Notable in the chemical composition of the proteins of the hair (and other epidermal structures) are the high contents of cystine and sulfur (1). In a previous study (2), we have been able to demonstrate that, in the young white rat, the amount of hair produced was related to the protein (and cystine) content of the diet, but that the demands for protein (and cystine) for the growth of the hair appeared to be secondary in importance to the demands for growth of the body with its more essential tissues.

In continuation of these studies, the present investigation is concerned with the composition of the hair as related to dietary factors. Are the amounts of cystine and sulfur present in hair influenced by factors of age or diet? Is the chemical composition of the protein of a tissue constant, or does the composition of a protein elaborated under conditions of a shortage of a specific amino acid reflect the lack of that particular amino acid in the diet? Since the hair is notably high in its content of cystine and since it has been demonstrated that cystine is used preferentially for general somatic development rather than for the growth of the hair under conditions of inadequate protein (and cystine) content of the diet (2), it has seemed that an attempt to solve this general question of the constancy of the composition of a tissue protein might well be made by a study of the composition of the hair produced by the young white rat on diets of varying protein and cystine content.
The general plan of the experiments was similar to that previously reported (2). Young white rats at the age of 30 days were selected from our colony in such a way that litter mates were distributed between the diets chosen, in order to eliminate so far as possible variations between litters. The animals were weighed weekly during an experimental period of 10 weeks. At the end of the 10 week period, the hair was collected by clipping. The samples were extracted with absolute alcohol and ether for periods of 24 hours each, dried at 60° in vacuo, and stored until analyzed in a desiccator over calcium chloride. Total sulfur was determined gravimetrically after oxidation of the sample in the Parr bomb; cystine, by the colorimetric method of Folin and Looney; and total nitrogen, by the Scales and Harrison modification of the Kjeldahl method (3). Duplicate analyses were carried out, when the amount of material available permitted; with the smaller animals, however, this was often impossible.

For the basis of the diets in which the amount of cystine present was the limiting factor, the milk powder-starch diet of Sherman and Woods (4) was used, modified as in previous studies (2). Groups 9 and 10 received diets in which 0.47 and 16 per cent respectively of casein replaced equivalent amounts of dextrin. In order to demonstrate more clearly that we were concerned with the quantity of cystine and not with the quantity of protein as such in these diets, the animals of Group 12 received a diet in which 6 per cent casein and 0.25 per cent of cystine replaced an equivalent amount of dextrin. The diets of Groups 10 and 12 should have contained approximately equal amounts of cystine, although differing considerably in their content of total protein (casein).

As further controls, collections of hair were also made from rats fed the stock diet previously described (2) for 10 weeks (Group 11), from rats at the age of 30 days (Group 15), from animals fed

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1 Sherman and Woods (4) in their biological test of the cystine content of casein found that 1 gm. of casein was equivalent in its effect on growth to 0.025 gm. of cystine. Hence the addition of 0.25 per cent of cystine to 6 per cent casein (Group 12) should give a cystine content equivalent in biological value to 16 per cent casein (Group 10). On the basis of the chemical analyses (5), however, in which casein was found to contain only 0.25 per cent of cystine, the diet of Group 12 would have included much more cystine than the diet of Group 10.
### TABLE I.

