tertiary syphilis in which the primary was ten years ago. It gave a positive Wassermann test with one antigen and negative with another. The Wassermann test was not tried with the antigen we employed in the Meiostagmin reaction. No. 52 was a case of early tabes probably on syphilic basis. No. 51 which gives a weak positive Meiostagmin, gave only a doubtful positive Wassermann test; this case had been treated for about eight months with mercury and potassium iodide.

The Meiostagmin reaction is evidently not quite as delicate as the Wassermann test, but it is a simpler test to make, for it only requires serum and antigen, while the Wassermann requires inactivated serum, antigen, guinea pig serum for complement, sheep blood corpuscles, and rabbit serum which has been injected with sheep corpuscles, all of which must be tested to see if they can be used together.

Summary.

The results of this investigation may be summarized very briefly as follows:

1. The surface tension of blood serum in an individual may change during the day, depending upon the food that is being absorbed by the blood.

2. The surface tension of the blood serum of different individuals and different species is approximately the same, if account be taken of the possibility of a daily change in any one individual.

3. The surface tension of blood serum seems to be abnormally high in certain diseases, especially those in which the kidneys are affected.

4. The Meiostagmin reaction was found to be positive in more than 80% of the cases of clinically positive syphilis investigated.

5. The clinically positive cases of syphilis in which the Meiostagmin reaction was not positive were those in which the Wassermann test was but weak or rather doubtful.

LABORATORY OF PHYSICAL CHEMISTRY.

[PAPER NO 24 FROM THE BIO-CHEMICAL LABORATORY OF THE STATION FOR EXPERIMENTAL EVOLUTION, THE CARNEGIE INSTITUTION OF WASHINGTON.]

STUDIES ON MELANIN V. A COMPARISON OF CERTAIN NITROGEN RATIOS IN BLACK AND IN WHITE WOOL FROM THE SAME ANIMAL.

BY ROSS AIKEN GORTNER.

Received July 17, 1913.

Introduction.

Historical.—In earlier publications upon the chemical nature of melanin and the chemistry of melanin formation (Gortner, 1910 (*a*), (*c*), 1911 (*a*), (*c*), (*d*), 1912 (*a*), (*b*)) I have shown the following:

(1) The animal pigments are the result of the action of the enzyme, tyrosinase, upon some oxidizable chromogen.

(2) In no instance has the chemical nature of this chromogen been proven although I have shown that there are two distinct types of melanin.

(3) These types may be distinguished from each other by the fact that one is readily removed from the keratin structure by hot 0.2% sodium hydroxide solution, while the other type is only dissolved after long boiling with frequent changes of the alkalin liquid, the keratin structure being dissolved long before any solution of the pigment takes place.

(4) The melanin which is readily dissolved by the dilute sodium hydroxide is present in black wool and in horse hair, the other type of pigment being found in negro hair, black feathers, black rabbit hair, etc.

(5) Little is known of the chemical nature of the more insoluble pigment.

(6) The pigment of black wool is apparently a pigmented protein, or as I have termed it, a melano-protein. When this pigment is hydrolyzed with strong mineral acids the pigmented portion is split off and remains as a dark brown powder while the protein residue yields the usual amino acids.[1]

(7) The wool of white sheep is, in all probability, white because there is an inhibitor present which prevents the process of melanin formation from taking place (Gortner, 1910 (*b*), 1911 (*b*)) and not because either the enzyme or the chromogen is lacking.

The Problem.—It seemed probable that the melano-proteins might be formed by the oxidation of some component of the keratin structure. Acting on this assumption I have made an analysis of black and of white wool taken from the same animal in the hopes that I might be able to detect some notable difference which would enable me to determin whether or not the chromogen is a part of the normal wool structure, or whether it is a foreign substance, secreted in the hair follicle solely for the purpose of pigment formation and not utilized in the elaboration of the hair structure when there is no oxidase present to cause pigmentation, or when there is an inhibition of pigment formation.

