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An Improved Method for Testing the Safety of Hair Dye Preparations

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Synopsis—The Draize procedure for subacute dermal studies in rabbits was compared with a modified procedure in which certain of the critical parameters were modified for the evaluation of oxidation hair dyes. These included limitation of the duration of contact to one hour, clipping the hair to ⅛ to ¼ in. instead of complete depilation, and thorough washing of the application sites after the one-hour contact. The results revealed that the modified method was equally sensitive with respect to the toxicological parameters examined. A further modification was employed in which the sites of application were excised and analyzed for hemoglobin content as an index of erythema. Addition of this procedure made it possible to detect effects within one hour of application. Significant differences were demonstrated between the irritation potential of 3 and 12% hydrogen peroxide.

Introduction

Irritation is manifested by a tissue system in response to stimuli of either exogenous or endogenous origins. The characteristic reactions include rubescence (erythema), edema, inflammation, and possible impairment of the integrity of the associated vasculature, with necrosis and tissue degradation if the stimulus is sufficiently intense or prolonged.

In this report, two dermal test procedures employing rabbits have been compared for their utility in the evaluation of systemic safety and irritation potential of oxidation hair dyes. One of these methods, originally described by Draize (1), has been widely applied to the testing of cosmetics, topical pharmaceuticals, and industrial or agricultural chemi-

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cals. A modified test procedure was used which more closely approximates use conditions. Oxidation hair dye formulations characteristically contain ammoniated bases which for use are mixed with an appropriate quantity and concentration of hydrogen peroxide. These dyes are used under specified conditions which include limited contact with the hair, followed by thorough shampooing and immediate rinsing. These conditions are employed both in the home and in the beauty salon.

In the Draize test prolonged (six-hour) contact is maintained with the test material by means of either a rubber dam or plastic sleeve wrapped around the trunk of the rabbits. The condition of the skin is

TABLE I
Evaluation of Skin Reactions

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm.)	3
Severe edema (raised more than 1 mm. and extending beyond area of exposure)	4
Total possible edema score	4

scored daily in accordance with Draize's grading system (Table I) for evaluating skin reaction, whereby emphasis is placed on the degree of edema, erythema, and eschar formation.

Under these test conditions, in which daily (5 days per week) applications to the abraded skin are made for 6 hours for 21 days, or to the intact skin for 90 days, even the most innocuous materials elicit adverse changes in the skin of rabbits. As a result of this treatment regimen the skin becomes dry and scaly, cracks, and thickens, with subsequent sloughing. Relatively large body areas become denuded, but regrowth of hair is generally observed in these areas. Since the test procedure precludes washing of the application area, it is intrinsically responsible for many of the physical changes observed. The effects due to any active components in the formulation are superimposed on these background reactions.

As a consequence, serious doubts have arisen concerning the validity of such exaggerated exposure conditions. The problem is compounded in the case of formulations which contain active ingredients where the dosages recommended are based on multiples of the human dosage. These are often scaled to 1, 3, and 10 times the human dose on a mg. per kg. body weight basis. The higher dosages often require volumes of test material considerably greater than can be applied to the trunk of the rabbit in single applications. When the total dose is placed under the plastic sleeve, the quantity of test material in direct contact with the skin is considerably less than if the same total quantity were applied as a thin layer covering the entire trunk. To avoid this problem of dosage, many workers administer the total dosage as a series of divided doses. These volumes are without doubt unrealistic in relation to the total body surface of the animal.

Recently, the Food and Drug Administration reviewed a protocol prepared by a group representing manufacturers of hair dyes. This group submitted a realistic procedure for evaluating oxidation hair dyes which bore a closer relation to the conditions of use. The results of several studies employing the original and modified procedures are covered by this report.

Characterization of the irritation response is one of the single most important criteria in these studies. Up to this time, only the Draize scoring procedure and subsequent histomorphological evaluation of tissue pathology have been applicable. The Draize system is an attempt to reduce subjective evaluations to numerical terms. Microscopic examination, a critical aspect of the total assessment, has serious limitations in terms of differentiating subtle differences in response.

