CLV. THE SULPHYDRYL REACTION OF SKIN.

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IN a preliminary communication [Walker, 1924], describing a nitroprusside reaction given by skin, it was shown that this reaction is definitely located in the metabolically-active layers of the skin. At that time it was not realised that the nitroprusside reaction of skin had already been described by Buffa [1904], in a paper in which he deals with the colour reaction given by many tissues on treatment with nitroprusside and sodium hydroxide. There can be little doubt that the reaction is due to the presence of the sulphydryl grouping, and the view was advanced in the preliminary communication that the substance responsible for the reaction was closely allied to the thermostable sulphydryl constituent of muscle, but was not identical with it. The only reason for considering the two constituents as not identical was based upon a slight difference in their respective reducing powers. Since then it has become apparent that this difference is simply one of degree and hence there is now no reason to suppose that different substances are responsible for the nitroprusside reaction of skin and of water-extracted muscle. Another view, however, is taken by Kaye [1924] in a more detailed examination of the nitroprusside reaction given by skin. Kaye definitely attributes the reaction to the presence of glutathione, basing this conclusion on the statement that the substance responsible for the reaction can be washed out by water. This is a result which has never been obtained by me and, in fact, one of the main reasons for associating this skin constituent with the thermostable sulphydryl constituent of muscle was its resistance to extraction by water. It is the purpose of this paper to record the observations which have been made in the course of examining the sulphydryl reaction of skin.

General Observations.

The technique for examining skin for the sulphydryl reaction has already been described [Walker, 1924]. It is most conveniently demonstrated when the skin is in section, but a rough qualitative test may be carried out upon any piece of excised skin, the reaction showing along the cut edge. Many different types of skin have been examined; viz. mouse, rabbit, cat, sheep, pig, calf and human. The reaction is essentially the same in all cases, varying in degree somewhat with variation in the skin structure. In general, skins rich in hair give intense nitroprusside reactions. In human skin the sulphydryl layer is relatively thin; the only other skin examined which is comparable to

human skin in this respect is that of the pig. Nevertheless the sulphydryl layer of skin is to be found independently of the presence of hair, for it can be demonstrated in such hairless structures as the skin from the finger-tip, the female breast, and the eyelid. It is highly probable that a similar layer exists in all epithelial tissue and it is noteworthy that such a layer is readily demonstrable in the trachea (cat, rabbit), just as definitely located as in skin.

Stability.

Of all the skins examined calf-skin has proved the best for most experimental purposes and has been used throughout this investigation. The skin was clipped as free as possible of hair, and any subcutaneous tissue present was removed. The skin was used either fresh or after storage in alcohol. The skin-sections used varied in thickness between 0.25–0.5 mm. Thinner sections (20μ) had been tried but the nitroprusside reaction observed in them was only faint and such sections are especially susceptible to oxidation.

One of the earliest facts to become apparent was the general stability of the sulphydryl constituent of skin. In the first place, its post-mortem survival is very marked; skin examined three days after death gives a reaction not appreciably less intense than that of skin removed a few minutes after death. The skin may be stored in alcohol for several weeks without losing its sulphydryl reaction. It may be thoroughly dehydrated with alcohol and embedded in paraffin without its reaction being impaired. After this latter treatment it is, of course, necessary to dissolve away the wax and to steep the section in water before carrying out the sulphydryl test.

Resistance to extraction.

The sulphydryl constituent proved to be resistant to extraction with alcohol or ether. In my hands it has also proved equally resistant to extraction with water. Upon this latter point hinges the decision as to whether the sulphydryl reaction obtained is due to glutathione or not. It is certainly true that skin loses its sulphydryl reaction on prolonged immersion in water, but this is attributed to oxidation. It may be thought that the simplest way of testing for glutathione would be to extract a quantity of skin with water and to test the solution obtained for the nitroprusside reaction. This has been done several times without obtaining the slightest positive reaction in the fluid. The same negative result was obtained on extracting chopped skin with dilute sulphuric acid and with acidified saturated ammonium sulphate. It is significant that Kaye [1924] makes no mention of the extractant giving a nitroprusside reaction. In an endeavour to settle the point a number of sections (0.5 mm. thick) of fresh calf-skin were extracted six times successively with boiling water. The only effect upon the sulphydryl layer was to intensify slightly the nitroprusside reaction. Again, a number of similar sections were thrown into distilled water, previously boiled and cooled, covered with a layer of toluene and allowed to stand. Sections were removed and tested each day. The sulphydryl reaction was present until the fourth day. There-

after it could be shown that the oxidised compound (the —S—S—form) was still present in the skin at the end of 14 days. The test for the disulphide form is a modification of the ordinary nitroprusside reaction and is described in the preceding paper.

Oxidation.

The general stability of the sulphhydryl layer has already been discussed. In particular, it is noteworthy that the layer shows considerable resistance to atmospheric oxidation. For example, a number of moist skin sections, 0.3 mm. thick, were spread upon a strip of suspended muslin and were thus freely exposed to the air. Although the sections had shrivelled up within a few hours the sulphhydryl reaction could still be demonstrated in them (after soaking them in *M*/120 acetic acid to restore their shape) after 72 hours. In the thinner (20–30 μ) sections used by Kaye it is natural that oxidation should be more rapid. Even the thermostable sulphhydryl constituent of muscle succumbs to oxidation when the muscle powder is exposed to the air in fine layers [Dixon and Hopkins, 1922]. Further, according to Kaye [1924], the sulphhydryl layer of skin is "...stable to some degree against alkalies (lime-water and ammonia)...." This does not suggest glutathione.

One further point of interest arose in examining the effect of allyl isothiocyanate on the sulphhydryl layer of skin. Treatment of skin *in vitro* with allyl isothiocyanate, either by painting it on the surface of the skin or by shaking up sections with an aqueous solution of the oil, causes complete disappearance of the sulphhydryl reaction very rapidly. The skin so treated no longer gives a disulphide reaction, so that we are not dealing with a case of oxidation but probably of combination. Muscle thoroughly extracted with water is similarly affected by allyl isothiocyanate.

Altogether it appears more reasonable to associate the sulphhydryl layer of skin with the thermostable sulphhydryl constituent of muscle rather than with glutathione. It is not unlikely that small amounts of glutathione are present normally in the sulphhydryl layer of skin, analogous to the presence of glutathione in muscle.

SUMMARY.

No evidence has been obtained to show that the nitroprusside reaction of skin is due to the presence of glutathione. It is suggested that the reaction is due to the presence of a substance similar to (or identical with) the thermostable sulphhydryl constituent of muscle.

It has been found that skin rapidly loses its sulphhydryl reaction under the influence of allyl isothiocyanate.

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