My results, however, still afford no definit solution to this problem, for I have as yet been unable to apply a suitable correction for the quantity of melanin present in the black wool and it is obviously wrong to compare the keratin + melanin of the black wool with the keratin of the white wool. If it were possible to take the same number of hairs, of the same

[1] Piettre (1912) in a recent paper has criticized some of my findings, but, inasmuch as he worked with the pigment of the melano-sarcoma, a pigment which apparently belongs to the more insoluble type of melanins, his criticisms do not necessarily apply to my work on the pigment of black wool. They will be taken up in detail in a later paper.

length and diameter of each color, thus using the same quantity of keratin in each case, no correction would be needed, but that is impossible, and when weights are used, the weight of the melanin present *in the form which is present in the natural wool structure* must be deducted from the weight of the wool taken, before the actual weight of the unpigmented hair structure can be ascertained, in order to make a direct comparison with the unpigmented white wool. I feel that there must be some method to arrive at the desired result, and I am, therefore, publishing these data in the hope that some one can advise me how to proceed.

Aside from my problem as outlined above, the work is of some general interest in being, in so far as I am aware, the first comparative analysis on record, of pigmented and unpigmented hair structure taken from the same animal and analyzed under exactly duplicate conditions.

Experimental.

The Method.—Exactly 9 grams of each color of wool were placed in special 500 cc. Erlenmeyer flasks fitted with inverted Hopkins' condensers by ground glass joints, 100 cc. of hydrochloric acid of 1.115 sp. g. were added and the flasks were boiled on the same electric hot plate for exactly 48 hours. The outlet of the flask was so arranged that all fumes entering or leaving the apparatus passed over dilute hydrochloric acid in a wash bottle, thus preventing the absorption of traces of ammonia from the air of the laboratory. At the completion of the 48-hour hydrolysis the mixture was treated in the same manner as I have previously described[1] and analyzed by Van Slyke's method (1911, 1912) for the determination of the chemical groups characteristic of the different amino-acids.

The determinations of the ammonia nitrogen and the humin nitrogen were made, using the entire sample of wool. The filtrate from the humin was concentrated to 250 cc. and the remainder of the analyses were made in duplicate on 100 cc. portions.

The analytical data follow:[2]

Data on White Wool.

Weight of extracted wool taken = 9.0000 grams.

Ammonia N = 97.25 cc. 0.1 *N* acid, indicating 0.1362 gram N in the entire sample, or 0.0546 gram in the $^2/_5$ portion.

Humin N = 10.05 cc. 0.1 *N* acid, indicating 0.0141 gram N in the entire sample or 0.0056 gram in the $^2/_5$ portion.

Total nitrogen in the filtrate from the humin in a portion of 10 cc. = 37.20 and 37.90 cc. 0.1 *N* acid, indicating 0.5257 gram N in the $^2/_5$ portion.

Total N in the $^2/_5$ portion of the original 9 grams = 0.0546 + 0.0056 + 0.5257 = 0.5859 gram N.

[1] THIS JOURNAL, **35**, 632–45.

[2] All calculations were made with the aid of a four-place table of logarithms.

Arginine N = 19.0 and 18.10 cc. 0.1 *N* acid, indicating (1) 0.1064 and (2) 0.1014 gram N or 0.1016 and 0.0968 gram N when corrected for the presence of cystine.

Total N in the bases = 60.32 and 62.48 cc. 0.1 *N* acid, indicating (1) 0.1689 and (2) 0.1749 gram N in the bases.

Cystine N = 0.0445 gram $BaSO_4$ and 0.0432 gram $BaSO_4$, indicating 0.0134 and 0.0129 gram cystine.

Amino N in the bases = 29.40 cc. N at 22° and 761 mm. and 29.60 cc. N at 22° and 761 mm., indicating 0.0819 and 0.0836 gram N when corrected for the presence of cystine.