A new procedure was employed which quantitates the inflammatory changes associated with irritation in terms of the fluids and cellular elements present at the application sites. It reflects the increased numbers of erythrocytes present due to the increased leakage through the capillaries and possible impaired integrity of these vessels. Frank hemorrhage is not required for definition. This procedure has revealed differences in the tissue fluids where no discrete visual evidence of erythrocytes had been noted.

Methods

These modified procedures were designed to approximate normal usage patterns. Adult albino rabbits weighing between 2 and 3 kg. were distributed into groups of 10 animals equally divided as to sex.

On the day prior to the initiation of applications, the dorsal hair was clipped to a length of ⅛ to ¼ in. with an electric clipper, care being taken to avoid nicking or abrading the skin. One group served as the control, receiving all preliminary preparations without treatment. The *p*-phenylenediamine-resorcinol (P.P.D.-R.) type dye-peroxide mixture was prepared in accordance with label directions, all dilutions being discarded after a single use. Two graded concentrations of the mixture were applied within 20 minutes of preparation. The contact

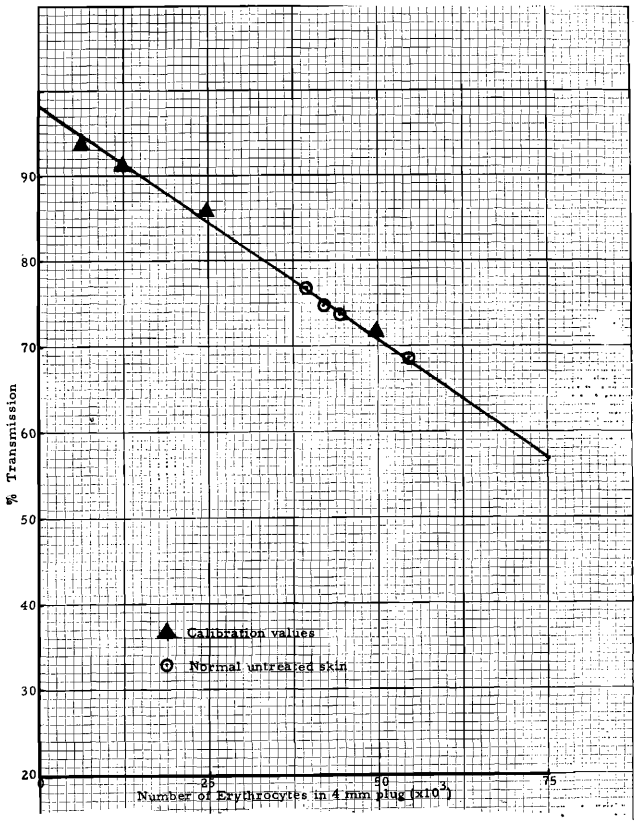


Figure 1. Standard values of rabbit blood cells-calibration curve for alkaline digestion procedure

time was fixed at 60 minutes. At the end of this exposure period, the dye mixture was rinsed off with lukewarm water. Four milliliters of a commercial shampoo intended for use after dye application was spread over the dyed area, worked into a rich lather, and then rinsed with lukewarm water until all traces of lather were gone. The rabbit was then

TABLE II
Primary Irritation Scores—Prolonged (6-hour) Daily Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose	Rabbit No.	Days 0	7	14	21
		Scores			
0	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
1x	1	0	2	2	2
	2	0	2	2	2
	3	0	2	2	2
3x	1	0	2	2	2
	2	0	2	2	2
	3	0	2	2	2
10x	1	0	Died		
	2	0	2	2	2
	3	0	2	2	2

thoroughly towel-dried, and a warm air stream, from a commercial 300-watt heater equipped with a fan, was directed over the rabbits for approximately five minutes. This procedure was repeated after each daily application of the test material. The effectiveness of this two-stage drying was demonstrated by the significantly reduced incidence of upper respiratory disease during these long test periods. One-half the animals were sacrificed 24 hours, and the remainder 14 days, after the last application; no further treatment was given during these terminal periods.

