CLXIX. OBSERVATIONS ON THE BEHAVIOUR OF A SUBSTANCE GIVING THE NITROPRUSSIDE REACTION IN SKIN AND IN HAIR.

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(Received October 20th, 1924.)

IN a preliminary communication to the Physiological Society, Walker [1924] has shown that a compound is present in skin which gives the well-known nitroprusside reaction for glutathione described by Hopkins [1921]. In view of this work it would appear of some interest to detail the results of experiments on somewhat similar lines carried out by the author during the last few months.

Walker does not consider the compound in skin giving this colour reaction to be glutathione. Other substances such as cysteine and proteins containing -SH groups also give this or a similar colour with sodium nitroprusside. Cysteine, however, has never been shown to be present in tissues to a sufficient extent to give the strong reactions observed, and proteins containing -SH groups would not be washed out with water from the cells in skin. The substance in skin which gives the reaction does wash out with water, and therefore cannot be a protein. On the other hand, Hopkins has isolated glutathione from muscle cells and observed it in practically every known tissue. The compound in skin is found in the erector pili muscles and is also associated in other parts of the skin with cells or living tissue, the reaction being particularly strong where metabolism is intense. Hopkins, in his original paper, suggested that glutathione is associated with active metabolism. Collagen and elastin fibres, the stratum corneum, hair shafts and other structures of extra-cellular origin do not give the reaction. It is significant that these structures are not in themselves living tissues but are only the products of active cells. It has, therefore, been assumed in this paper that the substance in skin which gives the nitroprusside reaction is glutathione.

The technique employed was histo-chemical, and essentially the same as that described by Walker. The sections, however, were about 20-30 μ in thickness, cut by a freezing microtome, as it was found to be much easier to localise the less intense reactions in definite regions of the skin in the thinner sections.

The skin of ox and calf and also hairs plucked from living subjects have been examined.

The parts of ox and calf skin which give the nitroprusside reaction and the intensity of this in each case are shown in Table I.

Table I.

Parts of skin	Intensity of reaction		
The epidermis (except the stratum corneum)	Intense colour (magenta)		
All cellular layers of hair follicles	Intense colour		
Roots of young hairs	"	"	"
Roots of mature hairs	"	"	"
Portion of hair just above the root	"	"	"
Walls of arteries	Pale pink colour: not intense magenta		
Walls of veins	"	"	"
Erector pili muscle	"	"	"
Cells lining sebaceous glands	"	"	"
Cells lining sweat glands	"	"	"
Groups of cells in the dermis (papillary layer)	"	"	"

It will be seen that the intense coloration is only found in certain structures. It is very fleeting, fades to pink and finally disappears. Other structures only show the pink colour.

The substance giving the reaction is stable for some time after death, and a good reaction is obtained up to three or four days after the skin has been removed from the animal.

Almost the whole of the compound is washed out of the tissues by running water within twenty-four hours, but if the skin is soaked in still water for the same length of time the tissues give a coloration almost as intense as that given by the fresh skin. After sixty-eight hours in still water, only those parts of the skin giving a strong coloration in the fresh condition show any reaction at all, and, with the exception of the portion of the hair just above the root which gives a strong coloration, these parts only show a pale pink colour with the nitroprusside reaction.

During drying, the substance is almost completely oxidised, and sections of dried skin examined after softening in water show only a slight coloration just above the hair root unless treated with a reducing agent. Zinc and dilute hydrochloric acid were used, sodium chloride being added to prevent excessive swelling of the skin in acid. After reducing, the coloration of the hair root and of the shaft just above the root is greatly intensified, and slight coloration also develops in the epidermis. It is obvious, therefore, that the substance in the oxidised form survives drying and can be detected by the nitroprusside reaction after being converted into the reduced form.

The substance is also stable to some degree against alkalis (lime water and ammonia), acid (hydrochloric acid) and sodium chloride.

Table II shows the coloration produced in skin with the nitroprusside reaction after previous treatment with the above reagents.

It can be seen from Table II that the position in the skin where the glutathione reaction can be demonstrated longest is in the portion of the hair shaft just above the hair root. This is probably due to the keratinous layer of the hair checking penetration.

Table II.

Reagent	Time	Colour reaction			
		Epidermis	Hair follicles	Hair roots	Hair shafts just above roots
Saturated lime water	14 days	0	0	0	+
Ammonia <i>N</i> /20	24 hours	+†	+†	+++*	+++*
Hydrochloric acid <i>N</i> /100	24 "	+	+	+++	+++
Sodium chloride sprinkled on skin	24 "	0	0	+	+++

* Before and after reduction.

† After reduction.

(+) = Very faint or doubtful reaction.

