Summary. Anesthesia in the rat by means of nembutal or ether produces a hemodilution, as shown by both a decrease in the red cell numbers and in the specific gravity of the blood.

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Possible Eczematous Cross-Hypersentitivity Between Paraphenylenediamine and Azo-Dyes Certified for Use in Foods, Drugs and Cosmetics.*

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In recent reports Dobkevitch and Baer[1,2] have analyzed in a number of patients the cross-sensitization between paraphenylenediamine and certain azo-dyes used in the manufacture of nylon stockings. It was found that this cross-sensitization represented a new example of the complex of hypersensitivity to compounds of quinone structure, as described by Mayer.[3,4] The present study was undertaken to ascertain, in subjects with allergic eczematous contact-type sensitization to paraphenylenediamine, whether this cross-sensitization extends not only to those azo-dyes used in the dyeing of various goods or as therapeutics for dermatological purposes, but also to those azo-dyes which are commonly used in the United States as dyes, for foods, drugs and cosmetics.

The use of synthetic dyes in foods, drugs and cosmetics is regulated under authority of the Federal Food, Drug and Cosmetic Act of 1938 and no coal tar colors other than those listed and described in these regulations may be used in foods, drugs and cosmetics. Among the 116 dyes listed, 18 are accepted for use in foods, 10 of which are azo-dyes. These 10 dyes are "straight" colors, free from all impurities other than a specified maximal allowance (5%) for chlorides and sulfates of sodium; for certain aromatic amines, as o-toluidine, 0.05%, permitted in FD&C Orange No. 1; or xylidine, 0.1%, in FD&C Red No. 32; for certain aromatic hydrocarbons, as betanaphthol, 0.05% in the above-mentioned dyes; and permitting only traces of metals such as arsenic (0.00014%) and lead (0.001%).

All colors used in foods have been examined by the Food and Drug Administration and found to be harmless in the toxicologic sense. The present regulations[5] do not set any standards for ascertaining the eczematogenic potential of dyes to be used in foods, drugs and cosmetics, although all of the certified colors have been tested for sensitizing capacity on the skin of groups of guinea pigs (Calvery[6]).

Experimental. Twenty-five subjects with known allergic eczematous contact-type hypersensitivity to paraphenylenediamine and 21 control subjects with no hypersensitivity to this compound but most of them with hypersensitivity to other non-related substances were given patch tests with the following substances:

* With the technical assistance of Mrs. Dorothy Furman.

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1 Dobkevitch, S., and Baer, R. L., *J. Invest. Dermat.*, 1947, **8**, 419.

2 Dobkevitch, S., and Baer, R. L., *J. Invest. Dermat.*, 1947, **9**, 203.

3 Mayer, R. L., *Arch. f. Dermat. u. Syph.*, 1928, **156**, 312.

4 Mayer, R. L., *J. Invest. Dermat.*, 1948, **10**, 389.

5 Federal Security Agency, Food and Drug Administration: Coal-Tar Color Regulations, September, 1940.

6 Calvery, H. O., *Am. J. Pharm.*, 1942, **114**, No. 9.

TABLE I.
Chemical Terminology of Dyes.

No.	Designation	Composition
1	FD&C Red No. 1	Disodium salt of 1-pseudocumylazo-2-naphthol-3,6-disulfonic acid
2	" " " 2	Trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid
3	" " " 4	Disodium salt of 2-(5-sulfo-2,4-xylylazo)-1-naphthol-4-sulfonic acid
4	" " " 32	1-xylylazo-2-naphthol
5	" Yellow No. 3	1-phenylazo-2-naphthylamine
6	" " " 4	1-*o*-tolylazo-2-naphthylamine
7	" " " 5	Trisodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-*p*-sulfophenylazopyrazole
8	" " " 6	Disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid
9	" Orange No. 1	Monosodium salt of 4-*p*-sulfophenylazo-1-naphthol
10	" " " 2	1-*o*-tolylazo-2-naphthol

(a) Ten azo-dyes certified for use in foods, drugs and cosmetics according to the Federal Food, Drug and Cosmetic Act, (b) paraphenylenediamine, and (c) a sample of nylon stocking material dyed, among others, with azo-dyes.

The procedure adopted for patch testing with these substances was that described by Dobkevitch and Baer.[2] All dyes were used in 2% concentrations in white petrolatum. In many instances the reading of the tests was difficult since the dyes, particularly the red, conferred upon the skin a color sometimes indistinguishable from simple erythema.