Histidine N calculated according to Van Slyke = 0.0308 and 0.0436 gram N.

Lysine N calculated = 0.0231 and 0.0216 gram N.

Total N in the filtrate from the bases ($^1/_8$) = (1) 30.85 and 31.15 cc. 0.1 *N* acid and (2) 30.35 and 30.95 cc. 0.1 *N* acid, indicating 0.3472 and 0.3444 gram N.

Amino N in the filtrate from the bases = (1) 29.50 cc. N at 19° and 749 mm. and 29.10 cc. N at 18° and 749 mm. and (2) 28.30 and 27.90 cc N at 18° and 749 mm., indicating 0.3313 and 0.3182 gram of amino N in the filtrate from the bases.

These data calculated to per cents. of the total nitrogen are shown in Table I, together with the average of both analyses and the corrections which are necessary when taking into account the solubility of the bases in the solution from which they were precipitated (see Van Slyke, 1911, p. 32).

Data on Black Wool.

Weight of wool = 9.0000 grams.

Ammonia N = 92.00 cc. 0.1 *N* acid, indicating 0.1288 gram N in the entire sample or 0.0515 gram N in the $^2/_5$ portion.

Humin (+ melanin) N = 46.15 cc. 0.1 *N* acid, indicating 0.0646 gram N in the entire sample or 0.0258 gram N in the $^2/_5$ portion.

Total N in the filtrate from the humin in a portion of 10 cc. = 33.15 and 33.55 cc. 0.1 *N* acid, indicating 0.4669 gram N in the $^2/_5$ portion.

Total N in the $^2/_5$ portion of the original 9 grams = 0.0515 + 0.0258 + 0.4669 = 0.5442 gram N.

Arginine N = 17.70 cc. 0.1 *N* acid (using 25 cc. of bases) and 9.40 cc. 0.1 *N* acid (using 15 cc. of bases), indicating 0.0935 and 0.0830 gram N when corrected for the presence of cystine.

Total N in the bases = 38.75 cc. 0.1 *N* acid (using 25 cc. of the bases) and 24.45 cc. 0.1 *N* acid (using 15 cc. of the solution of the bases), indicating 0.1581 and 0.1580 gram N in the bases.

Cystine N = (1) 0.0518 gram $BaSO_4$ (using 10 cc.) and 0.0213 gram $BaSO_4$ (using 5 cc.), indicating 0.0155 and 0.0128 gram cystine N.

Amino N in the bases = (using 10 cc.) 28.10 cc. N at 20° and 754 mm. and (using 5 cc.) 13.70 cc. N at 20° and 763 mm., indicating 0.0782 and 0.0774 gram N when corrected for the presence of cystine.

Histidine N (calculated) = 0.0280 and 0.0410 gram N.

Lysine N (calculated) = 0.0211 and 0.0212 gram N.

Total N in the filtrate from the bases = (1) 26.95 and 27.20 cc. 0.1 *N* acid and (2) 27.50 and 27.27 cc. 0.1 *N* acid, indicating 0.3032 and 0.3066 gram N.

Amino N in the filtrate from the bases = (1) 25.40 and 25.80 cc. N at 18° and 752 mm. and (2) 25.20 cc. N at 18.5° and 752 mm. and 25.40 cc. N at 19.5° and 749 mm., indicating 0.2910 and 0.2858 gram amino N.

These data calculated to per cents. of the total nitrogen are shown in Table II, together with their averages and the corrected percentage when the solubility of the bases is taken into account.

TABLE I.—THE DISTRIBUTION OF THE NITROGEN IN WHITE WOOL, IN PER CENTS. OF THE TOTAL NITROGEN.