Prior to starting treatment, a base line hemogram including hemoglobin concentration, hematocrit, platelet, and total and differential leukocyte counts was obtained on each animal. In addition, semi-quantitative determinations were made for specific gravity, pH, glucose, protein, and microscopic examination of the sediment of urine. These were repeated prior to termination after the last application. All animals that died and half the survivors, which were sacrificed after 20 applications, were autopsied. Nine tissues, lung, heart, liver, kidneys, spleen, pancreas, thyroid, gonads, and marrow were examined microscopically. The procedure was repeated on the survivors 14 days later, and target organs were examined.

Data obtained by this procedure were compared to a series in which the typical Draize procedure was followed. In this series, a commercially available oxidation hair dye similar to that used in the modified procedure was used. Groups of 10 rabbits (evenly divided as to

TABLE III

Primary Irritation Scores 1-Hour Contact with Graded Concentration H_2O_2

Rabbit No.	Application No.	H_2O_2, % 3	6	9	12
Males					
1	1	0	1	1	1
	2	0	0	0	2
	3	0	0	0	1
	4	1	1	2	1
	5	1	1	2	1
	6	H	H	H	H
2	1	1	1	1	1
	2	0	0	0	0
	3	0	0	0	0
	4	1	1	1	1
	5	H	H	H	H
	6	H	H	H	H
3	1	1	1	1	2
	2	0	1	1	1
	3	0	2	1	2
	4	2	2	2	2
	5	1	1	1	2
	6	1	1	1	1
4	1	1	2	2	2
	2	0	1	2	1
	3	1	1	1	1
	4	1	1	1	2
	5	H	H	H	H
	6	H	H	H	H
5	1	1	0	1	2
	2	0	1	1	2
	3	1	2	2	2
	4	1	1	2	2
	5	H	H	H	H
	6	H	H	H	H
6	1	1	0	2	2
	2	0	1	0	2
	3	1	2	2	2
	4	2	2	2	2
	5	1	1	1	2
	6	H	H	H	H
7	1	0	0	0	1
	2	1	1	1	1
	3	1	1	2	2
	4	1	1	1	1
	5	1	1	1	1
	6	H	H	H	H
8	1	1	1	2	1
	2	1	1	1	2
	3	0	2	1	2
	4	2	2	2	2
	5	1	1	1	1
	6	H	H	H	H

H = Indicates that hair regrowth was intense. No reading was made.

sex) weighing 2 to 3 kg. were prepared 24 hours prior to start of application. The entire dorsal surface was closely clipped by means of an electric clipper, removing as much hair as possible while avoiding nicking or abrading of the skin. The P.P.D.-R. dye-peroxide mixtures were prepared in accordance with the label instructions and applied five days a week for six hours to the body surface under plastic sleeves, following which the sleeves were removed. The excess was wiped off without shampooing. Skin scores were read daily. The physiological status of the rabbits was determined prior to and after the last applica-

TABLE IV
Body Weights 1-Hour Contact with P.P.D.-R. Dye + H_2O_2

Dose Factor	No. of Rabbits	Days 0	Days 21	Net Gain
0	5M	2.3–2.6	2.4–3.0	0–1.1
	5F	2.5–3.7	2.7–3.9	0–0.9
1x	5M	2.4–2.7	2.8–3.0	0.3–0.6
	5F	2.4–2.9	2.7–3.3	0.5–0.7
2x	5M	2.2–2.8	2.5–3.0	0.3–1.1
	5F	2.3–2.9	2.7–3.5	0.3–0.7

TABLE V
Body Weights—Prolonged Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose[a]	Days 0	Days 21	Net Gain
0	1.9–2.1	2.3–2.6	0.2–0.7
1x	1.7–2.3	1.8–2.5	−0.5–0.6
3x	2.4–2.6	2.4–2.8	−0.2–0.4
10x	1.8–2.6	2.0–2.7[b]	0.1–0.2

[a] Three rabbits per treatment group (males).
[b] One animal died in this group.

tion. Blood and urine examinations were made as above described. The animals were examined daily, and unusual signs were recorded. They were sacrificed by over-barbitalization at the end of the 21st day after 15 test exposures or at 35 days, examined grossly at necropsy, and tissues and organs examined microscopically with particular emphasis being placed on the skin and adnexal areas.