+ = Weak reaction.

++ = Fairly strong reaction.

+++ = Strong reaction.

++++ = Intense reaction.

The variation in the amount of the substance present or active in living hairs has been determined by this colour reaction. Human hairs, both strong and weak, were taken from a number of subjects. In each case the root bulb was surrounded by cellular tissue of the hair follicle which adhered to the root as the hair was plucked out. The reaction occurs in two stages:

(1) Firstly in the adhering cells around the hair root (these are the first to be penetrated by the solutions).

(2) Later in the hair root itself and the small portion just above the root. The root and hair are penetrated a little later than the follicle cells.

The two stages are quite definite. The coloration in the cells of the follicle of both weak and strong hairs is usually intense, but it is fleeting and fades rapidly. The coloration in the hair root and just above it varies considerably with the condition of the hair; it develops more slowly than that of the follicle cells, but is more permanent. In strong, healthy and pigmented hairs, the coloration of both the follicle and hair root is intense. In grey or white but apparently vigorous hairs, *i.e.* the root bulbs of which are large and well-shaped, the coloration in the follicle is intense, but in the hair root is not so strong as in the pigmented hairs. In grey or white hairs which are obviously weak or the roots of which are degenerating the coloration in the follicle is intense, but in the hair root it is either faint or absent.

It would appear from this that the reaction is intense where there is active metabolism, but where the metabolism may be considered to be reduced, the reaction is correspondingly weaker.

At the time that these experiments were being carried out, some rats which had been fed on experimental diets were given to the author by Miss Boas and Miss Luce. The rats supplied by Miss Luce [1924] were fed on a casein diet and the condition of the hair in some was normal, whilst others showed loss of the long hairs and patches of baldness covered only with weak downy hairs.

The rats supplied by Miss Boas [1924] were fed on an egg white diet, and were in a much poorer condition than those fed on the casein diet. The animals showed very poor growth of long hairs, and an almost universal development

of baldness except for the weak downy hairs. Bran was included in the diet of some of these rats, but not in that of all. A distinct development of scurf and a thickening of the stratum corneum was noticeable, but this was far greater in the case of those rats from whose diet bran was excluded.

Hairs taken from all these animals were examined for the nitroprusside reaction.

The results are given in Table III.

Table III.

A. *Normal rats on casein diet.*

	Reaction			
	Long white hairs	Long black hairs	Short white hairs*	Short black hairs*
Reaction given by hair follicles	+++	+++	++	++
Reaction given by hair roots	+++	+++†	++	+++

B. *Rats fed on casein diet, showing loss of hair and development of bald patches.*

Reaction given by hair follicles	++	+++	0	0
Reaction given by hair roots	++	+++	0	(+)

C. *Rats fed on dried egg white diet + bran.*

Reaction given by hair follicles	++	++	0	0
Reaction given by hair roots	+	++	0	(+)

D. *Rats fed on dried egg white diet and no bran.*

Reaction given by hair follicles	++	++	0	0
Reaction given by hair roots	+	+	0	(+)

* In C and D taken from bald patches.

† Stronger than in white hairs.

In sections of skin from C and D the reaction given by the various structures was as follows:

	C	D
Hair roots of long hairs	++	+
" " short "	0	0
Hair follicles	++	++
Epidermis except stratum corneum	++	(+)

The stratum corneum in C was little thicker than in the normal rat; in D it was enormously thickened.

The reason for the shedding of hair in animals fed on these diets is not yet known. It is quite evident, however, that little or no substance giving the nitroprusside reaction of glutathione is present in the hairs which have a very low metabolism.

Again, the substance is present or active on the whole to a greater degree in pigmented than in white hairs.

From the reactions given by the epidermis in the two cases of scurfy skin, it is suggested that in excessive or pathological metabolism the substance may only be present, or at any rate active, to a very reduced degree.

SUMMARY.

1. The substance which gives the nitroprusside reaction of glutathione is present in the cellular structures of the skin, *e.g.* in parts of the epidermis and hairs, in the hair follicles, the lining of the sebaceous and sweat glands, the erector pili muscle, and in the walls of arteries and veins.

2. It is not present or active to the same degree in all these tissues, and is absent in structures of extra-cellular origin such as collagen fibres, hair shafts, stratum corneum, and elastin fibres.

3. It is present to a larger extent in the roots of healthy pigmented hairs than in those of healthy white hairs, and it decreases in quantity or activity as the vitality of the hair decreases.

4. The substance is intimately connected with active metabolism and varies with the variations in metabolism.

In conclusion, the author wishes to extend her thanks to the Director (Dr R. H. Pickard) and Council of the British Leather Manufacturers' Research Association for permission to publish this work.

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