	I.	II.	Av.	Av. corr. for solubility of the bases.
Ammonia N	9.32[1]	...	9.32	9.32
Humin N	1.20[1]	...	1.20	1.20
Arginine N	17.34	16.52	16.93	17.46
Lysine N	3.94	3.69	3.82	3.90
Histidine N	5.26	7.44	6.35	7.00
Cystine N	2.29	2.20	2.25	2.70
Amino N in filtrate from the bases	56.53	54.30	55.41	54.54
Non-amino N in filtrate from the bases	2.71	4.47	3.59	2.76
Total	98.59	99.14	98.87	98.88

TABLE II.—THE DISTRIBUTION OF THE NITROGEN IN BLACK WOOL, IN PER CENTS. OF THE TOTAL NITROGEN.

	I.	II.	Av.	Av. corr. for solubility of the bases.
Ammonia N	9.46[1]	...	9.46	9.46
Humin (+part of melanin) N	4.74[1]	....	4.74	4.74
Arginine N	17.18	15.25	16.22	16.81
Lysine N	3.88	3.90	3.89	3.97
Histidine N	5.15	7.53	6.34	7.04
Cystine N	2.85	2.35	2.60	3.09
Amino N in filtrate from the bases	53.47	52.50	52.99	52.04
Non-amino N in filtrate from bases	2.24	3.82	3.03	2.13
Total	98.97	99.55	99.27	99.27

Discussion.

From a comparison of the data in Tables I and II it appears that the distribution of the nitrogen in the black wool is very similar to that in the white wool, only two points of difference greater than the experimental error being observed, *i. e.*, an excess of 3.54% in the humin nitrogen, this gain being due to the presence of pigment in the wool, and a deficiency of 2.50% in the amino nitrogen of the filtrate from the bases. This deficiency is greater than the experimental error and at first glance might be taken to indicate that the excess of humin nitrogen originated in some compound which appears in the amino nitrogen fraction in the white wool. It seems probable, however, that this is not the case and that other explanations will answer as well.

A second very noteworthy feature is the fact that my analyses show that white wool has a nitrogen content of 16.27% while the corresponding content of the black wool is only 15.11%. The nitrogen content of the white wool is near the expected figure (16.0%) for a pure protein

[1] Only one determination made, the entire sample being used in that determination.

($N \times 6.25 = 100$). The low nitrogen content of the black wool can only be explained by (*a*) the presence of a foreign compound with a lower nitrogen content than that of the keratin structure, or (*b*) by the oxidation of all or a part of the keratin structure in the process of pigment formation. The latter assumption would necessitate the addition of oxygen to an amount equivalent to 8.43% of the weight of the keratin structure (0.70 gram of oxygen to each 8.30 grams of white wool) and it seems highly improbable that oxidation would take place to such an extent in the process of pigment formation.

The only clue we have as to the presence in the keratin structure of a compound with a lower nitrogen content that the original keratin is the gain of 3.54% in the humin nitrogen. I have already shown (Gortner, 1912 (*c*)) that the melanin residue, isolated from the pigment of black wool by boiling with concentrated hydrochloric acid, contains 8.84% of nitrogen. We have, therefore, in the 9 grams of black wool taken for analysis, 0.5955 gram of this black "melanin residue." This is the compound which other authors have studied as the true pigment but which I believe to be a decomposition product of the true melanin. If we subtract this 0.5955 gram from the original 9 grams we have 8.4045 grams remaining, which, if my "melanin residue" represents the true melanin, should be the unpigmented keratin structure. Then subtracting the 0.0505 gram of nitrogen in the melanin residue from the 1.3405 grams of nitrogen present in the entire sample we have 1.2999 grams of nitrogen in the 8.4045 grams of keratin structure. This accounts for a nitrogen content of only 15.46%.

It can be readily seen that this figure is too far a divergence from the white wool percentage (16.27%) or even from the theoretical 16.00% to be entirely due to an experimental error. It seems far more probable that in the calculations given above *the "melanin residue" does not represent the true quantity of pigment* but that the melanin molecule is broken down by the acid hydrolysis and only a portion of the melanin nitrogen is obtained as humin nitrogen, so that the above correction did not take into account a very considerable portion of the melanin molecule with a much lower nitrogen content than that of the keratin structure. Such a supposition is in agreement with my previous work.

