budding of the dying cells which is a feature of the early stages of apoptosis in other situations (15). Phase-contrast microscopy has shown that the cellular condensation and budding phases of apoptosis take only a few minutes (15). This, combined with the avidity with which viable cells engulf the apoptotic fragments, accounts for the difficulty in finding the early stages of the process by electron microscopy.

This has undoubtedly contributed to its confusion with autophagic vacuole formation as the later stages of intracytoplasmic digestion are the most prominent feature. It was only after prolonged searching, aided by our knowledge of apoptosis in other circumstances, that we found early apoptosis in lichen planus (15) and in this study of catagen follicles.

Since Olson & Everett (10) first suggested that apoptosis might be involved in the involution of hair follicles during catagen, we have examined hair follicles for its presence in biopsies from patients with alopecia. We have found that apoptosis is a useful marker of catagen in follicles of human scalps. Interestingly, apoptosis has been demonstrated ultrastructurally in alopecia areata, although it was not recognized as such (12).

It remains to be determined what is responsible for inhibiting mitosis and initiating apoptosis in catagen transformation under various circumstances. In spontaneous catagen, some genetically programmed phenomenon is probably involved, but studies of molting in animals (7) would seem to indicate that numerous other factors, including climate, nutrition and light also have some influence on this process. Further work is needed to clarify these aspects.

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Formation of Glutathionedopa in Albino Rats after DOPA Injection

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Abstract. 5-S-Glutathionedopa was demonstrated in a homogenate of the choroid and the retinal pigment

Table I. *5-S-Glutathionedopa in organs of albino rats 1 h after injection of L-dopa (50 mg/kg)*

In 3 rats receiving saline injections no 5-S-glutathionedopa was detected

Rat no.	5-S-Glutathionedopa (ng/g)		
	Skin	Spleen	Kidney
1	17	50	0
2	13	45	0
3	5	29	0

epithelium of bovine eyes. When albino rats were injected with dopa, 5-S-glutathionedopa appeared in the spleen and in the skin, but not in the kidney.

Key words: 5-S-Cysteinyldopa; Dopa; Glutathione; Albinism; Pigment; Eye

Cysteinyldopas, especially 5-S-cysteinyldopa, have in recent years been the subject of great interest in melanogenesis (7, 9). 5-S-Cysteinyldopa is present in all melanin-forming tissues, in serum, and in urine. 5-S-Cysteinyldopa is also present in certain internal organs in which the cellular localization is unknown.

Since glutathione is the quantitatively most important free thiol in the body, we have for several years been investigating the role of this tripeptide in the formation of cysteinyldopa. It has been demonstrated that 5-S-cysteinyldopa can be formed by enzymatic hydrolysis of glutathionedopa (1). This formation depends on a γ-glutamyltransferase and a peptidase (3). Such enzymes are widespread in the body and it has been demonstrated that melanoma tissue itself can produce cysteinyldopa from glutathionedopa (1).

Cysteinyldopas are formed by nucleophilic addition of cysteine to dopaquinone. When dopa is oxidized in the presence of glutathione, formation of glutathionedopa occurs.

In spite of a pronounced capacity to metabolize glutathionedopa, melanoma tissue contains small amounts of this substance (3). So far, glutathionedopa has not been demonstrated in normal tissues. We now report the occurrence of this substance in normal bovine eyes and the appearance of glutahionedopa in tissues of albino rat after injection of dopa.

MATERIAL AND METHODS

Glutathionedopa was prepared as previously described (3, 9).

The amount of 5-S-glutathionedopa and of 5-S-cysteinyldopa was determined by the high performance liquid chromatography technique previosly described (6). A reversed-phase octadecyl-silica column (Nucleosil C_{18}, 5 μm, 250×4.5 mm) was used and the aqueous mobile phase contained 3.0 g phosphoric acid and 1.0 g methanesulphonic acid. The pH of the mobile phase was set either at 1.75 (A) or at 3.0 (B), depending on the purpose of the analysis. Detection was performed by an electrochemical detector with the anodic potential set at 0.85 V vs. Ag/AgCl reference electrode. This chromatographic system has smaller capacity factors for catecholic amino acids such as dopa, 5-S-cysteinyldopa and 5-S-glutathionedopa than the previously described eluents and is for that reason not suitable for more polar compounds such as 5-OH-dopa. The detection limit for 5-S-glutathionedopa is about 1 pmole per injected sample.

