reduces its biological activity by 97% or more[13]. It has been suggested that sialic acid could be involved in the transport of the hormone or have some action on activating the receptor sites at the target organs[14]. There are indications that sialic acid may have a role in the spatial configuration of glycoproteins which is drastically changed by releasing sialic acid residues[15]. Furthermore, the ability of certain glycoproteins to inhibit viral hemagglutination is related to their sialic acid content[16,17]. The cell surface of transitional epithelium is covered by a thick carbohydrate coat or glycocalyx rich in sialic acid[2]. This led to postulate that the glycocalyx of transitional epithelium is a source of certain urinary complex carbohydrates, such as the mucoprotein of TAMM and HORSFALL[2]. The present results, indicating the high carbohydrate content, particularly of sialic acid, in sheep transitional epithelium are followed by fractionation studies and comparison of its electrophoretic behaviour with the TAMM-HORSFALL mucoprotein[18].

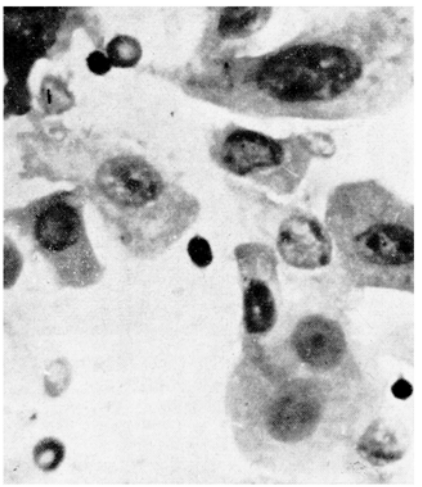

Fig. 3. Transitional epithelial cells from pellet I (see text). H + E. ×1600.

Resumen. El epitelio de transición tiene alto contenido de carbohidratos. Este tejido posee una de las concentraciones más elevadas de ácido siálico en el organismo. Se describe un procedimiento simple para exfoliar epitelio de transición.

A. CANDIOTTI, N. IBAÑEZ and B. MONIS

Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Casilla Postal 362, Córdoba (Argentina), 19 October 1970.

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Irreversible Depigmentation of Hair by N-Methyl-N-Nitrosourethane

Recently it has been reported that skin application of a solution of 8-hydroxyquinoline 0.5% in acetone causes striking, but reversible depigmentation of the subsequent hair growth in several strains of pigmented mice[1]. Several other copper reagents, however, failed to inhibit hair pigmentation in female C57BL mice. It has been suggested that possibly a complex of 8-hydroxyquinoline with the metal is involved.

In the course of testing the carcinogen, N-methyl-N-nitrosourethane (MNU) by various routes in several animal species, single s.c. injections of MNU were observed to cause sometimes irreversible depilation at the site of injection, or irreversible depigmentation of the hair when the dark CBA mice were used (Figure 1). The depigmentation effects on the hair were particularly striking after a single dose, 0.5 ml of a 2.5% solution of MNU in 30% aqueous ethanol was injected into the marginal ear-vein of pigmented 'Dutch' rabbits ears. The hair overlaying the veins through which MNU had pressumably passed (before being diluted in the blood stream), became depigmented and showed the pattern of the underlaying veins (Figure 2). The depigmentation was noticed a few weeks after the injection and remained unchanged for more than 1.5 years.

Melanine is known to occur in melanocytes, specialized cells in the basal layer of the epidermis, probably derived from the neural crest. Its formation involves several steps, the first of which is the oxidation of tyrosine to L-Dopa (3,4-dihydroxyphenyl alanine) by tyrosinase, a copper protein complex present in melanocytes. This enzyme is believed to catalyse also some of the subsequent reactions leading from Dopa to melanine. These involve the oxidation of L-Dopa to the respective *ortho*-quinone, cyclization to 5,6-dihydroxy-dihydro-indole-carboxylic acid, oxidation of the latter to the respective red *ortho*quinone, hallachrome, its decarboxylation and eventually the polymerization of indole-5,6-quinone, to melanins, which probably form complexes with proteins[2].

The structures of melanins and the effects of various agents on their formation have been intensely studied. The melanins give ESR signals for free radicals; they appear to contain besides indole-5,6-quinone, small numbers of units of some of its precursors[3].