From the observations made upon the differential pattern of erythema and injection of the vascular network of the dermal surface, it was ascertained that the gross scoring of the epidermal surface failed to reflect apparent differences between products or graded concentra-

TABLE VI

Hematological Findings (Rabbits) 1-Hour Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose	Sex	Hemoglobin, g./100 ml. 0	15	Hematocrit, % 0	15	Platelets, × 10^3 0	15	Leukocytes, × 10^3/mm.^{3} 0	15	Differential Count, %[a] P 0	15	L 0	15	E 0	15	M 0	15	B 0	15
0	M	8.6–13.3	10.9–12.2	28–44	30–40	111–170	111–210	3.9–8.3	6.1–9.1	29–30	70–85	67–70	15–30	0	0	0–3	0	0–2	0
	F	10.6–12.2	11.2-12.2	33–38	35–41	90–180	190–240	4.6–9.1	5.9–8.2	24–28	69–82	72–75	18–31	0–1	0	0–1	0	0	0
1x	M	10.1–12.7	10.6–12.9	32–45	36–41	100–170	140–240	4.5–8.3	4.5–8.7	13–23	71–82	67–87	18–29	0	0	1–6	0	0–1	0
	F	9.6–11.2	10.9–13.2	33–40	35–43	111–161	100–216	5.9–9.6	5.0–10.9	20–30	77–82	70–80	18–23	0	0	0–2	0	0–2	0
2x	M	11.6–12.6	10.9–12.9	37–40	36–41	140–220	111–200	3.8–12.8	5.3–8.2	17–40	65–80	55–83	20–35	0	0	0–6	0	0–1	0–1
	F	9.9–11.7	10.3–12.2	30–38	35–39	117–161	120–210	6.2–9.7	4.2–6.9	16–22	78–83	76–83	17–22	0	0	0–2	0	0–2	0–1

[a] P = polymorphonuclear neutrophils; L = lymphocytes; E = eosinophils; M = monocytes; B = basophils.

TABLE VII

Hematological Findings—Prolonged Contact with P.P.D.-R. Dye + H_2O_2

Multiple of Human Dose[a]	Hemoglobin, g./100 ml. 0	15	Hematocrit, % 0	15	Erythrocyte, × 10^6/mm.^{3} 0	15	Leukocyte, × 10^3/mm.^{3} 0	15	Differential Count, %[b] P 0	15	L 0	15	E 0	15	M 0	15	B 0	15
0	12.4–14.0	10.7–14.0	39–44	33–42	5.4–6.2	4.6–6.5	5.8–8.9	6.9–8.8	17–44	32–45	55–82	55–65	0–1	0	0	0	0	0
1x	12.1–12.7	10.3–13.1	39–47	33–43	4.6–5.7	4.8–5.8	6.3–9.3	6.4–11.7	15–52	13–61	48–85	39–86	0	0	0	0	0–4	0–1
3x	11.9–14.3	12.0–13.1	40–43	40–43	5.1–5.7	5.7–6.0	7.6–8.4	6.3–12.3	10–33	19–47	65–90	49–63	0	0	0–2	0	0–1	1–4
10x[c]	11.9–14.8	13.1–13.6	38–43	39–43	4.8–4.9	5.4–5.5	7.9–9.3	6.1–7.9	17–43	50–53	57–82	47–52	0	0	0	0	0–1	0–1

[a] Three rabbits per treatment group (males).

[b] P = polymorphonuclear neutrophils; L = lymphocytes; E = eosinophils; M = monocytes; B = basophils.

[c] One animal died in this group.

tions of a product. To differentiate these effects, a more definitive modification of the Ascheim (2) method was used. At autopsy, the skin was immediately stripped, and plugs 4 mm. in diameter were taken, using a carbide steel punch or a uterine biopsy forceps. This punch biopsy material was then placed in 1N NaOH for digestion overnight and examined for alkaline hematin content the next morning. The results could be expressed in terms of known erythrocyte counts (Fig. 1) or compared against standard curves for hemoglobin and expressed as mg. per 100 g. of skin. The latter alternative was preferred.

