the virus was recovered from the experimental plumbing system which had been contaminated with it by back-siphonage. Although the viruses were suspended in chlorinated municipal drinking water, they survived in

TABLE 1

CONTAMINATION OF AN EXPERIMENTAL PLUMBING SYSTEM WITH NEUROTROPIC VIRUSES BY BACK-SIPHONAGE

Type of specimen	No. of experiments	Viruses employed				Ident. of virus
		L.C.M.	Polio (Lansing strain)	W.E.E.	St. Louis	
Original	3	15/15*	12/18	18/18	18/18	Pos.
From faucet	3	32/36	28/36	35/36	36/36	Pos.
From fountain	3	34/36	31/36	36/36	32/34	Pos.

* Mice dead/mice used.

the plumbing system for several days in each set of experiments. Survival time for the different viruses in Experiments 1, 2, and 3, respectively, was as follows: lymphocytic choriomeningitis—7, 3, and 4 days; poliomyelitis (Lansing strain)—4, 1, and 2 days; Western equine encephalomyelitis—5, 2, and 4 days; and St. Louis encephalitis—4, 3, and 2 days.

These experiments show that a plumbing system may be contaminated with the neurotropic viruses studied by a simple form of back-siphonage. They also show that, when these viruses were suspended in chlorinated drinking water and retained in the plumbing system at room temperature, they remained infectious for several days.

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Role of Sulfhydryl Compounds in Pigmentation

PETER FLESCH[1] and STEPHEN ROTHMAN

Departments of Pharmacology and *Medicine, Section of Dermatology, University of Chicago*

In recent studies (*8*) it was found that aqueous extracts of human epidermis inhibit the oxidation of *l*-tyrosine and *l*-dihydroxyphenylalanine (dopa), thus preventing the formation of melanin. Since this effect is counteracted by iodoacetamide, it has been assumed that the inhibition is due to the presence of sulfhydryl compounds in the extracts.

Further evidence in support of this assumption is submitted in this communication. The inhibitory principle is water soluble, dialyzable, and heat stable and is counteracted by *p*-chloromercuribenzoic acid and by cupric ions. Direct evidence is supplied by *in vitro* experiments demonstrating the relationship between the inhibitory activity of epidermal extracts and their sulfhydryl content. It is found that the degree of inhibition of melanin formation varies directly as the logarithm of the molar concentration of –SH.

A similar relationship is demonstrated *in vivo* following ultraviolet irradiation of the skin. In these experiments an increase in melanin formation is found to be preceded by a decrease in the –SH content of the skin. Immediately following exposure of the shaved skin of rabbits to large doses of ultraviolet light there is a decrease of from 24% to 83% in the concentration of –SH in the skin. This suggests that pigment-producing stimuli act by eliminating the –SH inhibition, allowing the enzymatic oxidation of pigment precursors to occur.

A clue as to the possible mechanism of this elimination is provided by another defense reaction of the tegument to ultraviolet irradiation and to other injurious stimuli, namely, an increased production of keratin, manifested as a thickening of the horny layer in man (*7*) and as excessive hair growth in animals (*6*). The production of keratin is characterized by the formation of disulfide (–S–S–) bridges from sulfhydryl groups of native proteins. Possibly the same biochemical process, the oxidation of sulfhydryl groups to disulfides, is responsible for both intensified keratinization and pigmentation.

Additional information concerning the role of sulfhydryl compounds in melanin formation is provided by two observations: first, that a copper-containing enzyme, tyrosinase, is apparently essential for the formation of melanin in mammalian tissues (*2, 4, 5*), as it is in plants and lower animal forms; and second, that sulfhydryl-containing epidermal extracts neutralize the effect of both tyrosinase and cupric ions in pigment production. Copper is known to be an essential dietary factor in the maintenance of the color of fur (*3, 10*). Moreover, our analyses have confirmed and extended earlier data (*1, 9*) which show that in most cases the black and gray hair of rabbits, guinea pigs, and rats contains significantly

[1] American Cancer Society Fellow, 1948–49.

more copper than the white hair of the same animals. We have also found that among heavy metal ions, cupric ion has by far the strongest catalytic effect on the autooxidation of dopa. Expressed in molar concentration, cupric ion is 70 times more active than manganous and 100 times more active than ferrous ion.