Summary.

1. Comparative analyses of black and white wool, taken from the same animal, have been made, using Van Slyke's method for the determination of the chemical groups characteristic of the different amino-acids.

2. The analyses were undertaken to determin, if possible, whether the chromogen utilized in the formation of melanin is a part of the normal keratin structure (white wool) or whether it is secreted solely for the pur-

pose of pigment formation and is not utilized in the hair structure when there is no oxidase present or when there is an inhibition of oxidative processes. No definit answer has been found to this question, but the evidence tends somewhat to support the latter hypothesis.

3. The averages of the two analyses agree with each other remarkably well, with the exceptions that the humin nitrogen from the black wool is 3.45% in excess and the amino nitrogen in the filtrate from the bases is 2.50% less than that in the white wool.

4. The excess of humin nitrogen is due to the presence of pigment. There is no necessary relationship between the lack of amino nitrogen in the filtrate from the bases and the excess of humin nitrogen.

5. The nitrogen content of white wool was found to be 16.27% while there is only 15.11% of nitrogen in the black wool.

6. The low nitrogen percentage of the black wool is probably due to the presence of melanin, which has a lower nitrogen content than the keratin structure. I have shown that the nitrogen of the melanin which appears in the humin fraction can only be a part of the true melanin nitrogen present in the wool. Apparently, therefore, hydrolysis with strong acids breaks down the melanin molecule. This observation is in agreement with my previous work.

Literature Cited.

Gortner, R. A., 1910: (*a*) The Origin of the Brown Pigment in the Integuments of the Larva of *Tenebrio Molitor*, *J. Biol. Chem.*, **7**, 365; (*b*) Spiegler's "White Melanin" as related to Dominant or Recessive Whites, *Amer. Nat.*, **44**, 497; (*c*) Studies on Melanin I. Methods of Isolation. The Effect of Alkali on Melanin, *J. Biol. Chem.*, **8**, 341. 1911: (*a*) Melanin II. The Pigmentation of the Adult Periodical Cicada (*Tibicen septendecim, L.*), *J. Biol. Chem.*, **10**, 89; (*b*) Melanin III. The Inhibitory Action of Certain Phenolic Substances upon Tyrosinase. (A Suggestion as to the Cause of Dominant and Recessive Whites.) *J. Biol. Chem.*, **10**, 113; (*c*) On Melanin, *Biochem. Bull.*, **1**, 207; (*d*) Melanin IV. The Origin of the Pigment and the Color Pattern in the Elytra of the Colorado Potato Beetle (*Leptinotarsa decimlineata* Say), *Amer. Nat.*, **45**, 743. 1912: (*a*) The Occurrence and Significance of Tyrosinase in the Reproductive Organs of Certain Amphibians, *Proc. Soc. Exper. Biol. Med.*, **9**, 118; (*b*) On Two Different Types of Melanin, *Proc. Soc. Exper. Biol. Med.*, **9**, 120; (*c*) Sur les pigments mélaniques d'origine animale, *Bull. soc. chim.*, [4] **11**, 498.

Piettre, M., 1912: Recherches sur la constitution chimique du pigment mélanique, *1st Congr. Internat. Compar. Pathol.*, Paris. Reprint, 44 pp.

Van Slyke, 1911: (*a*) A Method for the Quantitative Determination of Aliphatic Amino Groups, *J. Biol. Chem.*, **9**, 185; (*b*) The Analysis of Proteins by Determination of the Chemical Groups Characteristic of the Different Amino-Acids, *Ibid.*, **10**, 15. 1912: The Quantitative Determination of Aliphatic Amino Groups. II. *Ibid.*, **12**, 275.

Cold Spring Harbor.
Long Island, N. Y.