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KERATINIZED CELLS AS A POSSIBLE YELLOW-STAIN RECEPTOR FOR GOLD-ORANGE

Astrid Höglund

From the Department of Medical Biophysics, University of Umeå, Umeå, Sweden

Abstract. Using a previously described staining procedure, it was found that gold-orange stains some epithelial cells yellow, both in skin sections and in cytologic smears from skin and various other epithelia. A correlation seems to exist between the degree of keratinization and the yellow colour obtained with this staining method.

Key words: Keratin; Gold-orange; Staining

It has been reported (4) that a combination of gold-orange and Chinablue stains vaginal smears so that mainly yellow and blue cells occur. The yellow cells were concluded to be more mature, since they had significantly smaller nuclei and larger cytoplasm than the blue cells. This finding indicates that the compound responsible for the visualization of the yellow stain should increase during cell maturation. Such compounds are glycogen, lipids, and keratin-like substances (1).

In order to decide which type of compound is associated with the yellow staining, investigations were extended to other epithelia, including epidermis. These studies are reported in the present paper.

MATERIAL AND METHODS

108 smears from human epidermis and other epithelia (Table I) and 61 histological sections (5 μm) of normal human skin (6 sections including hair follicles) were studied. Powdered samples of RNA and keratin were also smeared on slides and stained for comparison. The epithelial smear usually adhered to the slide. Occasionally, minute amounts of albumin-glycerin had to be used as an adhesive. Staining with gold-orange (4), with Chinablue as a counterstain, was undertaken as described below and the samples were examined under the microscope. The gold-orange (also called methyl orange, Merck Co.) has the Colour Index number 13025; the formula is presented in Fig. 1.

Staining procedure

Ethanol, in decreasing concentrations, 99, 95 and 70%. Nuclear staining with Harris hematoxylin for 4 min. Rinse in tap water. Dip in acidified ethanol (1 ml 10 M HCl + 100 ml 70% ethanol). Rinse in tap water. Dip in Li_2CO_3 solution (saturated Li_2CO_3) 10 drops per 100 ml water. Running tap water for 10 min. Mordant 1 min in 5% phosphotungstic acid. Distilled water. Stain 3 min in solution of 6 g Chinablue in 300 ml distilled water and 4.5 g gold-orange in 300 ml distilled water, mixed together to form 600 ml. Rinse in distilled water. Ethanol, increasing concentrations, 70, 95 and 99%. Xylene, mounting.

RESULTS

A. *Comparison between keratin and yellow staining*

Histological sections from skin and hair. In histological sections it was found that the cells of stratum Malpighii and stratum granulosum were stained blue. The cells of stratum corneum were stained yellow. The borderline at the stratum granulosum was sharply defined. Hairs were stained yellow.

Smears from various types of epithelia. Epidermal smears from palmar skin, hairs, nails, and hyperkeratosis of portio represented presumably highly keratinized cells. They all showed a high degree of yellow staining. The yellow stain was easily distinguishable from the hair pigment. Smears from the tongue and gingiva are reported to be moderately keratinized epithelia, and showed the presence of some yellow-stained cells. The buccal epithelium has been reported to contain few

Table I. *Comparison between degree of keratinization and fraction of yellow cells*

Type of epithelium	Number of samples	Degree of keratinization	Ref. for keratin contents	Fraction of yellow cells
Epidermis	14	high	(1) p. 376	high
Hair	5	high	(10) p. 237	high
Nail	14	high	(3)	high
Hyperkeratosis of portio	7	high	(11) p. 164	high
Tongue	14	moderate	(1) p. 397	moderate
Gingiva	10	moderate	(12) p. 57	moderate
Bucca	10	few	(12) p. 57	few
Lower conjunctival sac	6	0	(2) p. 356	0
Cervical canal epithelial cells	14	0	(11) p. 40	0
Cervical canal leucocytes	14	0	(11) p. 40	0
Total	108			

keratinized cells (12) and thus showed few yellow cells. Smears from endocervix and conjunctival sac represent presumably keratin-free cells. They did not take on yellow staining (Table I).

B. *Comparison between lipids and yellow staining*

In order to study whether lipids in the stratum corneum could induce the yellow staining, 25 skin sections were treated for various time intervals up to half an hour in a methanol-cloroform mixture (1 : 1 volume). The yellow stain still persisted in the stratum corneum after this treatment. This indicates that the yellow stain does not depend on the presence of lipids.

C. *Comparison between glycogen and yellow staining*

Five vaginal smears containing less than 15% yellow cells were selected for study. Five duplicates were stained with iodine vapour for demonstration of glycogen, and the smears were then compared. These smears, containing very few yellow cells, showed a considerable number (more than 50%) of glycogen-containing cells, as evidenced by the brown colour in iodine vapour staining. Thus there is no relation between yellow staining and glycogen.