When the enzymatically prepared 5-S-glutahionedopa was chromatographed on a semipreparative HPLC prepacked column (Microbondapack 18), Waters Ass.), using B as mobile phase, a peak appeared after 24 min. The effluent corresponding to the peak was collected. The sample was incubated with a rat kidney homogenate for 2 hours (1) and then analysed for 5-S-cysteinyldopa on the HPLC-system. A distinct peak corresponding to 5-S-cysteinyldopa appeared. Acid hydrolysis in 6 M HCl at 120° for 15 h in a sealed tube under a nitrogen atmosphere of the compound was performed on another sample collected after 24 min. The hydrolysate was analysed for glutamic acid and glycine by gas chromatography – mass spectrometry after pentafluopropionic acylation of the methylester derivatives prepared according to a previously described method (2). The amounts of 5-S-cysteinyldopa, glutamic acid and glycine in the hydrolysates proved the identity of 5-S-glutathionedopa in the fractions analysed.

The amount of 5-S-glutathionedopa in tissue extracts was calculated from the HPLC chromatogram by comparison with injected external 5-S-glutathionedopa standard solution. The mobile phase B was generally used for quantitative analysis of 5-S-glutathionedopa. Standard 5-S-glutathionedopa was prepared by enzymatic oxidation of ^{14}C-labelled tyrosine in the presence of glutathione (9). The 5-S-glutathione-^{14}C-dopa formed was isolated by semipreparative HPLC and adsorption onto alumina. The amount of 5-S-glutathionedopa was determined by liquid scintillation counting.

Glutathionedopa has so far been described in malignant melanoma, a pathologic pigmented tissue. We wanted to know if glutathionedopa could be detected in a normal pigmented organ. Therefore, the choroid and the retinal pigment epithelia from 30 brown-black bovine eyes were pooled. The tissue was homogenized in 0.4 M perchloric acid, centrifuged for 10 min at 15 000 r.p.m. and the supernatant was filtered. The filtrate was adsorbed onto Al_2O_3 in the presence of EDTA and sodium metabisulphite and eluted with 0.3 M HCl. The eluate was then injected onto the semipreparative HPLC column. When the elution was followed by means of an UV-detector (210 nm), a peak appeared with the same retention time as the 5-S-

Table II. *Experiment demonstrating 5-S-glutathionedopa in extracts of the skin and the spleen after dopa injection*

Purified extracts were chromatographed on semipreparative HPLC and the effluents corresponding to the peak of synthetic 5-S-glutathionedopa were collected. Analytical HPLC showed that the effluents from dopa-injected rats contained 5-S-glutathionedopa before hydrolysis and 5-S-cysteinyldopa after enzymatic hydrolysis

Treatment of rats	Tissue	Treatment of semipreparative 5-S-glutathione-dopa fraction	Analysis on HPLC	
			5-S-cyste-inyldopa	5-S-gluta-thionedopa
NaCl injection	Skin	No hydrolysis	0	0
		Hydrolysis	0	0
	Spleen	No hydrolysis	0	0
		Hydrolysis	0	0
Dopa injection	Skin	No hydrolysis	0	+
		Hydrolysis	+	0
	Spleen	No hydrolysis	0	+
		Hydrolysis	+	0

glutathionedopa standard. The effluent corresponding to the peak was collected and hydrolysed by a rat kidney homogenate as described above for synthetic 5-S-glutathionedopa. 5-S-Cysteinyldopa appeared in the hydrolysate.

Three adult albino rats were injected intraperitoneally with L-dopa (50 mg/kg bodyweight) and 3 control animals received saline injections. The spleen, kidney and skin were removed 1 hour after injection and homogenized in 0.4 M perchloric acid, purified as previously described (4) and analysed on HPLC for 5-S-glutationedopa.

For confirmation of the formation of 5-S-glutathionedopa after dopa injection, 5 albino rats injected with saline and 5 with dopa (50 mg/kg bodyweight) were killed after 1 h. The skin and the spleen were pooled and homogenized in phosphate buffer, pH 7.4. The homogenates were purified on Al_2O_3 and chromatographed on semipreparative HPLC. The fractions corresponding to synthetic glutathionedopa were collected and incubated with homogenates of rat kidney, dialysed against 0.5 M phosphate buffer (pH 7.4) over night. The incubates contained 30 mg metabisulphite and 10 mg glutamine per ml. To the control incubates perchloric acid was added immediately. The incubates for enzymatic hydrolysis of glutathionedopa were interrupted after 2 h by perchloric acid addition. The perchloric acid extracts were analysed by HPLC for 5-S-glutathionedopa and 5-S-cysteinyldopa.