The above observations suggest that within resting melanoblasts sulfhydryl compounds keep balance with a copper-containing enzyme, in analogy with *in vitro* experiments where epidermal extracts and cupric ions neutralize each other's effect on the substrate dopa. Under the action of pigment-producing stimuli, such as radiation, this equilibrium is disturbed by the oxidation of –SH groups, the enzyme thus being enabled to act freely on the substrate to form pigment.

Further evidence for a balance between enzyme and inhibitor is found in the more stable conditions of genetically induced pigmentation. In mottled rabbits white skin samples containing no enzyme, as shown by negative dopa reaction, yield from 25% to 44% lower –SH values than pigmented skin samples of the same animal.

Detailed experimental data will be presented elsewhere.

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Detection of Sulfur-containing Amino Acids on Paper Chromatograms[1]

HERBERT M. WINEGARD and GERRIT TOENNIES

Institute for Cancer Research and *Lankenau Hospital Research Institute, Philadelphia*

RICHARD J. BLOCK

Department of Biochemistry and Physiology, New York Medical College, New York City

The recent description, in a paper of Chargaff, *et al.* (*1*), of a method for the detection of cystine, cysteine, and methionine on paper chromatograms has prompted us to present a method employing potassium iodoplatinate as the reagent which we have used for similar purposes. Cystathionine, lanthionine, djenkolic acid, methionine sulfone and sulfoxide, cystine disulfoxide, and cysteine sulfinic acid, in addition to the first-mentioned amino acids, react with this compound. While, to our knowledge, it has not been used for chromatographic purposes, Sease and co-workers (*4*) have recently given a partial review of the literature on the reaction between organic sulfides and iodoplatinate and have described a quantitative procedure for analyzing organic sulfides and disulfides with iodoplatinate. Bleaching of the reagent by urine, albumin, tannic acid, gallic acid, pyrogallic acid, KCN, KCNS, and saliva, but not by urea, uric acid, starch, dextrin, sucrose, glycerol, gelatin, oxalic acid, tartaric acid, citric acid, carbon disulfide, and alcohol, was apparently first described in 1881 (*3*).

The reagents used are 0.066 *M* KI and 0.0033 *M* H_2PtCl_6. Mixing of equal volumes of these solutions yields a deep red solution, the color of which may be attributed to the ion $(PtI_6)^=$ (*3*). This reagent has been found stable for at least two weeks at room temperature. For spraying purposes, a 1: 6 dilution has given the best compromise between contrast and sensitivity.

TABLE 1

Amino acid	R_F	Bleaching time	Amount detected (γ)
Cystathionine	0.30	Immediate	24
Cysteine	*	2 min	12
Cysteine sulfinic acid	0.21	Immediate	12
Cysteic acid	0.10	Does not bleach	..
Cystine	0.25	2 min	12
Cystine disulfoxide	0.21	Immediate	12
Djenkolic acid	0.30	"	12
Lanthionine	0.27	"	24
Methionine	0.76	"	12
Methionine sulfone	0.65	"	18
Methionine sulfoxide	0.81	"	18

* Cysteine could not be detected on the chromatogram when phenol was used as a solvent.

In practice, the completed chromatogram is dried as usual and then washed thoroughly in a 1: 1 acetone-ether solution to remove traces of the chromatographing solvent. The paper is dried at 90° and sprayed evenly and lightly with the diluted reagent. Although all of the amino acids mentioned can be detected by bleached areas against a pink background when tested on filter paper without chromatographing, on a finished chromatogram the reagent is usually bleached throughout the entire area. However, it has been found that suspending the sheet or strip, still damp from spraying, in a covered crock or cylinder containing a layer of concentrated hydrochloric acid on the bottom causes a redevelopment of the pink color by the HCl vapors, except in the areas occupied by the sulfur amino acids, which remain colorless. Air drying of the redeveloped sheet (heat will cause charring) yields a chromatogram on which the bleached areas are stable for many days. Eventually, bleaching of the entire sheet occurs. In the case of methionine sulfoxide and cystine disulfoxide, the bleached spots were faintly yellow, and the application of a starch solution revealed the presence of iodine. This was not

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