ministration to account for the latter's pharmacologic effects. The amount of free gentisic acid present in the body cannot be changed by alkalinization or acidification and therefore the effects noted are of pharmacologic but not clinical interest.

Conclusions. 1. The recovery of gentisic acid and salicylate were similar, approximating 62 and 68% respectively of the administered drugs, and 84 and 88% respectively, following alkalinization. 2. Alkalinization increased the combined and free forms of gentisic acid eliminated but did not alter their relative proportions. 3. The amount of gentisic acid recovered from salicylate administration was increased with alkalinization without alteration in the proportion of free to combined gentisic acid obtained with salicylate alone. 4. Whereas the free gentisic acid represented approximately 42% of the total gentisic acid recovered when administered alone, the amount of free gentisic acid obtained from salicylate administration represented approximately 10% of the total gentisic acid. 5. Acidification with dilute hydrochloric acid, but not ammonium chloride decreased the total excretion of gentisic acid at the expense of the combined, the free remaining unaltered. 6. Acidification with both dilute hydrochloric acid and ammonium chloride decreased the total excretion of salicylate and the combined gentisic acid metabolite, the proportion of free to combined gentisic acid remaining unaltered.

1. Neuberg, C., *Berl. Klin. Wschr.*, 1911, v48, 798.
2. Meyer, K., and Ragan, C., *Science*, 1948, v108, 281.
3. Batterman, R. C., Kryle, L. S., and Dann, S., *N. Y. State J. Med.*, 1951, v51, 1152.
4. Lester, D., Lolli, G., and Greenberg, L., *J. Pharmacol.*, 1946, v87, 329.
5. Lutwak-Mann, C., *Biochem. J.*, 1943, v37, 246.
6. Kapp, E., and Coburn, A., *J. Biol. Chem.*, 1942, v45, 549.

Received December 29, 1952. P.S.E.B.M., 1953, v82.

Histochemical Studies of Thermal Injury on Rat Skin.* (20122)

O. A. Ross† AND C. J. WALKER. (Introduced by A. R. Moritz.)
From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland.

During the course of an histochemical study of thermally injured rat skin, a unique differential staining property of heat-altered collagen was observed.

Methods. The abdomen of young adult male rats was exposed to various temperatures between 50°C and 80°C for 1-2 minutes in a thermostatically controlled water bath. The area exposed was limited by a shielding device. Immediately following the thermal exposure, skin blocks were excised which included both the exposed and shielded areas. These were immediately fixed in Carnoy's fixative to prevent solution of water soluble substances present as normal constituents of skin or as a result of the heat treatment.

Results. Paraffin sections (4 μ) were stained for 24 hours at room temperature by an aqueous solution of aniline blue (C.I. No. 707) and a mordant, phosphomolybdic acid. Both the normal and the heat-altered collagen of the rat skin stained an intense blue. When the hyperthermic episode ranged between 50 and 60°C, the heat-altered collagen was indistinguishable from that of normal unheated skin. At higher temperatures (70°C and 80°C) the heat-altered collagen showed a variable loss in structure, due to homogenization and swelling of the individual bundles. Orange G (C.I. No. 127) added to the above

* Aided by a contract between the Office of Naval Research, Dept. of the Navy, and Western Reserve University.

This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U.S.P.H.S.

† Public Health Service Fellow, National Cancer Institute.

TABLE I. Staining of Rat Skin Collagen. Effect of solvents and dye combinations.

Exposure, °C	Stain soln.* Dyes	Stain soln.* Solvent	Treatment	Collagen stain Normal	Collagen stain Burn
50	1, 2, 3	M	0	B†	B
55	1, 2, 3	M	0	B	B
60	1, 2, 3	M	0	B	B
70	1, 2, 3	M	0	B	Y
80	1, 2, 3	M	0	B	Y
50–80	1, 2, 3	W	0	B	B
70	1, 3	M	0	0	Y
80	1, 3	M	0	0	Y
70	2, 3	M	0	B	0
80	2, 3	M	0	B	0
50–80	1, 2, 3	M	Saline phosphate	B	B
50–80	1, 2, 3	M	Trypsin	B	B (a)

* 1 = Orange G; 2 = Aniline blue; 3 = Phosphomolybdic acid; M = Methanol; W = Distilled water.

† B = Blue; Y = Yellow; (a) = Any collagen remaining, following tryptic digestion.

aqueous staining solution serves as a cytoplasmic and keratin stain.

When, however, the above stains (aniline blue, phosphomolybdic acid) were dissolved in absolute methanol and duplicate tissue sections stained for 24 hours, there was a striking difference in the staining properties of normal collagen and those of collagen which has been brought to temperatures of 70°C or above. In these circumstances, the thermally-altered collagen stained intensely with Orange G in contrast to the normal unheated collagen, which is still stained dark blue by the aniline blue. At the periphery of the heat-altered area, many of the collagen bundles appeared structurally unchanged to the extent that they were not swollen and had not lost their fibrillar appearance. In this region, where the temperature rise was not as great, collagen stained blue-green. On inspection at high magnification, this was seen to be due to partial uptake of both Orange G and aniline blue by individual collagen bundles. Sections stained with either dye plus phosphomolybdic acid in absolute methanol showed the dye uptake of normal and heat-altered collagen to be mutually exclusive, *i.e.*, normal collagen was unstained by Orange G and heat-altered collagen was unstained by aniline blue. Where tissues were prepared in aqueous fixatives and stained by the methanol staining method, differential staining of normal and heat-altered collagen did not occur. These sections stained blue throughout.