The chemical terminology of these dyes is given in Table I.

Results. The results of the patch tests are summarized in Tables II and III.

In the group of subjects who were hypersensitive to paraphenylenediamine (Table II), 4 of the 10 azo-dyes tested elicited strongly positive reactions (2+ to 3+) in several patients: FD&C Red No. 32, FD&C Yellow No. 3, No. 4 and No. 6. Occasional reactions were produced by FD&C Red No. 2 and FD&C Orange Nos. 1 and 2.

In the group of control subjects, not sensitive to paraphenylenediamine, (Table III), 3 of the 10 azo-dyes elicited marked positive (2+) reactions: FD&C Red No. 32, FD&C Yellow No. 3 and FD&C Orange No. 2. Slightly positive reactions ((+) and 1+) were occasionally produced by the dyes FD&C Red Nos. 1 and 4 and FD&C Yellow No. 4.

The 3 dyes which produce the strongest primary reactions in the group of patients not sensitive to paraphenylenediamine are among the 4 dyes which produce the strongest reactions in the group of patients sensitive to paraphenylenediamine. Our inference is that in the concentration used, these dyes are primary irritants.

However, an analysis of the results reveals certain differences between the reactivities of the two groups of patients towards these dyes. Generally, the reactions produced in the non-paraphenylenediamine-hypersensitive control group are not only fewer in number, but they are definitely weaker, 10 1+ to 2+ and 4 2+ reactions being elicited in 210 tests. In the group of the paraphenylenediamine sensitive patients there were 13 1+ to 2+, 5 2+ and 14 stronger positive reactions in 250 tests. Dye FD&C Yellow No. 3, for instance, gave the following results: In the control group, 3 patients had 2+ reactions; all the others showed a weaker reaction or were negative (9 out of 21). In the group of patients sensitive to paraphenylenediamine, 8 patients had 2+ or stronger reactions and only 6 out of 25 were negative. Similar results were obtained with the other dyes.

Contrary to the 3 aforementioned dyes which had produced many positive reactions in both groups, FD&C Yellow No. 6 is not a primary irritant, since it produced one 1+ reaction in only one of the controls but produced 8 reactions, 4 of which were 2+ to 3+, among the patients sensitive to paraphenylenediamine.

Discussion. The tests reported in Table III on patients not sensitive to paraphenylenediamine show that the dyes FD&C Yellow No. 3, Orange No. 2, Red No. 32 and to a lesser extent, Yellow No. 3 must be considered as primary irritants in the concentra-

TABLE II.
Patch Tests in Subjects Hypersensitive to Paraphenylenediamine.

Patient	FD&C Red No. 1	FD&C Red No. 2	FD&C Red No. 4	FD&C Red No. 32	FD&C Yel. No. 3	FD&C Yel. No. 4	FD&C Yel. No. 5	FD&C Yel. No. 6	FD&C Or. No. 1	FD&C Or. No. 2	Para-phenylene-diamine	Nylon Stock-ings
F21768	0	0	0	+	+++	(+)	0	++-+++	0	+	++++	+++
F26922	0	0	0	(+)	+-++	(+)	0	+-++	0	++-+++	++++	++++
C17908	0	0	(+)	+	++-+++	+	0	++-+++	0	+-++	++++	+++
F23260	?	?	?	+-++	++-+++	?	0	++-+++	0	(+)	++++	+++
F27324	0	0	0	+-++	0	0	0	0	0	0	+-++	+++
E91914	0	0	0	+	+-++	0	0	0	0	0	++++	+++
F34347	0	+	0	+	+	0	0	+-++	(+)	(+)	+++	++
F33237	0	0	0	0	0	++	++	+	0	0	+++	++
F37037	0	0	0	+	+++	++-+++	0	+	0	+-++	++++	++
F32987	0	0	0	0	+	0	0	0	0	+	+	++
F29973	0	0	0	+	+	0	0	0	0	+-++	+-++	++
F33388	0	0	0	0	0	0	0	0	0	+	++	++
E51513	0	0	0	+++	+++	0	0	0	0	++	++-+++	++
F39596	0	0	0	+	(+)	0	0	0	+	+-++	+++	++
F35345	0	0	0	+	0	0	0	0	0	0	+++	+-++
F29970	0	0	0	+	(+)	0	0	0	0	+	++	+
F26266	0	0	0	0	++	0	0	0	0	0	+++	—
F27324	0	0	0	(+)	+	+	0	0	0	+	+-++	—
D10124	0	0	0	(+)	+	?	0	0	0	+-++	+++	—
F44406	0	0	0	+	(+)	?	?	++-+++	0	?	++-+++	0
D22965	0	0	0	?	0	(+)	0	0	0	(+)	++-+++	0
F44385	0	0	0	?	(+)	(+)	0	0	0	+	++	0
F44384	0	?	?	++-+++	++-+++	+	?	?	?	+-++	++	?
F44400	0	0	?	?	0	0	0	0	0	0	++-+++	?
F44932	0	0	0	+	++	0	0	0	0	+-++	++	0