Irradiation, whether with ultraviolet rays, or small doses of X- and γ-rays as well as local application of carcinogenic polycyclic aromatic hydrocarbons are known to induce increased skin pigmentation[4]. These agents have been shown to activate the usually non-pigmented melanocytes and also to cause their proliferation. This

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effect is explained by the removal of the reducing SH-groups (in-S-S-bonds) though other (e.g. hormonal) factors may also be involved. In albino animals, which lack the genetically determined tyrosinase, pigmentation of melanocytes can not take place.

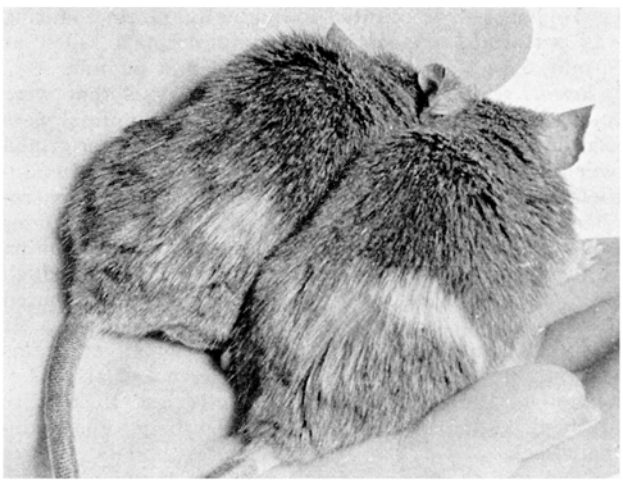

Fig. 1. Local depigmentation of hair in CBA mice, persisting for many months after a s.c. injection of MNU (0.25 mg in 0.05 ml 30% aqueous ethanol).

Fig. 2. Depigmentation of hair covering the veins of 'Dutch' rabbit's ear after a single dose of MNU (12.5 mg in 0.5 ml of 30% aqueous ethanol) injected into the left peripheral vein of the left ear. The right ear is shown for comparison.

A temporary inhibition of melanin formation can be caused either by compounds that combine with copper, or with *o*-dihydroxy groups and *o*-quinones, by certain analogues of tyrosine (which can act as competitive substrates for tyrosinase) and also by various reducing substances, ascorbic acid, hydroquinones, sulphydryl compounds etc. Hormonal and nutritional factors, and micro-elements play also a role. Depigmentation due to copper deficiency can be precipitated by excess molybdenum in diet[5], and by certain compounds that bind copper (including sulphydryls)[2]. In such cases addition of copper sulphate to the diet can often reverse the inhibition of melanin formation.

However, when the melanocytes degenerate and atrophy, irreversible depigmentation will follow. This happens after large doses of X-rays[6] or during treatment with alkylating agents, of which MNU is a good example. Similar permanent depigmentation of hair was observed in pigmented mice also after s.c. injections of N-ethyl-N-nitrosourethane and of elaiomycin.

The question arises why melanocytes are more sensitive to the necrotizing action of MNU (and of the other agents) than other cells in the skin and in the hair follicles. It is possible that this may be due to a low content of glutathione and of other protective sulphydryl (or selenohydryl) compounds.

Melanocytes are likely to have a low concentration of cytoplasmic sulphydryl-compounds, otherwise melanin would not be formed. In the case of MNU, it is known that it rapidly interacts with sulphydryls at physiological pH[7] and that depending on its concentration it can form a number of derivatives from cysteine[8]. Small molecular sulphydryl compounds (cysteine, glutathione) will protect the more important sulphydryls present in enzymes and in the structural elements of cells from the irreversible action of MNU, as they also do in the case of radiation[9]. The known resistance of the liver to the carcinogenic action of radiation and of MNU[10] is probably due to its high concentration of glutathione.

Very many metabolic processes depend on the integrity of the appropriate cellular SH-constituents, the sensitivity of which may depend on steric and other factors. The concentration of MNU reaching the site critical for melanin formation will be of paramount importance. The paradox of the situation that dosing with sulphydryl compounds usually prevents melanin formation, but can sometimes prevent the opposite effect, depigmentation, may be only apparent. With the increase in our knowledge of the fundamental biochemical mechanism involved in the process, this paradox will, no doubt, be resolved.

Résumé. Une injection du carcinogène N-méthyl-N-nitrosouréthane (MNU) entraine dépigmentation permanente des poils des animaux pigmentés (ceux des souris CBA et des oreilles du lapin «Dutch»). Le rôle des groupements sulphydryles qui réagissent avec MNU est discuté.

R. Schoental

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