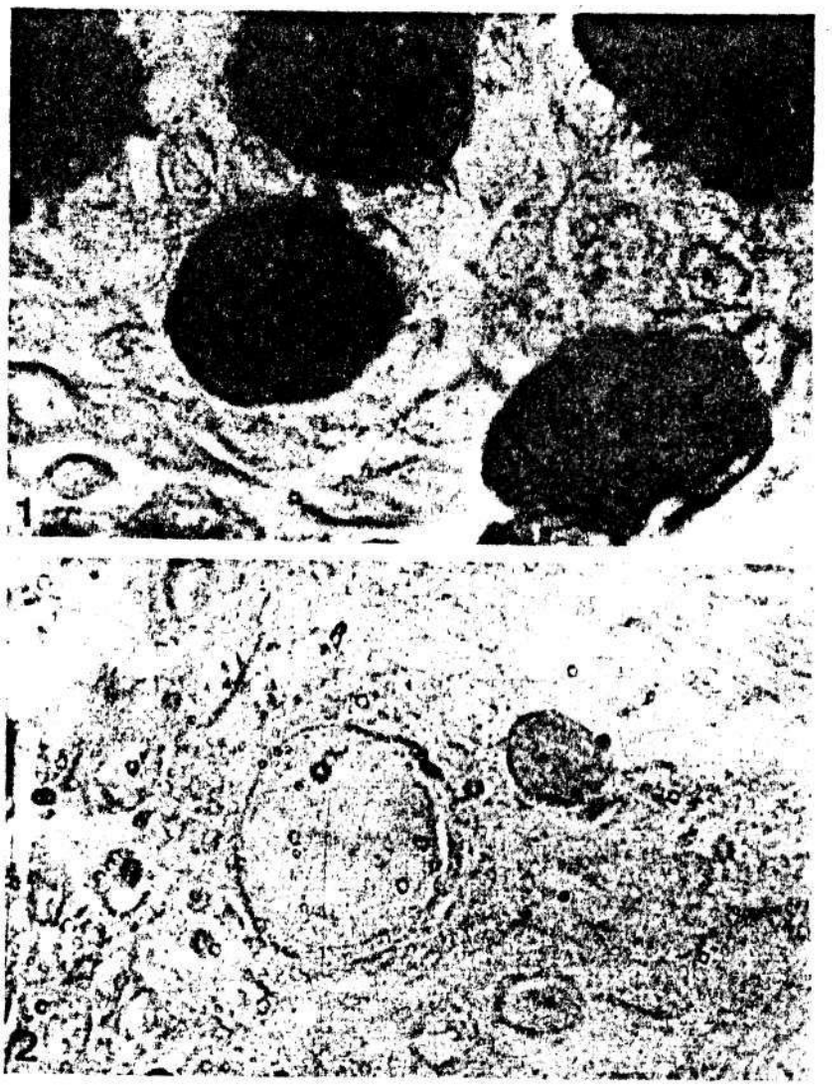

Fig. 1. Mucous skin of the ventral region of the adult trout (length 30 cms) Hotchkiss' reaction. Scale 1000x.

Fig. 2. Mucous skin of the ventral region of the adult trout (length 30 cms) Hotchkiss' reaction with prior digestion using α-amylase. Scale x1000.