TABLE VIII
Incidence of Histopathological Findings[a] in Rabbits, 1-Hour Contact with P.P.D.-R. Dye + H_2O_2

	None		P.P.D.-R. Dye + H_2O_2			
	Dose, ml./kg./day					
	0		2		4	
	5M	5F	5M	5F	5M	5F
Organ and Findings	Incidence					
Lungs						
Interstitial inflammation	2					2
Focal chronic inflammation	2	2				2
Focal acute congestion					1	
Focal thickening of pleura					1	
Kidneys						
Focal chronic inflammation						1
Testes						
Occasional abnormal germinal epithelial cell	1					
Brain						
Perivascular cuffing		1				
Skin—Treated						
Chronic inflammation in dermis, focal	1			1	1	
Epidermal thickening	1			1	1	1
Hyperkeratosis					1	1
Epidermal inflammation						1
Acute inflammation in dermis	1		1	1		
Abscess in keratotic layer	1		1	2	1	
Dermal fibrosis				1	1	2
Edema in dermis				1		
Atrophy of adnexal structures in dermis				1		
Acute and chronic ulceration in dermis						2

[a] Only positive findings are shown.

Four graded concentrations of aqueous hydrogen peroxide solutions of 3, 6, 9, and 12% (equivalent to 10, 20, 30, and 40 volumes per cent) were applied to groups of 10 rabbits each. These materials were applied

TABLE IX

Incidence and Severity of Histopathological Findings[a] in Rabbits[b] Prolonged (6-hour) Daily Contact with P.P.D.-R. Dye + H_2O_2

	P.P.D.-R. Dye + H_2O_2 Dose, ml./kg./day			
	0	1	3	10
Organ and Findings	Incidence			
Liver				
Focal acute necrosis				1
Focal lymphocytic accumulation	1			
Kidneys				
Focal chronic pyelonephritis				1
Chronic interstitial nephritis			1	
Marrow				
Congestion				1
Skin—Untreated		2^{2+}, 1^{3+}	1^{2+}	2^{2+}
Epidermal thickening		1^{2+}		
Skin—Treated				
Chronic inflammation in dermis	2^{1+}	1^{2+}, 2^{4+}	2^{2+}	
Epidermal thickening		1^{3+}, 1^{5+}	3^{4+}	
Hyperkeratosis	1^{1+}	2^{2+}, 1^{5+}	3^{3+}	
Acute inflammatory dermis			1^{3+}, 1^{4+}	
Abscess in keratis layer		1^{3+}, 1^{4+}	1^{3+}	
Dermal fibrosis		2^{4+}, 1^{5+}	2^{2+}	2^{5+}
Telangiectasia in dermis		1^{2+}, 1^{3+}, 1^{4+}		
Abscess in hair follicle		1^{4+}		
Atrophy of adnexal structures in dermis		1^{5+}	3^{5+}	
Foreign body material in dermis				1
Diffuse necrosis in epidermis				2
Early ulceration				1

[a] Only positive findings are shown.

[b] Groups of 10 rabbits received each treatment.

once weekly for six weeks using a one-hour contact period. The skin was stripped at autopsy, a sample prepared for microscopic examination and another prepared for hemoglobin analysis.

In another series, groups of rabbits were placed on test to compare the results after 48-hours or two applications, each of one hour's duration. A third comparison was based on a single acute continuous 24-hour application of four commercial oxidation dyes purchased on the open market and of a vehicle common to each of the bases. These tests were terminated immediately after the period of application, and the sites were treated in the manner described.

Results

Primary irritation scores are shown in Table II for the daily six-hour contact. The maximum scores were 2 and indicative of slight ery-

thema. In the Draize system, effects scored as 2 or less are considered only mildly irritating, products eliciting scores of 2 to 5 are moderate irritants, and those with scores above 6 are considered to be severe irritants. There was no evidence of irritation in this modified procedure.

Primary irritation scores for the graded peroxide concentrations are shown in Table III. For this study, samples were applied on each animal in a randomized fashion so that in every instance each concentration used was placed adjacent to a different concentration.

Comparison of the data in Tables II and III indicates that the intensity of response to oxidative irritants of equal peroxide concentration is not a function of contact time. This may be due to the rapid decomposition of the hydrogen peroxide in contact with organic matter and air.

