PRELIMINARY AND SHORT REPORT

STUDIES ON DOPA REACTION

II. Effect of Chemicals on the Reaction*

Susumu Iijima, M.D. and Kazu Watanabe

Recently Iijima and Watanabe (1) described a simple method for the dopa reaction which had certain advantages over the conventional methods.

The present authors applied this method to test the effects of various chemicals on the dopa reaction of human skin. In the following, some of the results are reported.

MATERIALS AND METHOD

The technic of the dopa reaction is same as originally described. The pH of the reaction mixture was adjusted to 7.4. The difference in the intensities of the reaction between control slides and test slides after three hours' reaction time was recorded as minus (no inhibition); one plus (slight inhibition), two plus (moderate inhibition) and three plus (complete inhibition).

Materials consisted chiefly of the skin of the axillary region surgically removed for the treatment of osmidrosis.

RESULTS

The results are given in the table. Generally speaking, the dopa reaction of human epidermis is relatively insensitive to the action of chemicals. Although it was originally stated that the darkening of leukocytes did not occur, it was actually noticed in some instances.

Hydroxyphenyl compounds: It is interesting to note that hydroquinone, which in manometric experiments inhibits only the tyrosine-tyrosinase reaction and not dopa-tryosinase reaction (2), inhibits the dopa reaction of the skin. 4-Chlororesorcinol, which is the most potent inhibitor among the substances tested by Kull *et al.* (3), is not much more effective than others.

Substances which combine with copper: All SH compounds tested were found to be more or less inhibitory, the most powerful inhibitor being p-thiocresol. Sodium diethyldithiocarbamate inhibited the reaction completely at the final concentration of $\frac{1}{6000}$ M, while ethylenediamine tetraacetate cannot abolish the reaction even at the final concentration of $\frac{1}{60}$ M.

Salts of metals: Cupric sulfate and ammonium molybdate inhibited the reaction completely at the higher concentration. In the case of the former, the deposition of dopa melanin was less than the control at all concentrations tested. The inhibitory action of ferrous, lead and silver salts was less remarkable. Sodium chloride and potassium chloride have little or no effect on the reaction.

Urea derivatives: In view of the fact that guanofuracin (5-nitro-2-furfurylidene aminoguanidine hydrochloride) causes depigmentation of the eyelid and eyelash after topical application (4), the effects of several derivatives of urea on the dopa reaction were tested. Aminoguanidine and diphenylguanidine were found to be inhibitory. The effect of guanofuracin itself is somewhat complicated and varies according to the mode and the length of the time of action and will be reported separately.

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* From the Department of Dermatology, Fukushima Medical College, Fukushima, Japan. (Director: Prof. Susumu Iijima.)

TABLE I

Reagents*	Final Concentrations (molar)					
	$1/6 \times 10^{-1}$	$1/6 \times 10^{-2}$	$1/6 \times 10^{-3}$	$1/6 \times 10^{-4}$	$1/6 \times 10^{-5}$	$1/6 \times 10^{-6}$
Hydroquinone		+++	+	+	+	+
Monobenzylether of hydroquinone†		+++	−	−		
Resorcinol			+	+	+	−
4-Chlororesorcinol		++	++	−	−	−
Thiourea	++	++	+	+	−	−
Benzylthiourea		+++	++	++	−	
Diphenylthiourea†		+	+	−	−	
p-Thiocresol†	+++	+++	+++	++	+	−
2-Mercaptobenzothiazole†		+++	++	++	++	
Cysteine		+	+	+	+	
Ethylcysteine		++	+	+	+	
Benzylcysteine			+	+	+	
Dibenzoylcysteine†		+++	++	+	+	
Cysteine sulfuric ester	+	+	+	+	−	
Cysteine ethyl ester†		+++	++	+	−	
Tetramethylthiurarum disulfide†			++	++	++	
Dibenzothiazole disulfide‡			−			
Sodium cyanide	+++	+++	+	+	−	
EDTA	++	+	−	−	−	
Sodium diethyldithiocarbamate			+++	+	−	
8-Hydroxyquinoline†		++	+	+	+	
INAH	++	+	+	+	−	
2-Amino-4-methylthiazole†		+	+	+	+	
Cupric sulfate	+++	+++	+	+	+	+
Ammonium molybdate		+++	−			
Sodium tungstate		+	−	−		
Ferrous sulfate		++	−	−	−	
Lead acetate		−	−	−	−	
Lead nitrate		+	−	−		
Silver nitrate	+++	−	+	+	−	−
Sodium chloride	−	−	−	−	−	−
Potassium chloride	+	−	−	−		
Aminoguanidine			++	+	−	
Guanidine hydrochloride	−	−				
Diphenylguanidine†	++	+	−	−	−	
Phenylurea		−	−	−		
Diphenylurea†		+				
Phenylsemicarbazide†	++	+	+	+		

* Some of the reagents were supplied by Taiyo Pharmaceutical Co., Tokyo.

† Dissolved in absolute ethyl alcohol. Same amount of the solvent was added to the controls in place of distilled water.

‡ Saturated solution in absolute ethyl alcohol.

DISCUSSION

The effect of chemicals on the activity of tyrosinase preparations of plant or mammalian origin has been investigated by many workers (2–16). However, no comparable work has been made on the dopa reaction of human skin. Duijin's report (17) is concerned with the

dopa reaction of rabbits hair-matrix melanocytes which may not always coincide with that of human epidermal melanocytes.

Although the relatively small numbers of the compounds tested do not permit us to draw general conclusions concerning the relation between the chemical structure and the inhibitory action, some of the results which may be of special interest are discussed briefly below.

It has been known that hydroquinone inhibits melanin production *in vivo* (18). In *in vitro* experiments using mammalian tyrosinase preparation, Denton *et al.* (2) found that hydroquinone inhibited only the tyrosine-tyrosinase reaction and not dopa-tyrosinase reaction. In this experiment, hydroquinone and its monobenzylether were found to inhibit the dopa reaction of the skin. It may be that these compounds act directly on the enzyme system.

The insensitiveness of the dopa reaction to cyanide is remarkable. Thus the presence of sodium cyanide in the reaction-mixture at the final concentration of $1/6000$ M affects the result only slightly. However, since sodium diethyldithiocarbamate at the same concentration inhibits the reaction completely, it may be too early to assume the participation of other enzyme systems than tyrosinase in the reaction.

The ineffectiveness of sodium chloride is noteworthy, since it has been claimed that this compound inhibits melanin production *in vitro* and that the increase of melanin in Addison's disease is partly due to the release of this inhibition (19). Such a hypothesis is untenable from the results of present investigation.

Compounds with marked inhibitory action are now being tested for the treatment of melanodermas and the results will be published later.

SUMMARY

The effects of 38 chemicals on the dopa reaction of human epidermis were investigated using a simple slide glass technic.

Among the substances tested, p-thiocresol and sodium diethyl-dithiocarbamate inhibited the reaction completely at the final concentration of $1/6000$ M, and hydroquinone, monobenzylether of hydroquinone, 2-mercaptobenzothiazole, cysteine ethyl ester, sodium cyanide, copper sulfate, ammonium molybdate and silver nitrate, at the final concentration of $1/600$ M.

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