pounds are formed artificially if cysteinyldopas present are not oxidized before the analysis for trichochromes.

Dopa and 5-S-cysteinyldopa have previously been observed in guinea-pig hairs (9). The finding of these catechol amino acids in hair and feathers indicates that a third path of distribution of these compounds from the melanocytes exists. Firstly, they may form pigments, which are transferred to keratinocytes or phagocytes. Secondly, they may be excreted as such into the connective tissue and into the circulation. Thirdly, they may be directly ingested by keratinocytes, where they remain non-oxidized.

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Free and Bound 5-S-Cysteinyldopa and Dopa in Human Malignant Melanomas

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Abstract. Free dopa and 5-S-cysteinyldopa were extracted from two human melanomas. Subsequent hydrolysis of carefully washed melanoma tissue released dopa and 5-S-cysteinyldopa, indicating the presence of these catechol amino acids in proteins. Cysteinyldopa-containing proteins may represent the antigens previously demonstrated in human melanomas.

Key words: Melanoma; Cysteinyldopa; Dopa; Protein

Studies on patients with malignant melanoma have produced evidence of tumour-directed serum antibodies and also of cell-mediated allergy to melanoma cells (3, 6). Antibodies to cytoplasmic components of the tumour cells have been reported, but no tumour-specific antigens have so far been defined.

Melanomas contain dopa and cysteinyldopas, amino acids which are precursors of melanin, but which may also leave the cell, enter the circulation, and be excreted in the urine.

Tyrosine in a protein may be oxidized to dopa and we assume that this dopa can also be oxidized, with resultant formation of immobilized dopaquinone. By nucleophilic addition of cysteine to this dopaquinone, cysteinyldopa in a protein may be produced. Cysteinyldopa as part of a protein may also be formed by the addition of free dopaquinone to a sulfhydryl group of cysteine in a protein.

If dopa or cysteinyldopas are formed in a protein this may have immunological implications. It seems quite possible that such a protein when exposed to immunocompetent cells, is registered as "non-self". An immune reaction against the melanocytes may result.

The present study was performed with the object of demonstrating dopa and cysteinyldopas as components of peptides or proteins in human melanomas.

MATERIAL AND METHODS

Melanoma tissue obtained at necropsy of 2 patients was analysed. For determination of free dopa and 5-S-cysteinyldopa, 1.4–1.5 g of tumour tissue was homogenized and extracted with perchloric acid, adsorbed onto Al_2O_3 and eluted with 0.1 M HCl. Purification and fluorimetric determination of dopa and 5-S-cysteinyldopa were performed by previously described methods (2, 7).

In order to detect dopa and 5-S-cysteinyldopa in proteins, 4–5 g of melanoma tissue was homogenized in 10% trichloroacetic acid (TCA) and centrifuged. The pellet was washed four times in 10% TCA and three times with ether, then suspended in H_2O, and finally divided into two aliquots, one of which was examined for residual free dopa and 5-S-cysteinyldopa, and the other hydrolysed and examined for dopa and 5-S-cysteinyldopa.

For demonstration of residual free dopa and 5-S-cysteinyldopa, 4 N perchloric acid (PCA) was added to the first aliquot to render the sample 0.4 N. Catechol compounds were adsorbed onto Al_2O_3 and eluted with 0.1 N HCl. Dopa and 5-S-cysteinyldopa were demonstrated by fluorimetry (2, 7).

The second aliquot of melanoma tissue was evaporated to dryness and 12.5 ml of 6 N H_2SO_4 was added. The tube was gassed with N_2 sealed by melting, and heated for 17 h at 110°C. Demonstration of free dopa and 5-S-cysteinyldopa after hydrolysis was performed as described above.

The identity of dopa and 5-S-cysteinyldopa obtained in free form by hydrolysis was confirmed after chromatography on a Dowex 50W-X4 column by gas chromatography and mass spectroscopy (GC-MS) of the derivatives previously described (1).

RESULTS AND DISCUSSION

Both tumours contained free dopa and 5-S-cysteinyldopa in large amounts (Table I). The free catechol amino acids were washed away from the pellets. Significant amounts of dopa and 5-S-cysteinyldopa could then be detected after hydrolysis (Table I). The figures presented should be considered as minimum values for bound catechol amino acids, since model experiments have shown that, on hydrolysis, dopa and cysteinyldopa form products that cannot be detected by the fluorimetric methods used (unpublished observations).