TABLE X
Incidence—Peroxide Studies, 1-Hour Contact Histopathological Findings in Skin[a]

Findings		Per cent H_2O_2			
		3	6	9	12
Chronic inflammation in dermis	1+	4	2	3	3
	2+		1		
	3+	1			
Epidermis thickening	1+	1			1
Epidermis inflammation	1+	1			
Hyperkeratosis	1+		1		1
Epidermis abscess	1+			1	
Dermal abscess	1+	1			
Dermal fibrosis	1+	3			
	2+		1		

[a] Two groups of 6 rabbits, each treated with each concentration.

Examination of a variety of physiological factors failed to demonstrate any evidence of systemic toxicity, following subacute dermal applications. The data shown in Tables IV and V indicate no adverse responses in body weights. No deleterious changes were seen (Tables VI and VII) in hemoglobin concentration, hematocrit, or total erythrocyte counts with either treatment groups. The variance in poly: lymphocyte ratio seen at all levels in the one-hour group (Table VI) indicates a complete reversal. This is commonly noted in rabbits and occurs spontaneously in nontreatment groups. Literature values for the ratio of these two leukocytes confirm the spontaneity of this shift. Critical examination of the differential slides revealed no change in the

incidence of juvenile or adult forms, indicating no treatment-related shift either to the left or right.

The histopathological assessments of the vital organs and of the skin of rabbits in which the two test methods were employed are shown in Tables VIII to X. The increase in number of skin lesions characterized by dermal fibrosis and ulceration in the 4 ml. per kg. group underscores the usefulness of the one-hour contact procedure for safety evaluation of this type of formulation. In general, these findings were observed in one or two rabbits in each group. Therefore, there were no differences between the treated and control groups or between the two treatment levels. The lack of significant findings in the viscera in both control and treated groups is indicative of the generally innocuous nature of the daily shampooing in this species.

TABLE XI
Hemoglobin Concentration in Skin Peroxide Studies, 1-Hour Contact

H_2O_2 Concentration, %,				
Untreated	3	6	9	12
		mg./100 g.		
		Males		
60	230	550	520	330
20	180	230	640	840
10	270	20	220	350
60	230	210	20	330
30	270	270	250	710
10	240	240	470	290
20	230	290	350	290
60	460	340	310	360
		Females		
50	370	230	180	250
20	510	580	350	430
90	270	320	220	170
10	230	230	250	340
20	220	300	230	170
10	240	210	380	270
<10	200	200	200	270
20	220	500	320	400

The observations (Table IX) in both the abraded and intact skin treated for six hours each day appeared to have no adverse significance. Telangiectasia, atrophy of adnexal structures in the dermis, epidermal thickening, focal or diffuse chronic inflammation of dermis, hyperkeratosis, and occasional ulceration have been frequently seen in control

groups in both 21- and 90-day tests. The abrading process intensifies these changes. In these studies, epidermal thickening, inflammatory responses, and even hyperkeratosis were often noted near or adjacent to treatment zones. These changes which often appear as a consequence of the effects induced at the site of application may be seen 1 to 3 cm. away from a treated or abraded area.

The blood hemoglobin levels in the 3, 6, and 9% H_2O_2 groups did not differ from each other. The results of the hemoglobin determinations in the skin biopsies (Table XI) did reveal a significant difference ($p = 0.05$) between the groups receiving 3 and 12% H_2O_2. This is in contrast to the histopathological findings (Table X), where the incidence of these findings failed to indicate any differences related to the concentration of peroxide.

TABLE XII
Comparison of Hemoglobin Values in Rabbit Skin at 48 Hours

	P.P.D.-R. Dye + H_2O_2, Depilated (no wash)	Depilated and Abraded (no wash)	Clipped ¼–⅛ in. (wash at 1 hour)
		mg./100 g.	
	1630	2830	1330
	2170	3550	860
	2350	3380	950
Means	2050	3250	1050

TABLE XIII
Erythrocyte Counts ($\times 10^3$) Estimated from Alkaline Hematin Analysis of Skin Plugs (Rabbit)

