WATER EXTRACTABLE CONSTITUENTS OF HAIR*

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In the process of hair formation deep-reaching rearrangements occur during the transformation of the epidermal cells into keratin fibres. For example, the numerous cell nuclei degenerate; and the tyrosine, and particularly the cystine content of the keratin fibre is greater than that of the tissues from which they are derived.

In such a complicated process it may be expected that metabolic by-products are formed, some of which are probably of no further use in the new structures formed. These by-products may be reabsorbed by the skin or may be partly or wholly stored in the keratin fibre formed. Hitherto, the storage of such by-products in the keratin fibre has been given little attention, in contrast to the many studies on the main constituent of hair, the insoluble keratin.

Recent work in this laboratory, however, has shown that the extradermal fur of mammals contains varying amounts of water-soluble organic nonprotein substances in addition to the insoluble keratin (1). For example, a simple extraction with hot water of clipped rabbit fur and evaporation of the extract yields about 3.5 gm.% of a protein free solid, which contains a number of organic substances. Many of these substances have been identified and their approximate amounts determined (Table 1). The unexpected occurrence of comparatively large amounts of certain of these compounds throws new light on our conception of the mechanism of keratinization.

Uric Acid and Other Purines. Of the individual nitrogen-containing substances so far identified and estimated in the aqueous extract, uric acid is the greatest in quantity, namely, 400 mgm.%, according to colorimetric methods confirmed by gravimetric determinations (2). This high uric acid content of rabbit fur is remarkable indeed because, in common with most mammals, the rabbit possesses an effective uricase which destroys practically all uric acid entering the circulation. It is for this reason that the blood, other tissues, and secretions of the rabbit contain practically no uric acid. The anomaly of large amounts of uric acid in rabbit fur suggests that the metabolism in the fibre-producing hair follicle, where in all probability the uric acid is formed, is largely independent of the general circulation.

By analogy with mammalian metabolism in general, the high uric acid content is probably derived from nucleic acid. Accordingly a search was made for other breakdown products of nucleic acid, especially as it could be shown that the skin of rodents contains enzymes such as guanase, adenase and xanthine oxidase which according to the classical theory are involved in uric acid formation from nucleic acid. The aqueous extract was therefore examined for further purine

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compounds after removal of the uric acid. For this purpose the aqueous extract was treated with copper sulphate, the precipitate decomposed with hydrogen sulphide, and the filtrate evaporated. A white solid containing 43% of nitrogen remained. This high nitrogen content suggested a probable mixture of adenine, guanine, hypoxanthine, and xanthine compounds, all of which possess a high nitrogen content in the vicinity of 40% or more. Up to the present, however, only xanthine (N = 37%) could be identified definitely in this mixture. Adenine and hypoxanthine are believed to be present because characteristic crystals of adenine picrate and hypoxanthine silver nitrate could be seen in the precipitate after appropriate chemical treatment.

Pentoses. Another nucleic acid decomposition product, a pentose, was found in comparatively large amounts of 200 mgm.% in the aqueous fur extract (3). The nature of this pentose has not yet been determined and conceivably it may

TABLE I

Approximate content of substances identified in the aqueous extract of clipped rabbit fur

	mgm. %
Uric acid	400
Other purines	100
Amino acids	300
Urea	100
Ammonia	60
$C_6H_{11}O_4N_3$	50
Creatine	9
Creatinine	6
Glycogen	400
Pentose	200
Total phenols	60
Citric acid	50
Lactic acid	10

consist of a mixture of one or more riboses. Since the usual fate of endogenous pentoses is their conversion to glucose the accumulation of pentose in fur may have a significance similar to that of uric acid, that is to say, it is independent of the physiological laws which govern metabolism in the general circulation.

On this analytical and enzymatic evidence nucleic acid seems to be the logical precursor of the large amounts of uric acid and pentose present in the hair fibre. Consequently a search was also made in the hair fibre extract for pyrimidines also characteristic components of nucleic acid, but none have yet been isolated. It must be pointed out, however, that in general these decomposition products of nucleic acid can usually not be isolated in any appreciable amounts from tissues, probably because they become oxidized and converted to urea. The urea found in the water extract of hair (100 mgm.%) may well have been derived originally from the pyrimidines present in nucleic acid.