**Composition of Hair.**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet.</th>
<th>Final weight, average.</th>
<th>Composition of hair of individual animals, per cent.</th>
<th>Average for group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Casein, 0.47 per cent.</td>
<td>106.5</td>
<td>S. 3.80, 3.86, 3.98, 4.21, 4.03, 3.74, 3.74</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cystine. 10.96, 10.82, 11.79, 11.99, 11.47, 11.36, 11.50</td>
<td>11.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N. 15.8, 16.0, 17.2, 17.0, 16.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Casein, 16.0 per cent.</td>
<td>203.9</td>
<td>S. 4.27, 4.58, 4.31, 4.22, 4.43, 4.21, 4.68</td>
<td>4.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cystine. 12.36, 12.61, 12.89, 12.68, 12.99, 13.02, 12.79</td>
<td>12.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N. 16.3, 16.1, 15.5, 16.2, 16.2, 16.0, 16.4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Stock diet.</td>
<td>199.5</td>
<td>S. 4.58, 4.28, 4.11, 3.89, 3.93, 4.64, 4.96</td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cystine. 12.83, 12.68, 12.99, 12.27, 12.88, 12.52, 13.02</td>
<td>12.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N. 15.5, 15.9, 15.2, 15.9, 15.7, 15.9, 16.1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Casein, 6.0 per cent. Cystine, 0.25 per cent.</td>
<td>181.7</td>
<td>S. 4.47, 4.59, 4.21, 4.30, 4.25, 4.52, 4.41, 4.34, 4.97, 4.73</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cystine. 12.48, 13.15, 12.78, 12.01, 12.24, 12.82, 12.87, 12.02, 11.82, 13.75</td>
<td>12.59</td>
</tr>
<tr>
<td>13</td>
<td>Low lysine.</td>
<td>124.9</td>
<td>S. 4.21, 3.98, 4.53, 4.50, 3.89, 4.96, 3.98, 4.15, 4.88</td>
<td>4.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cystine. 12.70, 12.46, 12.75, 12.88, 12.75, 12.40, 12.23, 12.29, 12.29 12.77</td>
<td>12.59</td>
</tr>
<tr>
<td>14</td>
<td>Average weight, 120 gm.</td>
<td>118.9</td>
<td>S. 3.87, 3.96, 3.43, 3.22, 3.55, 3.37, 4.24</td>
<td>3.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cystine. 10.01, 10.80, 10.82, 11.01, 10.95, 11.67, 10.97, 10.90</td>
<td>10.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N. 16.0, 17.0, 16.0, 10.8, 10.9, 10.0, 16.6, 16.4</td>
<td></td>
</tr>
</tbody>
</table>

*Note: S, C, N, and S represent the percentages of sulfur, cystine, nitrogen, and sulfate, respectively.*
the stock diet (for a period of 2 to 4 weeks) until they reached an approximate body weight of 120 gm. (Group 14), and from a group of stock adult rats (Group 16). In order to further control our experiments with diets varying in their content of cystine, hair was collected for analysis from a group of animals (Group 13) which were fed a diet which retarded growth as did the cystine-deficient diet, but which was inadequate in respect to a different amino acid, lysine. The lysine-deficient diet (6), upon which these animals were maintained for a period of 10 weeks was made up of rolled oats (60.0 per cent), dextrin (30.3 per cent), salt mixture (4.7 per cent), and butter fat (5.0 per cent). Animals on this diet showed a retarded growth similar to that of the animals of Group 9, which received a diet in which cystine was the factor limiting the rate of growth.

As shown in Table I, the analytical data fall into two general groups. All of the animals fed a diet adequate in its cystine content over an experimental period of 10 weeks had produced hair which contained nearly the same percentage of cystine (Groups 10 to 13), a figure which was comparable also to the cystine content of the hair of the adult animals (Group 16) which were from 12 to 18 months old. On the other hand, the animals of Group 9,
whose growth was limited by the low cystine content of the diet
and which in a period of 10 weeks on the experimental diet were
unable to make as great a gain in weight as animals on the stock
diet in 2 to 3 weeks (Group 14), have produced hair which was
distinctly lower in its cystine content than that produced in the
same period of time by the animals fed diets adequate in their
contents of cystine as already discussed.

That the lower cystine content of the hair of the animals receiv-
ing amounts of cystine insufficient to permit a normal rate of
growth was not the result of retardation of growth per se, how-
ever, is demonstrated by the cystine content of the hair of the
rats which also received a diet inadequate for a normal rate of
growth, but in which the amount of lysine rather than of cystine
in the diet was the limiting factor (Group 13). Although the
animals of this group were able to make increments in weight
only slightly greater than the animals of Group 9, the hair pro-
duced during this period was similar in its cystine content to that
of those animals which received amounts of dietary protein and
cystine such that marked growth resulted.

The cystine content of the hair of the rats receiving the diet
inadequate in its cystine content was similar to and only slightly
higher than the cystine content of the hair of the young rats
(Group 15), animals whose hair was obtained for analysis before
the development of the adult type of hair (7). The animals of
Group 14 from which hair was collected during the period of
transition from the "puppy" coat to the adult type, and which
were fed the stock diet for 2 to 3 weeks only also showed a lower
content of cystine in the hair.