TABLE III.
Patch Tests in Subjects *Not* Hypersensitive to Paraphenylenediamine (Control Group).

Patient	FD&C Red No. 1	FD&C Red No. 2	FD&C Red No. 4	FD&C Red No. 32	FD&C Yel. No. 3	FD&C Yel. No. 4	FD&C Yel. No. 5	FD&C Yel. No. 6	FD&C Or. No. 1	FD&C Or. No. 2	Para-phenylene-diamine	Nylon Stock-ings
F31491	0	0	0	0	0	0	0	0	0	0	0	0
F31702	0	0	0	0	0	0	0	0	0	+	0	0
F19511	(+)	0	+	+	++	(+)	0	+	0	+-++	?	0
F40196	+	0	0	0	0	0	0	0	0	0	0	0
C46823	0	0	0	0	+	(+)	0	0	0	+	0	0
F40521	(+)	0	0	(+)	0	+	0	0	0	+	0	0
F41148	0	0	0	+	+	(+)	0	0	0	+	0	0
F41871	0	0	0	+	+	?	0	0	0	+	0	0
F43607	0	0	0	0	0	0	0	0	(+)	0	0	0
A54758	0	0	0	0	?	0	0	0	0	?	0	0
F44451	0	0	(+)	+	+-++	+	0	0	0	+	0	0
F44021	0	0	0	(+)	+-++	0	0	0	0	+	0	0
F44304	0	0	0	?	0	0	0	0	0	(+)	0	0
F45131	?	0	0	+-++	0	0	0	0	0	+	(+)	0
F44868	0	0	(+)	(+)	++	0	0	0	0	?	0	0
F15719	0	0	+	+	+	0	0	0	0	+-++	0	0
F44462	0	0	0	+	+	0	0	0	0	+	0	0
F38568	0	0	0	+-++	++	+	0	0	0	++	0	0
F44014	0	0	0	?	?	0	0	0	0	+	0	0
F45131	0	0	0	+	+	0	0	0	0	+-++	0	0
F45521	0	0	0	+-++	+-++	?	0	0	0	+-++	0	0

tion chosen for the tests. One could therefore question whether positive skin reactions elicited with these dyes in the group of patients sensitive to paraphenylenediamine are expressions of an allergic cross-sensitivity to compounds of quinone structure and not merely coincidental reactivities produced by primary irritants.

Two facts, however, favor the supposition that these reactions are etiologically associated with the sensitivity to paraphenylenediamine and not merely coincidental. (1) Both the number and the strength of the skin reactions produced by these dyes are definitely greater in the group of patients sensitive to paraphenylenediamine than in the control group consisting of patients not sensitive to paraphenylenediamine, but sensitive to other allergens. These dyes elicited 14 reactions stronger than 2+ in the patients hypersensitive to paraphenylenediamine; and no reactions stronger than 2+ in the control group. (2) Although dye FD&C Yellow No. 6 was not a primary irritant in the concentration employed, it produced skin reactions in 8 of the patients sensitive to paraphenylenediamine. In 5 of these cases the strength of the reactions closely paralleled their reactions to paraphenylenediamine. We therefore believe that among the positive reactions produced by the various dyes in paraphenylenediamine sensitive patients, there are several elicited by a specific skin sensitivity to compounds of quinone structure.

The results obtained suggest that the eczematous hypersensitivity to paraphenylenediamine not only crosses over to some azo-dyes which are used for dyeing leather, fabrics and other goods, but also to certain food dyes, such as FD&C Yellow No. 6 which is commonly used for coloring beverages, FD&C Red No. 32 used in external coloring of oranges and FD&C Yellow Nos. 3 and 4, both used in oleomargarine and butter.

It is by no means surprising that certain azo-dyes employed in coloring food are capable of producing cross-reactions in patients sensitive to paraphenylenediamine. From the chemical standpoint these food colors are not fundamentally different from those azo-dyes which are used for technical or pharmaceutical purposes, their only distinctive property being their specific purpose and the fact that they are more highly purified.