D. *Comparison between XPS and sulphide bonds*

A biophysical method for direct determination of sulphide bonds in biological samples is photoelectron spectroscopy (XPS). This technique has recently been applied (7) and (5) for demonstration of keratin or keratin-like compounds. In the latter study, presumably keratinized cells (skin, barb, nails and estrogenic vaginal smears) proved to contain significant amounts of sulphide bonds and stained yellow. Presumably non-keratinized cells (cervical cells, buccal mucosa and gestagenic vaginal smears) did not contain sulphide bonds and did not stain yellow. The results are presented in Table II and indicate that yellow-stained material contained large amounts of sulphide bonds, claimed to be characteristic of keratin (6) and (7).

DISCUSSION

All the results obtained in this investigation are consistent with the conclusion that keratin (or keratin-like compounds) is associated with, or is the receptor for, the yellow colour obtained in the gold-orange staining procedure. It is of interest to discuss the present method in relation to other identification procedures for keratins. A discussion of this subject is preferably based on the molecular properties reported as being specific for keratins.

Keratins are characterized (A) by their large number of S–S, S–H and –S– groups, due to cystein, cystine and methionine, (B) by their contents of certain other amino acids, (C) in part by their specific molecular α-helical structure (6), and (D) by properties not specified.

(A) Regarding S–S and S–H groups, they can be

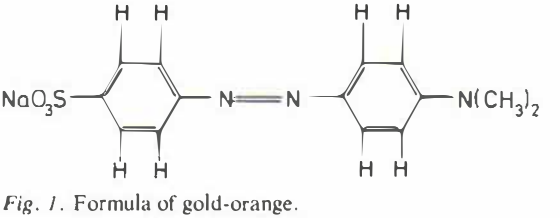

Fig. 1. Formula of gold-orange.

Table II. *Comparison between amount of sulphide bonds from XPS and staining*

Sample	Sulphide bonds from XPS	Stain
Keratin	+++	Yellow
Nail	+++	Yellow
Barb	+++	Yellow
Skin	+++	Yellow
Estrogenic vaginal epithelia	+++	Yellow
RNA	0	Blue
Bucca	0	Blue
Cervix	0	Blue
Gestagenic vaginal epithelia	0	Blue

demonstrated by histochemical methods. A comprehensive review of such methods was given by Pearse (10).

The main methods are the following:

1. The performic acid-Schiff method for S–S groups. (10), page 624, 150–152.

2. Dihydroxy-dinaphthyl-disulphide reaction for S–H. (10), page 625, 154–156.

3. Performic acid–Alcian blue technique for S–S groups. (10), page 624.

4. Mercury orange method for S–H. (10), page 622.

Other methods are also described and discussed by Pearse (10). Another method specific for the S–S, S–H and –S– bonds is XPS (5). This method detects the electronic bonding energy specific for divalent sulphur, and is independent of the molecular environment of keratins.

All the methods mentioned above are based on the presence of S–S, S–H and –S– groups in keratin, but other tissues and compounds containing these chemical groups may also be recorded, depending on the sensitivity of the method. This circumstance limits their specificity.

(B) Regarding other specific amino acids, it has been claimed (10) that keratin should contain large quantities of basic amino acids. However, no specific keratin stains seemed to be based on this property.

(C) The α-helical structure of keratins (9) gives rise to typical X-ray diffraction patterns, especially meridional reflections at 1.5 Å, 5.2 Å and 197 Å and equatorial reflections at 27 Å, 41 Å and 80 Å (3). These reflections can be used for the identification of keratins. The periodic structure of keratins also gives rise to a typical electron microscopic pattern, with closely packed fine filaments embedded in a fairly dense matrix.

(D) A cytologic staining method which is claimed to indicate keratinization or cornification is the Papanicolaou stain (8). The so-called acidophilic cells which are stained pink or orange are said to be cornified. The typical colour probably arises from the Orange G present in the Papanicolaou stain. The molecular basis for the stain reaction is not known.

For the gold-orange stain, the situation is at present similar, i.e. the molecular mechanism of the staining is not known so far. Nor is it possible to decide if the yellow staining is associated with keratin proper or with some supposed precursor to keratin, such as prekeratin or keratohyalin. Therefore, it will at present be most appropriate to conclude that some "keratin-like compounds" accept or are associated with the yellow stain of the gold-orange method. In this department, studies are presently being performed, utilizing various biophysical methods such as nuclear magnetic resonance (NMR), electron spin resonance (ESR), molecular orbital calculations (MO), optical spectrophotometry and extended XPS studies, in order to further elucidate the staining mechanism of the gold-orange stain. Certain progress has been made, which will be reported in forthcoming papers.

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A. Höglund, Chief technician
Department of Medical Biophysics
University of Umeå
S-901 87 Umeå
Sweden