Dopa has previously been reported in the hydrolysate of melanoma tissues (4, 5, 8), but this is the first observation of 5-S-cysteinyldopa after hydrolysis of melanoma. The most probable explanation for the presence of dopa detectable in free form after hydrolysis is the formation of this compound by oxidation of tyrosine in a peptide.

Table I. *Free and bound dopa and 5-S-cysteinyldopa in two human melanomas*

Melanoma patient	Dopa (μg/g)		5-S-cysteinyldopa (μg/g)	
	Free	Bound	Free	Bound
HN	3.4	6.9	380	7.5
JE	4.8	14	83	1.9

Peptide or protein-bound cysteinyldopa can be obtained by enzymatic oxidation of dopa formed in a peptide chain with subsequent nucleophilic addition of cysteine, or by binding of free dopaquinone to cysteine in a peptide.

Dopa is present in many tissues, including the adrenals, central nervous system, peripheral nerves, and melanocytes. Cysteinyldopas, however, are unique in that they originate only in melanocytes, and their formation in a protein could from an immunological viewpoint prove to be equivalent to the formation of a complete antigen by binding of a hapten to a protein.

It seems possible that antibodies to cytoplasmic components of melanocytes are directed to proteins containing cysteinyldopas, which amino acids are specific for these cells.

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Deposits of Complement and Immunoglobulins in Dermal and Synovial Vessels in Psoriasis

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Abstract. Deposits of complement C 3 and/or immunoglobulin were found in the vessel walls and/or at the dermal–epidermal junction in skin lesions of all of 11 patients with psoriatic arthritis and guttate psoriasis. Similar deposits were seen in 6 out of 15 patients with psoriasis vulgaris. Synovial tissue available from 2 patients revealed deposits in the vessel walls.

Key words: Psoriasis; Immunofluorescence; Vessel walls; Dermal–epidermal junction

Burnham and co-workers demonstrated immunoglobulin at the dermal–epidermal junction in psoriasis (2). In pustular psoriasis complement C 3 has also been found at this site (3). We used immunofluorescence microscopy to look for deposits of immunoglobulins and complement C 3 in the skin of patients with psoriasis.

MATERIAL AND METHODS

Biopsies from lesions as well as clinically uninvolved skin were obtained from 26 patients. Fifteen had psoriasis vulgaris, and 6 had acute guttate psoriasis with histories of recent sore throat and fever. Five patients had psoriasis and psoriatic arthritis, 2 of whom underwent synovectomy and synovial biopsies were examined concomitantly with the skin biopsies.

The biospies were examined for deposits of IgG, IgM, IgA and complement C 3 by a direct immunofluorescence technique. The procedure has been described in detail elsewhere (5).

RESULTS

Deposits of complement C 3 were seen in the vessel walls of skin lesions in 15 patients (Table I) (Fig. 1).

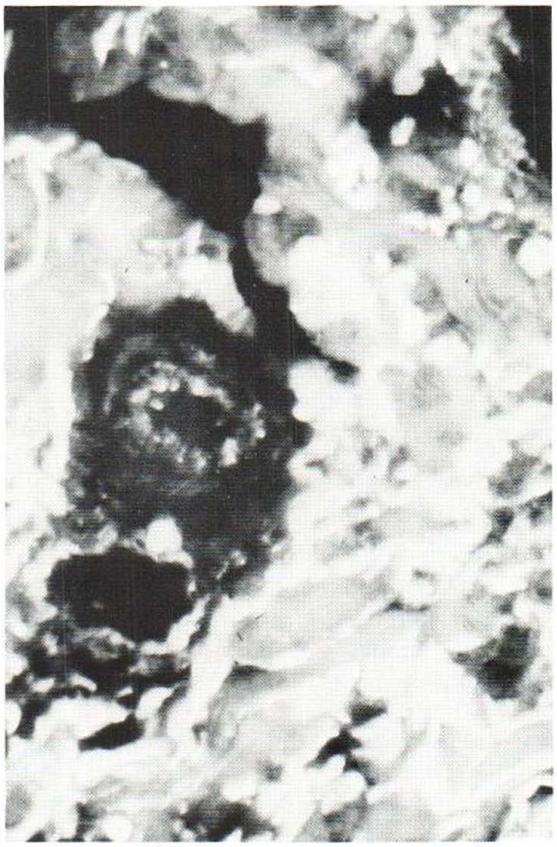

Fig. 1. Granular deposits of complement C 3 in dermal vessel.