For obvious reasons these azo-dyes are less conspicuous as general allergens than the related azo-compounds which are used for other purposes. The production of sensitizations and the elicitation of the reactions of hypersensitivity depend to a great extent not only on the intrinsic sensitizing power of the antigen, but also on the concentration and on the intensity with which the material comes in contact with the susceptible cells.

There are indeed quantitative differences in the amounts of dye usually absorbed by the body from foods and those coming in contact directly with the skin from dyed goods. The quantities of azo-dyes ingested with each meal and then reach the cells of the skin must be much smaller than those which reach the skin from dyed fabrics, furs or from medicinal preparations.

As is often the case under similar circumstances, the question arises whether the positive patch tests elicited by the food dyes in patients sensitive to paraphenylenediamine are of practical importance. It remains to be shown that patients presenting these positive reactions will react with clinical symptoms to the ingestion of foods colored with the incriminated dyes.

We have had one patient, sensitive to paraphenylenediamine, eat within 48 hours, one pound of oleomargarine dyed with dye FD&C Yellow No. 3 but the ingestion was not followed by any clinical manifestations of hypersensitivity. However, further trials of this type and particularly chronic and repeated exposures by ingestion are necessary; only then will it be known whether the amount of azo-dye ingested with food is sufficient to elicit reactions in individuals sensitized to compounds of quinone structure or will cause sensitizations in normal individuals. This problem is worthy of serious consideration since a very large portion of the population of the United States is continually exposed to many of the certified azo-dyes by contact, ingestion and probably also inhalation and

the possibility of their reacting to these substances may constitute a serious problem.

Certain other problems are intimately related to this question. It is known, for example, that a number of cases of dermatitis from paraphenylenediamine and related compounds used in hair and fur dyes, photo developers, leather dyes, nylon stockings, etc. occasionally show flare-ups which cannot be explained on the basis of a new exposure to the causative agent. Our results suggest the possibility that the ingestion of certified azo-dyes, which have been shown to produce reactions in patients hypersensitive to paraphenylenediamine, may be responsible for these recurrences. Furthermore, it may be possible that certain eczematous eruptions now attributed to various foods themselves are actually due to the azo-dyes contained in these foods. A study of the influence of continuous exposure to these dyes on the causation and persistence of non-eczematous and possibly non-cutaneous allergies as, for example, asthma due to paraphenylenediamine and related compounds, would prove of great interest.

In evaluating the results shown in Table II it seems at first glance inexplicable that not all aminated azo-dyes used in foods, cosmetics and drugs produce irritations. While certain of the food dyes listed in Table II are seemingly harmless, even for patients highly sensitive to paraphenylenediamine, other dyes of closely related constitution elicit strong skin reactions in a high percentage of cases. The data gathered to date in various studies indicate that the presence or absence of a response to a given azo-dye in a case of cross-sensitization to compounds of quinone structure depends essentially upon the chemical structure of the dyes. Indeed, the capacity of an azo-dye to produce skin reactions in patients sensitive to paraphenylenediamine seems to be dependent in most cases upon the ease of its transformation into compounds of quinone structure and upon the ability of the quinone compound thus formed to couple with certain body constituents. We believe that only those azo-dyes capable of undergoing these changes have sensitizing power and elicit reactions in an appreciable number of cases.[4]

Conclusions. Skin tests with certain certified food dyes in patients sensitive to paraphenylenediamine suggest that eczematous hypersensitivity to paraphenylenediamine may not only cross over to azo-dyes used for the dyeing of leather and fabrics, or in ointments, but also to certain azo-dyes certified by the Food, Drug and Cosmetic Act of 1938 for use in foods, drugs and cosmetics. The implications of these findings are discussed.

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Effect of Pneumonectomy and of Lung Extract on Experimental Renal Hypertension.*

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Katz and Steinitz[1] have demonstrated in dogs that pulmonary arterial pressure is not altered in experimental renal hypertension. This may be explained by the inability of the pulmonary arterioles to respond adequately to the renal pressor mechanism, inasmuch as Brenner[2] found that the walls of these vessels average 5.7% of the external

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[1] Katz, L. M., and Steinitz, F. S., *Am. J. Physiol.*, 1940, **128**, 433.

[2] Brenner, O., *Arch. Int. Med.*, 1935, **56**, 211, 457, 724, 976, 1189.