From these studies it was inferred that the hyperthermic episode had rendered some component of the dermal collagen water soluble. In addition, this component was altered in its staining reaction to aniline blue and failed to be stained by it. Rather, it now was stained by Orange G. When microscopic sections were extracted with distilled water, M/15 Sörenson Buffer plus M/50 NaCl at pH 7.7, or M/6 NaCl alone, the normal and heat-altered collagen both stained blue in the methanol dye solution given above. The heated areas, however, showed some attenuation and loss of stainable collagen. These observations further substantiated the presence of an aqueous soluble collagen derivative, the result of the thermal exposure. That the previously differentially stained heat-altered collagen bundles became aniline blue positive following various aqueous extractions suggested a surface alteration of the collagen bundles consistent with partial denaturation. As such, this reaction might be interpreted as a partition of the effect of heat on collagen.

A further partition was observed by the staining of sections which were treated with trypsin (1 mg/ml in M-15 Sörenson Buffer-M/50 NaCl at pH 7.7) for one hour at 37°C. Sections of skin previously exposed at temperatures from 60°C to 80°C showed degradation and attenuation of collagen bundles which were most marked at the highest temperature (80°C). In some cases, normal collagen structures were completely obliterated by the tryptic action. On the other hand, the staining reaction of normal unheated portions of

TABLE II. Differential Staining of Heat-Altered* Collagen by Azo Dyes.

Dye	C.I. No.	No. of chemical groups Azo	$SO_3^=$	Differential stain
Orange I	150	1	1	+
II	151	1	1	+
III	142	1	1	+
IV	143	1	1	+
G	127	1	2	+
Amaranth	184	1	3	+
Sudan III	248	2	0	—
Biebrich scarlet	280	2	2	+
Chlorazol black E	581	3	2	—

* Heat exposure 70°C or more. Specimens excised 90 sec. after heating.

collagen on the sections was deep blue and similar to that seen in the untreated controls. In both portions, all remaining collagen stained blue. These observations served to verify the specificity of the Orange G for staining heat-altered areas of collagen.

In addition, the effect of trypsin further partitions the heat-induced alterations of collagen. In sections failing to stain differentially with Orange G (*e.g.*, 60°C), there was, nevertheless, a loss of collagen substance by enzyme treatment. This effect perhaps demonstrates collagen that has been sufficiently altered by the thermal exposure to render it hydrolyzable by trypsin but still quantitatively not degraded enough to yield the Orange G aqueous soluble products formed at higher temperatures or times of exposure.

Preliminary studies of the specificity of the azo dye uptake in methanol solutions are presented in Table II. These results showed that mono-azo and di-azo dyes with mono, di, and, trisulphonated end-groups all stain heat-altered collagen differentially. The triazo, disulphonated dye, chlorazol black E, failed to stain the heat-altered collagen. In this situation, the heated area was completely unstained. Non-sulphonated azo dyes did not stain collagen at all, while the trisulphonated diazo dye, amaranth, appeared to differentially stain heat-altered collagen, even more intensely than Orange G (di-azo, disulphonated).

These preliminary studies are presented at this time because of their possible usefulness in the understanding of collagen degradation during thermal injury.

Received December 29, 1952. P.S.E.B.M., 1953, v82.

Circulatory Changes in the Hamster's Cheek Pouch Associated with Alimentary Lipemia.* (20123)

ROY L. SWANK AND CHESTER F. CULLEN.†

From the Department of Neurology and Neurosurgery, McGill University and the Montreal Neurological Institute.

Swank(1) showed that the red blood cells in humans and dogs, when observed in whole blood smears in dark field illumination, have a tendency to aggregate, be adhesive to one another, and be distorted 4 to 9 hours after a large fat meal (2 to 4 g/kg). These changes in the suspension stability of the blood were usually first detected about an hour after the chylomicron response had passed its peak, and they increased, sometimes becoming very marked, as the the visible lipemia lessened. With complete clearing of the visible lipemia 9 to 12 hours after the fat meal the suspension stability of the blood returned to normal. The altered suspension stability of the red blood cells was accompanied by a tendency of the chylomicrons to aggregate, by changes in the sedimentation rate and hematocrit(1), and by alterations in the pattern of paper chromatograms (Swank, Franklin and Quastel)(2).

In the present paper the effects of large fat meals on the intact circulation of the blood

* Supported jointly by the Multiple Sclerosis Society of Canada and the Department of National Health and Welfare, Ottawa.

† Aided by a Fellowship from the National Foundation for Infantile Paralysis.