The total sulfur content of the hair showed variations similar to
and parallel to the cystine content, since it was possible in this
case also to distinguish between two types of hair, the low sulfur
type (Groups 9, 14, 15) and a higher sulfur type (Groups 10 to
13, 16). That these differences in sulfur and cystine content were
not due to differences in content of moisture, ash, or other consti-
tuents, was shown by the analyses for total nitrogen which varied
little throughout the series. As a matter of fact, the nitrogen
content of the hair of Group 15, which showed the lowest average
cystine content of all the groups, would appear to be slightly
higher than that of any other group.
From these results, it would seem that the initial coat of hair ("puppy" coat) contains a lower content of cystine than the adult coat; that during the early period of rapid somatic development, although a heavier coat of hair (adult hair) may be produced (2), the cystine content of the hair does not increase significantly; but that in the later stage of somatic development, in which growth proceeds less rapidly, if adequate amounts of cystine are available in the diet, the cystine content of the hair is increased. During the longer period of time, if the cystine content of the diet is not sufficient for either normal somatic development or normal growth of the hair, the percentage of cystine in the hair changes little from that of the "puppy" coat of hair. Retardation of growth, due to a shortage of dietary factors other than cystine, e.g. lysine, does not affect the cystine content of the hair, which resembles that of the adult type of hair.

In the series considered, the animals fed experimental diets were for the most part, of the same age but of widely different body weights at the time the hair was collected for analysis. It might be argued that age and body weight were more important factors in determining the cystine content of the hair than were dietary factors. In a second series, the animals were placed on a series of experimental diets, but the hair was collected from each animal when it had reached a body weight of approximately 120 gm. In other words, we were concerned with the analysis of hair produced by animals of the same body weight but of different ages. The stage of somatic development corresponding to a body weight of 120 gm. was chosen since, in our colony, this weight in control animals was reached at the age of 6 to 7 weeks, the time at which according to Greenman and Duhring (7) the transformation from "puppy" to adult hair has occurred.

The diets of the second series were similar to the preceding. A control group (Group 17) received the stock diet, a second group (Group 18) received the Sherman-Woods diet in which 16 per cent of casein had been substituted for an equivalent amount of carbo-

=During (8) observed that the total sulfur content of the hair of rabbits remained relatively constant until the 3rd month, and that the total sulfur increased slightly thereafter as the animals became older. The changes, 4.00 per cent to 4.65 per cent of total sulfur, are similar to those we have observed in the composition of the hair of rats.
hydrate, a third group (Group 19) received the same diet as Group 18 with the further addition of 0.75 per cent of cystine to insure an excess of cystine in the diet. The rate of growth of these three groups, as shown in Table II, was essentially the same, since all animals of these groups had attained a weight of about 120 gm. at the age of 6 to 7 weeks (i.e., after 2 to 3 weeks on the experimental diets).

Two groups received diets which retarded growth. The animals of Group 20 received the basal Sherman-Woods diet as modified

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Diet.</th>
<th>Age.</th>
<th>Final weight, average</th>
<th>Cystine content of hair of individual animals, per cent.</th>
<th>Average for group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Stock diet.</td>
<td>7</td>
<td>122.8</td>
<td>11.40, 11.44, 12.26, 12.52, 11.38, 12.41</td>
<td>11 90</td>
</tr>
<tr>
<td>18</td>
<td>Casein, 16.0 per cent.</td>
<td>6-7</td>
<td>125.0</td>
<td>13.51, 13.82, 15.44, 15.28, 14.29, 14.07, 15.03</td>
<td>14.49</td>
</tr>
<tr>
<td>19</td>
<td>Casein, 16.0 per cent; cystine, 0.75 per cent.</td>
<td>6-7</td>
<td>121.4</td>
<td>14.06, 15.14, 14.29, 14.80, 14.51, 15.16, 14.76</td>
<td>14.67</td>
</tr>
<tr>
<td>20</td>
<td>No added casein.</td>
<td>11-15</td>
<td>119.0</td>
<td>10.08, 10.05, 10.77, 9.85, 10.93, 10.72, 10.48</td>
<td>10.41</td>
</tr>
<tr>
<td>21</td>
<td>Low lysine.</td>
<td>12-14</td>
<td>123.8</td>
<td>12.51, 12.46, 12.75, 12.09, 12.50, 12.46, 12.20</td>
<td>12.42</td>
</tr>
</tbody>
</table>