Amino Acids. Free amino acids were also present in large quantities in rabbit hair extract. Approximately 60 mgm.% of amino acid nitrogen were found as

estimated by Van Slyke's gasometric method. So far, however, only three amino acids, glutamic acid, valine, and leucine have been detected by paper chromotography in the aqueous hair extract. All these are familiar components of keratin, but the most characteristic amino acid of keratin, cystine is apparently absent.

Other Nitrogen-containing Compounds. Clipped rabbit fur contains approximately 60 mgm.% of water extractable ammonia, and creatine and creatinine in amounts of 9 and 6 mgm.% respectively. The total nitrogen content of the aqueous extract of rabbit fur varies from 500 to 600 mgm.%. The nitrogen containing substances mentioned so far, however, would account for only about two-thirds of this amount of nitrogen. Accordingly it should be possible to isolate further compounds of fairly high nitrogen content, but only one other nitrogen-containing compound has been obtained in the pure state. This was derived by first hydrolysing the glycogen of uric acid free hair extract and then adding alcohol. The substance, insoluble in alcohol, could be recrystallised from a small amount of water. Analysis gave the empirical formula $C_6H_{11}O_4N_3$, which agrees with that of the triamide of citric acid, citramide. The identity of the compound has not yet been proven, however, owing to lack of a reference specimen of citramide.

Glycogen. Histologic skin studies by several workers have demonstrated the presence of large amounts of glycogen in the lower half of the outer root sheath of the hair follicle, suggesting that glycogen plays an important role in the process of hair formation (4). It may be assumed that some of the histologically demonstrable glycogen in the outer root sheath is deposited in or on the keratin of the hair fibre. Chemical analysis demonstrated remarkably large amounts of glycogen in the clipped fur of the rabbit which was identified by its solubility, its reaction with iodine and by hydrolysis to glucose with mineral acids or enzymes. In different rabbit furs, glycogen was found to vary from 300 to 500 mgm.%, (5).

However, the glycogen found in the hair fibre cannot be demonstrated by staining methods as in the outer hair root sheath. In other words the recognized stains for tissue glycogen such as carmine, and fuchsin after treatment with periodic acid, fail to reveal glycogen in hair sections. This is unfortunate because no histological method has yet been found to demonstrate the location of glycogen or any of the other non-protein substances in the hair fibre. Nevertheless glycogen isolated from fur extracts reacts with tissue glycogen stains.

Phenols. The aqueous extract of rabbit fur gives a weak Millon's reaction, and Folin's phenol reaction shows the presence of approximately 60 mgm.% of total phenols. However, none of the compounds in rabbit fur responsible for Folin's phenol reaction have been isolated. The fraction obtained by ether extraction of the acidified aqueous extract amounts to only about 10 mgm.%, a value probably representing true phenols. The main interest of the total phenols is the fact that some components seriously interfere with uric acid determination, causing too high values. Therefore, phenols have to be removed before attempting a uric acid determination by Folin's colorimetric method. This becomes

particularly necessary with furs which have large phenol contents such as those of certain marsupials (6).

Other Non-protein-containing Substances. Citric acid, partly in the form of calcium citrate, occurred in the aqueous extract in amounts of 50–60 mgm.% and lactic acid in an approximate amount of 10 mgm.%.

Findings on Other Mammals. Uric acid, glycogen, pentose and phenols were the only constituents determined on a number of widely different animals including birds and reptiles. The amounts present varied greatly even in closely related species (Table 2). In fact, the values varied as much as the composition of keratin derived from different animals and seem to be characteristic of individual species. The greatest amounts of uric acid, pentose and glycogen occurred in the fur of the rabbit. The reason for this is not known but it may be

TABLE 2

Approximate amounts of uric acid, pentose, glycogen and total phenols in the aqueous extract of clipped mammalian hair

