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Oxidation-Reduction Potentials of Inflammatory Lesions of the Skin.

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The low oxidation-reduction potential of the vitamin-C molecule has suggested that the function of this substance may be concerned with the regulation of the Eh in the animal body. Fluctuations in the ascorbic-acid concentration of inflammatory lesions of the skin induced by diphtheria toxin and by heat have been reported from this laboratory.[1] Such lesions, therefore, offered a means of studying the effect of alterations in the vitamin-C concentration of tissues upon their oxidation-reduction potential. Fildes[2] reported on the oxidation-reduction potential of the subcutaneous tissue fluid, as determined by indicator dyes. His findings indicated that while the Eh determined in the normal animal was above that at which tetanus spores could germinate, the injection of such substances as 5% calcium chloride or aleuronate caused the potential to fall to levels at which growth of these microörganisms was possible.

Purdy[3] and her colleagues investigated the electrical potentials of the skin of persons and reported a correlation between the oxidation-reduction potential of the skin and the basal metabolic rate in the range of from —10 to +13% of the normal metabolism. They reported, however, that these relationships did not hold when the circulation of the area under investigation was markedly accelerated or retarded.

Methods of Investigation. In the present studies, guinea pigs with white skins were clipped over the back and injected intradermally with diphtheria toxin diluted for the intracutaneous test of susceptibility to diphtheria in persons, or with the toxin inactivated by heat. The dose of active toxin used in these experiments, 0.1 cc, contained 0.02 of a minimum lethal dose. It induced erythematous lesions in twenty-four hours, which extended and became necrotic by the end of the third day. Similar lesions were induced by holding against the skin for 60 seconds the end of a Nessler tube, 2 cm in diameter, which contained water heated to 70°C.

1 Torrance, C. C., *J. Infect. Dis.*, 1940, **67**, 53.

2 Fildes, P., *Brit. J. Exp. Path.*, 1929, **10**, 197.

3 Purdy, C., Johnson, A. F., and Sheard, C., *Science*, 1931, **73**, 46.

The Eh values of the extracellular fluid of such lesions and of uninflamed skin were estimated by injecting a series of oxidation-reduction dyes[4] in both the oxidized and reduced states. The animal was destroyed one-half hour after the injection of the dyes and the injected areas were incised and examined. If the blue color which was first observed did not increase in depth on exposure to the air or on the addition of potassium ferricyanide solution, the dye was considered completely oxidized. If the original color observed when the test area was incised increased appreciably on exposure to the oxidizing solution, the dye was estimated as reduced by half, and as completely reduced if the area which contained no blue color when first observed became blue on swabbing with potassium ferricyanide. Gradations of color between were evaluated on this basis. Occasionally, if the reduced methylene blue solutions had been exposed to light prior to injection, the leuko dye was destroyed and the color did not return when the oxidizing solution was applied. In such instances the tests were repeated.

Results. The oxidation-reduction potentials of the extracellular fluid of the skin of 11 normal guinea pigs were determined. The tests were made at different times during a period of 2 years. Three or more dyes were used on each animal. In 5 of 7 guinea pigs thionine, one of the dyes injected, was almost completely reduced (Eh +0.002 volts). In the remaining 4, cresyl blue was reduced similarly (Eh —0.01 volts). Methylene blue was never reduced more than half (Eh +0.01 volts) in the three animals in which it was used. Indigo trisulfonate (Eh —0.02 volts) was very slightly reduced in 2 animals. While there appears to be some fluctuation in the Eh of the extracellular fluid of the skin of normal guinea pigs, the range in the animals tested never fell below —0.02 volts and the available data suggest that it is usually higher.

Heated diphtheria toxin was given intracutaneously to 5 guinea pigs. One was tested with the dyes within the first hour; the remainder at 24-hour intervals for 4 days. In 4 there was almost complete reduction of methylene blue (Eh —0.05 volts). In the fifth tested at the end of 2 days, not more than 50% reduction was observed (Eh +0.01 volts). However, the readings of the reactions on all animals with indigo tetrasulfonate were in agreement and consistent with an Eh at which methylene blue would be nearly completely reduced. The Eh of the extracellular fluid of the skin of guinea pigs injected with heated diphtheria toxin which

[4] Torrance, C. C., *J. Bact.*, 1938, **35**, 339.

induces no anatomical lesions would appear to be reduced below that of the uninjected animals, at least for the first 4 days after injection.