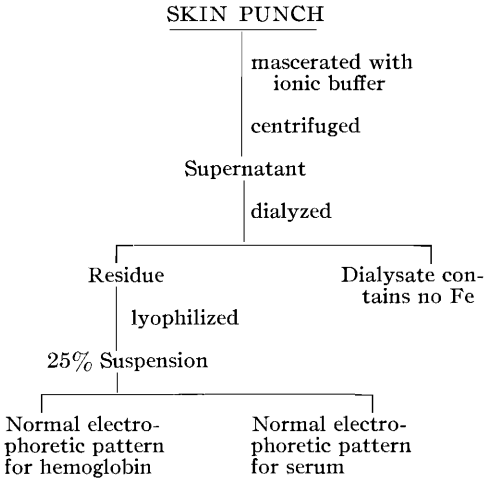
Dye sample →	1[a]	2	3	4	5
pH →	9.0	9.7	9.5	8.8	9.3
	67.5	95.0	83.0	86.5	85.0
	48.5	90.0	74.0	77.5	98.7
	40.5	83.0	85.0	81.3	91.3
	44.0	73.0		73.0	83.0
Means	51.0	85.2	81.0	79.6	89.6
Saline, pH	8.0		9.0		7.0
	67.5		62.5		56.3
	41.0		48.0		42.5
	33.7		46.3		42.5
Means	47.4		52.1		45.3

[a] Product 1 = vehicle for products 2, 3, 4, and 5.

To ascertain effects at 48 hours, in rabbits treated by both the prolonged and the one-hour contact procedures, animals were sacrificed, the application sites examined, and skin plugs cut and prepared for hemoglobin analysis. The results are shown in Table XII. Marked differences in the values were observed between the untreated and treated skin. In each case, the treatment elicited increased quantities of hemoglobin-bearing red cells at the sites of application. The quantitative differences suggest that those animals treated by prolonged contact showed the more marked responses.

Data from the comparison of commercial oxidation dye preparations are shown in Table XIII. Product "1" was the vehicle for the various dyes. Products 2, 3, 4, and 5 were dye mixtures to be evaluated. The vehicle was included in order to assess the effects of the mechanics of the treatment. All, including product "1," were prepared with appropriate peroxide solution and applied for a one-hour period. The animals were sacrificed immediately thereafter and the skin subjected to the alkaline hematin test. For each product, replicate applications were made to a series of sites. Untreated controls and pH-adjusted saline were included. Data for these analyses are shown as red cell counts, read from a standard curve (Fig. 1). The values for products 2, 3, 4, and 5 are significantly higher than those for product "1," the vehicle, the pH-controlled saline, or the untreated skin (Table XI). These data generally correlated with the edema and erythema seen on exposure of the dermal surface of the skin.

To ascertain whether these alkaline hematin determinations are



indicative of the presence of blood *per se* or some foreign protein associated with injury, electrophoretic separations were made. The flow diagram for the procedure is shown in the scheme on page 760. No evidence of any unnatural or foreign protein was observed. The presence of hemoglobin protein was demonstrated by direct comparisons with an external standard.

Discussion and Summary

The modified procedure utilizing the one-hour application resulted in a marked diminution or absence of the characteristic cracking, thickening, sloughing, and necrosis so often seen with the original Draize procedure and which do not contribute to the safety evaluation. This was in large part due to the shampooing and drying of the rabbits after each daily exposure, thus avoiding drying of the residual test material. No significant differences were observed between the two methods being evaluated as toxicological tools. Not withstanding the similarity of findings with those of the Draize method, the one-hour procedure has distinct advantages with respect to utility and extrapolation to humans. Though both procedures yield comparable results, the one-hour contact more closely resembles use conditions with loss of sensitivity as a test method. Both procedures, though valuable in ascertaining irritation potential, failed to yield the means whereby more subtle differences between test preparations can be ascertained.

A revised method whereby the hemoglobin content of 4 mm. skin plugs taken from the application sites was determined by an alkaline hematin method permitted differentiation between graded concentrations of a hydrogen peroxide solution. Irritation and inflammation resulting from this contact is associated with the extravasation, through the capillaries, of red cells and their collection at these inflammatory sites. Electrophoretic identification of this material as hemoglobin was made. The value of the modified method as a means of differentiating potentially irritating materials is indicated by the comparison of commercial oxidation hair dyes and their common vehicle.

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References

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