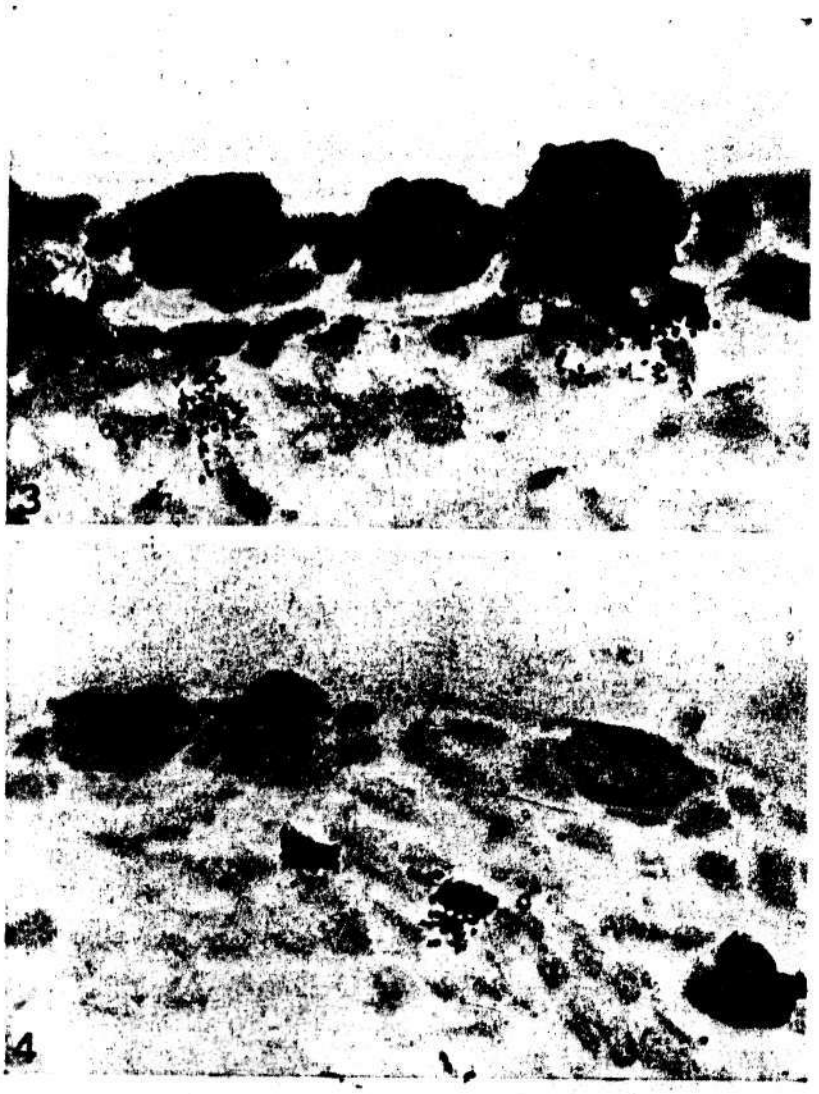

Fig. 3. Mucous skin of trout at the early posthatching stage. Stained with Alcian blue 8GN. Scale 1000x.

Fig. 4. Mucous skin of trout at the miniature posthatching stage. Stained with Alcian blue 8GN with prior digestion using sialidases preceeded by treatment with KOH. Sclae 1000x.