Diphtheria toxin in doses of 0.02 M.L.D. was injected similarly into 19 guinea pigs and the Eh of the extracellular fluid of the injected areas was determined at intervals of from one to 5 hours. In only 4 was an Eh recorded which was significantly different from that of the normal animals. The extracellular fluid of the skin of 2 of the 5 examined after 2 hours showed slight reduction of indigo disulfonate (Eh —0.065 volts). In one of 3 tested after 4 hours gallocyanine was completely reduced (Eh —0.04 volts), while in the only animal in which determinations were made after a 4½-hr interval indigo disulfonate was partially reduced (Eh <—0.065 volts).

The toxin was given similarly to 17 guinea pigs and the Eh of the extracellular fluid of the skin was determined at one-day intervals for 4 days. In 2 of the animals destroyed at the end of the first period, indigo monosulfonate (Eh —0.09 volts) and in one indigo disulfonate (Eh —0.065 volts) were slightly reduced, while in 3 others methylene blue was nearly completely reduced (Eh —0.05 volts). The Eh of the extracellular fluid of the skin of 2 remaining animals varied little from that of the uninjected guinea pigs. Two days after injection indigo monosulfonate was slightly reduced (Eh —0.09 volts) and indigo disulfonate nearly 50% reduced (Eh —0.125 volts) in one animal. In 2 others the potentials of the extracellular fluid did not appear to be significantly below that of methylene blue reduced (Eh —0.05 volts). On the third day in tests on one animal, indigo tetrasulfonate was completely reduced (Eh —0.10 volts), in another 50% reduced (Eh —0.08 volts). The extracellular fluid of the skin of a third at this time was found to have a potential between methylene blue reduced and indigo disulfonate oxidized. On the fourth day slightly less negative oxidation-reduction potentials were recorded (Eh —0.08, —0.06, —0.04 volts).

Reactions similar to the diphtheria toxin lesions were induced on the backs of 6 guinea pigs by heat and the oxidation-reduction potentials determined after one, 2, and 5 hours. In all 6 animals thionine was only 50% reduced (Eh +0.08 volts).

Discussion and Summary. While a certain fluctuation was found in the oxidation-reduction potential of the extracellular fluid of the skin of normal guinea pigs, the changes recorded following the injection of diluted diphtheria toxin or the application of sufficient heat to induce a mild inflammatory reaction may be significant.

The changes in the oxidation-reduction potential of the extra-

cellular fluid of the inflammatory reactions of the skin of the guinea pigs tested did not appear to be associated with the loss of ascorbic acid, as reported in previous work.[1] Comparable losses were found in the ascorbic-acid concentration of the skin lesions induced by diphtheria toxin and by heat, but the change in the Eh of the extracellular fluid of the skin in the reaction induced by heat was in the direction of a more positive potential while that of the toxin reaction was toward a more negative potential than was observed in the normal animals.

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Nature of the Change in Resistance of Red Cells to Hemolysis.

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It is known in hemolysis experiments that a suspension of red cells varies in its resistance to hemolysis, becoming more resistant the longer it stands. Whether this change is due to an inhibitory substance which is given off, or whether the cell membrane is altered, is not known.

We therefore did experiments to determine: (1) whether the phenomenon has any temperature dependence, and (2) whether an inhibitory substance can be detected in the supernatant saline of a red cell suspension which had been standing at 39° C for about 3 hours.

In the first experiment, a standard suspension (1 cc rabbit red cells in 20 cc of 1% saline) was hemolysed by varying dilutions of saponin (1-10,000 to 1-50,000) at 16-18°C and at 39°C immediately after making the suspension and again after the suspension had stood for one hour.

In the second experiment, a standard suspension was left in a water bath at 39°C for 3 hours. It was then centrifuged, and the supernatant saline was used as the 0.8 cc of saline in the hemolysis of a fresh cell suspension.

The first experiment showed that the resistance of the cell suspension increased by varying amounts (from 0.1% to 17%) during the hour experimental period. These values might be indirectly a