(2) with no supplementary casein. For the low lysine diet of this series, the gliadin diet of Osborne and Mendel (9) was used. This diet contained gliadin, 18 per cent; salt mixture, 4.5 per cent; dextrin, 50.0 per cent; lard, 24.5 per cent; and cod liver oil, 3.0 per cent. On these two diets growth proceeded slowly and an average body weight of 120 gm. was reached at the age of 11 to 15 weeks (i.e., after 7 to 11 weeks on the experimental diets).

The analytical data for this series demonstrate even more strik-

3 Vitamin B was supplied by Vegex in amounts of about 75 mg. daily per rat.
ingly the influence of the cystine content of the diet on the composition of the hair (Table II). The animals of Groups 18 and 19, which were supplied with amounts of cystine fully adequate for or in excess of the requirements for rapid growth, produced hair which contained a high percentage of cystine. There was no significant difference in the composition of the hair of those animals which received excess cystine in the diet (Group 19), thus demonstrating that amounts of cystine in excess of those necessary to meet the demands for growth and to insure the production of hair of normal composition did not result in the production of hair of proportionately greater cystine content. The animals of Group 20, which, in a period of 11 to 15 weeks, i.e. nearly twice the time required by the animals of Groups 18 and 19, had reached a body weight of about 120 gm. and which as shown previously had been able to add little hair to their coat in this period (2), had elaborated a coat of hair much lower in its content of cystine, similar in cystine content to the hair of young animals of the age of 30 days (Table I). The animals of Group 21 which also grew slowly due to a dietary deficiency of lysine and whose diet contained cystine in adequate but not excessive amounts, had produced a hair whose cystine content was significantly greater than that of the animals on a cystine-poor diet, a cystine content similar to but slightly higher than that of the stock diet group (Group 17) which made a normal rate of growth.

These results supplement the data of the first series of experiments (Table I). They indicate that in the period of rapid growth hair of a cystine content similar to that of adults may be produced if excess cystine is present in the diet, and that if an adequate but not excessive amount of cystine is furnished by the diet in this early period of growth, the hair may contain a higher percentage of cystine than that produced by young animals, but that under these circumstances, the cystine content of the hair is not as great as in the adult or in the younger animals which have received an excess of cystine in the diet.

It should be noted that Group 10 (Table I) and Group 18 (Table II), both of which received the same diet showed a considerable difference in the percentage of cystine present in the hair. We are unable to determine whether this is a variation due to individuals, season, or age. The experiments with the groups presented
in Table II were carried out subsequent to those in Table I. The rats of Group 18 were from 6 to 7 weeks of age while those animals of the earlier series were about 14 weeks of age. Experiments designed to study more in detail the effect of these and other factors on the composition of the hair have been planned.

**SUMMARY.**

1. Further evidence is presented which indicates that the cystine requirements for growth of the body take precedence over the cystine requirements for the production of hair in the white rat.

2. The cystine and sulfur contents of the hair of the white rat were found to vary with the cystine content of the diet and to some extent with the age of the animals.

3. The cystine and sulfur contents of the hair of young rats ("puppy" coat) were significantly lower than those of the hair of rats fed on diets high in their content of cystine.

4. Hair from animals which received a diet in which cystine was the factor limiting growth, resembled in its lower cystine and sulfur contents the first coat of hair produced in young rats.

5. Retardation of growth alone did not produce a hair low in its cystine content, since a diet deficient in some other factor than cystine, e.g. lysine, did not result in the production of a hair of abnormally low content of cystine or sulfur.

**BIBLIOGRAPHY.**

THE METABOLISM OF SULFUR: XVI. DIETARY FACTORS IN RELATION TO THE CHEMICAL COMPOSITION OF THE HAIR OF THE YOUNG WHITE RAT
Howard D. Lightbody and Howard B. Lewis


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