RESULTS AND COMMENTS

The analysis of the choroid and retinal pigment epithelium of bovine eyes showed the presence of small amounts of 5-S-glutathionedopa, 2.7 ng/g.

The tissues of control albino rats did not contain any glutathionedopa. In contrast, the rats wich had received injections of dopa demonstrated the presence of 5-S-glutathionedopa in the skin and still more in the spleen, but in the kidney no glutathionedopa was detected (Table I).

With or without hydrolysis the extracts from skin and spleen of NaCl-injected rats did not contain cysteinyldopa or glutathionedopa in the HPLC fraction corresponding to 5-S-glutathionedopa. In contrast, the same fraction from dopa-injected rats contained 5-S-glutathionedopa when no hydrolysis had been performed, and 5-S-cysteinyldopa after hydrolysis by the kidney homogenate (Table II).

The results indicate that dopa oxidation occurs also in albino animals and that 5-S-glutathionedopa is a quantitatively important addition product of dopaquinone. The high amounts in the spleen compared with the skin may be due to a retention mechanism for glutathionedopa formed elsewhere. It seems more likely, however, that glutathionedopa is formed in some structures in the spleen, which are able to oxidize dopa. It is tempting to speculate that glutathionedopa is localized in nerve terminals or in non-melanocytic cells of neural crest origin, since cysteinyldopa has recently been detected in ganglion stellatum of the cow (5). The absence of glutathionedopa in the kidney is remarkable, as this organ contains considerable amounts of 5-S-cysteinyldopa (4).

The present study has demonstrated 5-S-glutathionedopa to be a normal metabolite in pigmented animals. Furthermore, we have found that this compound is formed in albinos after dopa injections. The production of glutathionedopa may be the result of tyrosinase activity in melanocytes, but the finding of larger amounts of glutathionedopa in

the spleen may indicate that extramelanocytic, 'nonspecific' or 'accidental' oxidation of dopa may occur—at least after injection of large amounts of this compound.

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Flare-up of Contact Dermatitis to Picryl Chloride in the Mouse

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Abstract. In mice sensitized to picryl chloride, external challenge with the antigen was followed 2 weeks later by an intraperitoneal injection of picryl sulphonic acid. This resulted in a flare-up of the previous dermatitis.

Key words: Contact dermatitis; Mice; Picryl chloride; Flare-up

It is well known that many exacerbations of allergic contact dermatitis are caused by systemically administered antigen (2, 3). Pathogenetic studies on such reactions are difficult to perform in man. We report here an attempt to design an animal model for "endogenous contact dermatitis".

MATERIAL AND METHODS

Animals: Female NMRJ albino mice weighing about 30 g and 2 months of age were obtained from Anticimex AB, Stockholm. *Drugs:* Picryl chloride (PCh) purchased from BDH, Poole, U.K.; picryl sulphonic acid (PSA) obtained from Sigma Chemical Co., St. Louis, USA, dissolved in saline immediately before use; croton oil dissolved in ether.

Sensitization and external challenge were carried out according to Möller (6). Thus all mice were sensitized by a single painting on the abdomen with PCh 7% in ethanol and challenged one week later by painting their left ears with PCh 0.5% in olive oil (control: right ears, oil only).

Internal challenge was performed by intraperitoneal injection of PSA 5 mg/kg bodyweight in 0.25 ml saline, this being the highest tolerable dose. The interval between external and internal challenge was 2–3 weeks, except in one experiment with an interval of 11 weeks. The animals were sacrificed 24 h later and the wet weight of both ears was registered (6). For *controls*, see Table I. In one type of control ear, painting with croton oil substituted the external challenge with PCh in order to obtain a toxic dermatitis. Application of 0.1% was found suitable as it produced a dermatitis of similar wet weight and duration to the allergic contact dermatitis.

The Student's *t*-test was used for statistical evaluation.

RESULTS

As earlier shown (6) painting with PCh in sensitized mice regularly resulted in a contact dermatitis. Our