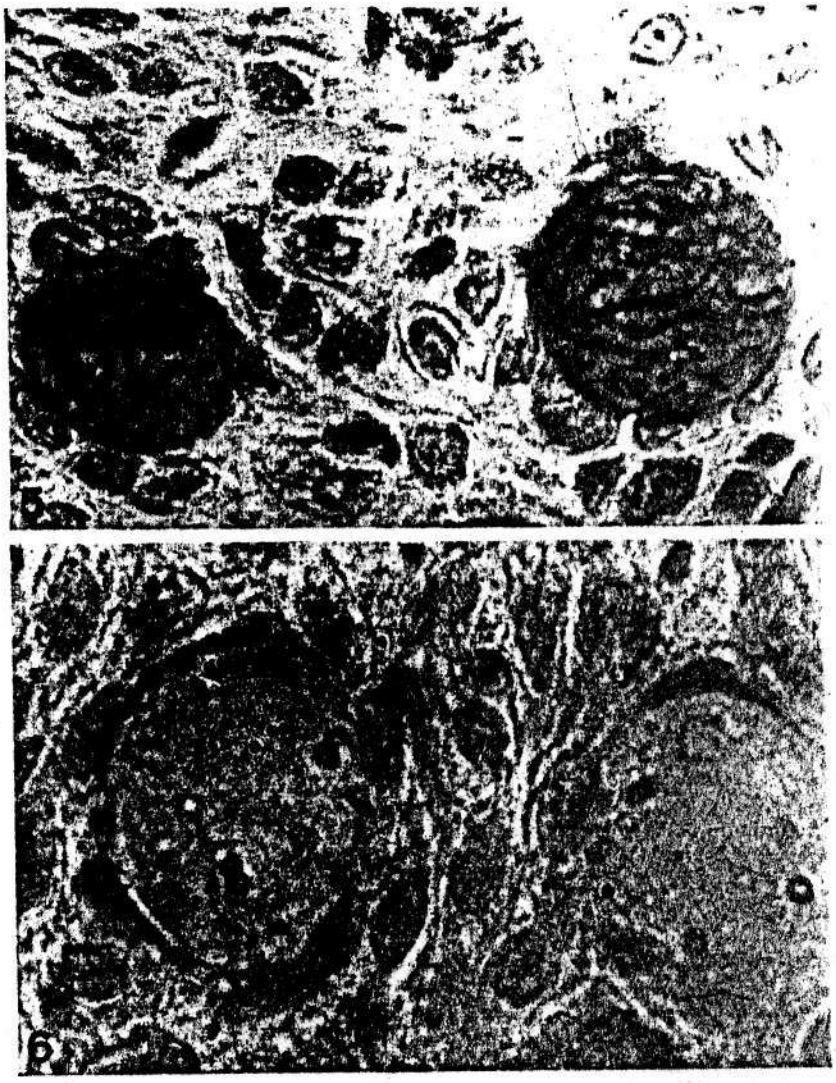

Fig. 5. Mucous skin of the ventral region of the adult trout (30 cms long). Stained with Alcian blue 8GN.

Fig. 6. Mucous skin of the ventral region of the adult trout (length 30 cms) Stained with Alcian blue 8GN and with prior digestion using sialidases preceeded by treatment with alcoholic KOH.

Stadi	Local.	G (1)	MPS n.	MPS a.n.s.	MPS (2) s.	Ac. jal.	An. sialico	Prot. g.	Prot.
pre. p.	c.	(•)	+	+	+−	−	+	−	−
	s. v.	(•)	+	+	+−	−	+	−	−
pre. t.	c.	(•)	+	+	+−	−	+	−	−
	s. v.	(•)	+	+	+−	−	+	−	−
po. t.	c.	+	+	+	+	−	+−	−	−
	s. v.	+	+	+	+	+−	+−	−	−
po. t.	c.	+	+	+	+	+−	+	−	−
	s. v.	+	+	+	+	−	+	−	−
fry.	c.	+	++	+	+	+−	++	−	−
	s. v.	+	++	+	+	+−	+	−	−
trota 15 cm. p. v.	s.	+	+	++	+−	+−	+++	−	−
	p.	++	+−	+	+	+−	++	−	−
p. d.	s.	++	+−	+	+−	+−	++	−	−
	p.	++	+−	++	+−	+−	+	−	−
trota 30 cm. p. v.	s.	++	+−	+	+−	−	+++	−	−
	p.	++	+−	+	+−	+−	+−	−	−
p. d.	s.	++	+	++	+	−	++	−	−
	p.	++	++	+	+−	+−	+	−	−

Table

pre. p. = early prehatching
pre. t. = later prehatching
po. p. = early posthatching
po. t. = late posthatching
c. = mucous cell from the skin of the fry.
s.v. = mucous cell from the yolk ectodermis
p.v. = ventral skin
p.d. = dorsal skin
s = superficial mucous cell
d = deep mucous cell
G = glycogen
MPS n. = neutral mucopolysaccharides incomparable with glycogen.
MPS a.n.s. = non-sulphated, acid-mucopolysaccharides.
Ac. jal. = hyaluronic acid.
Prot. g. = protein in the general sense
Prot. = protein containing the amino acid tyrosine.

(1) The demonstration of glycogen was obtained by comparing the slides from Hotchkiss' reaction and those after digestion with α-amylase. The histochemical diagnosis of glycogen was confirmed by the fact that in the examined locations Hotchkiss' reaction exists after pretreatment with dimedone. Langhams' reaction to iodine gives positive results only after 24 hrs of staining following Mancini's technique.

(2) For the sulphated mucopolysaccharides various grades of reactivity were observed between adjacent mucous cells (see text).

(•) There is no data on glycogen in the within-egg stages due to the extreme difficulty in cutting material fixed with Carnoy's liquid.

Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.