	URIC ACID	PENTOSE	GLYCOGEN	TOTAL PHENOLS
	mgm.%	*mgm.%*	*mgm.%*	*mgm.%*
Man	7–12	30	50*	28
Dog	70			
Cat	200	50		50
Guinea pig	100			60
Rat	200	60	50	60
Sheep	20	50	100	
Rabbit	400	200	300–500	60
Kangaroo	300	50	140	300
Phalanger	100	180	Trace	300† 600‡

* Young adult female.
† Dorsal fur.
‡ Ventral fur.

significant that the rabbit possesses no sweat glands and is endowed with but poorly developed sebaceous glands. It seems feasible that secretions, particularly from the sudoriferous glands, may dissolve constituents of the hair fibre such as the readily soluble glycogen or may produce enzymes which destroy some of these substances.

Definite but widely varying amounts of uric acid were encountered in 30 mammalian species examined. For example, the kangaroo yielded about 300 mgm.% of uric acid and thus is second only to the rabbit in the uric acid content of its fur. The rat yielded about 200 mgm.%, the guinea pig about 100 mgm.% and the dog about 70 mgm.% of uric acid. The hair of man has only about 7 to 12 mgm.% of uric acid. The phenol values exhibited similar species variations, the highest values occurring in marsupials such as the kangaroo (*macropus* spec.), and the phalanger (*Trichosurus vulpecula*). The lowest value

was found in human hair which contained 28 mgm.%. Rodents, such as the rat, guinea pig, and rabbit yielded about 60 mgm.% of total phenols. The glycogen and pentose contents were determined on a much smaller variety of animals and it is noteworthy that human hair may contain as much as 30 mgm.% pentose.

DISCUSSION

Each individual hair follicle and its fibre may be considered as a compact metabolic unit, in which a thin layer of the cellular epithelial tissue is ultimately converted into a keratin fibre, and a number of other products many of which are water soluble. In this process certain metabolic reactions normally taking place elsewhere in the body seem to play only a minor role. For example, uric acid and pentoses which are rapidly converted in other tissues, accumulate during the formation of the hair fibre.

The hair root is undoubtedly the factory where keratinization takes place, and whence the water soluble compounds such as uric acid, pentose, urea, ammonia, glycogen and phenols, etc. are derived. Nucleic acid seems to be the direct source of some of these products and apparently the origin of this nucleic acid is mainly the cell nuclei which degenerate in the process of keratinization. Obviously the loss of these nuclei entails the disposal of considerable amounts of desoxypentose nucleic acid. In the keratin itself no trace of this nucleic acid or its most characteristic degradation products could be found. This applies equally to the pentose nucleic acid of the cytoplasm, which also leaves no obvious traces in the protein keratin formed from it. Uric acid, the generally recognised end-product of nucleic acid metabolism, is found in comparatively large amounts in all aqueous extracts of mammalian fur. In the fur of the rabbit and the kangaroo it is present in comparatively enormous amounts, i.e. 300–400 mgm.%. Furthermore xanthine and probably adenine and hypoxanthine, precursors in the accepted scheme of uric acid production, have been detected in the hair fibre extract. The enzymes responsible for the above process have also been demonstrated. All these findings strongly suggest that the purines of nucleic acid derived both from extruded nuclei and the cytoplasm of the keratinized cells are ultimately converted into uric acid and may be deposited in the fibre in variable quantities. The question arises how substances like uric acid, glycogen, pentoses and phenols are attached to the hair fibre. Within the same species they are present in remarkably constant amounts and values in different animals of the same species kept under similar conditions do not differ more than plus or minus 20%. These substances, however, are removed in part at least even by water at room temperature without markedly altering the fibre. This would suggest that they are somehow attached to the cuticle of the fibre but so far it has been impossible to demonstrate histologically their position in relation to hair structure. It is hoped that electron microscopy of the native hair compared with that of the water treated fibre may shed some light on this problem.

SUMMARY

In addition to the insoluble keratin, the native extradermal hair fibre contains a number of water soluble organic compounds in physiologically large amounts. These include uric acid, glycogen, pentose, phenols, amino acids, urea and ammonia.

These compounds are considered to be by-products of the process of hair formation and there is some evidence that uric acid and pentose are derived mainly from the nucleic acid of degenerated nuclei.

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