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Researches on Turacin, an Animal Pigment Containing Copper. II

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XIII. *Researches on Turacin, an Animal Pigment containing Copper.*—II.

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Contents.

§ 1. *Touracos, Turacin, and Copper in Animals.*

Since the publication of my paper on Turacin,* read before the Society in May, 1869, several interesting facts have come to light in reference to the Touracos, and to the occurrence of copper in the animal kingdom. Before giving the results of my own further researches, I would first of all mention the book on "Angola and the River Congo" by the late J. J. Monteiro.† In the second volume of this work, pp. 75 to 79, will be found some curious particulars about two species of *Turacus* (*Corythaix*), as well as an account of Mr. H. Bassett's experiments with turacin, to which further reference will presently be made. Mr. Monteiro describes a singular trait manifested by one of his tame plantain-eaters, a *Turacus schalowi*, which showed marked delight in gaily coloured dresses and pictures. Several instances are given of Touracos in captivity having moulted and then renewed their crimson plumage with all its original richness of colour. Some feathers from these birds, which Mr. Monteiro handed to me for examination, were found, by optical tests, to contain a turacin identical with that which colours them in their native countries. Yet these feathers had been produced in captivity, and after the birds had been for several years in England, where they were fed upon imported bananas and other vegetable foods. Certainly they had not had the opportunity of picking up "the grains of malachite and of other copper minerals to which, it has been suggested, they may have had access in

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* 'Phil. Trans.,' vol. 152, Part II., pp. 627–636 (1870).

† London: Macmillan and Co., 1875.

28.10.92.

their African homes, and from which they might have derived the copper necessary for the formation of their cupreous pigment. Such a suggestion appears, however, of doubtful value, for the plantain-eaters are arboreal feeders; and it is unnecessary, since copper is now known to be very widely distributed in plants, and I have shown it to be present in decided traces in bananas, the chief food of many species of Touraco

In 1881 Dr. M. GIUNTI published a paper entitled "Ricerche sulla Diffusione del Ramo nel Regno Animale."[*] This memoir gives a *résumé* of the work previously done on this subject, but also contains many new observations. Dr. GIUNTI found in the ash of the animals and animal products named below the following percentages of copper oxide (CuO) :—

Chrysomela americana	1·293
Lizard skins, *Podarcis muralis.*	1·049
Blatta orientalis	0·826
Bat-guano (from Calabria)	0·817
Catonia hirtella	0·661
Julus terrestris	0·221
Swallows	0·217
Armadillidium vulgare	0·197
Helix pisana.	0·089
Hedgehog	0·016

These results are of value not only as confirming the view that copper is widely diffused in the animal kingdom, but because they render probable further discoveries as to the occurrence of definite organic cupreous compounds in nature.

The researches of Dr. C. F. W. KRUKENBERG, on "Die Farbstoffe der Federn,"[†] must now be considered. This investigator has described a green colouring matter obtained from the green feathers of *Turacus corythaix*, and of other plantain-eaters, by the employment of a 2 per cent. caustic soda-solution as the solvent. He calls this pigment "turacoverdin," and, although he did not obtain enough of it for quantitative analysis, states that it contains "much iron, but no great quantity of copper and manganese," and that "perhaps, like turacin, it is free from sulphur and nitrogen." Here I must observe that Dr. KRUKENBERG, on referring to my paper on turacin, with which he was acquainted, would have found that turacin contains between 6 and 7 per cent. of nitrogen. The Author goes on to say: "This colouring matter (turacoverdin) becomes of high interest through the results of the experiments made by CHURCH on turacin." And then he proceeds as follows—I quote the original German of Dr. KRUKENBERG—"In seiner ausgezeichneten Arbeit über das Turacin—einem, auf dem Continente durchaus unbekannt gebliebenen und von mir in der grossen Katakombe des 'Philosophical Transactions' deshalb auch erst so spät aufgefundenen Meisterwerke auf dem Felde der physiologischen Chemie—mit welcher

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[*] 'Accademia Reale delle Scienze,' Napoli, 1881.

[†] Heidelberg, 'Vergl.-physiol. Studien,' 1881.

ich leider erst zu einer Zeit bekannt geworden bin, wo diese Untersuchungen bereits abgeschlossen waren und ausgearbeitet vorlagen, theilt CHURCH Folgendes mit: 'Turacin, by long exposure to air and moisture, or by continued ebullition with water or alkaline liquids, acquires a colour closely resembling that of chlorophyll.'" Dr. KRUKENBERG decides, after comparing the spectrum of altered turacin, as given in my memoir (*loc. cit.*, fig. 4), with the spectrum of turacoverdin, that the presence of the absorption band on the less refrangible side of line D indicates the presence of his turacoverdin in my "altered turacin," and proves that I had, in 1869, transformed turacin into turacoverdin, although there remained, as I had at the time conjectured, some unaltered turacin in the preparation examined. Although I cannot but appreciate very highly the handsome way in which Dr. KRUKENBERG speaks of my work and his confirmation of my results, I am unable to agree with Mr. F. E. BEDDARD* when he states that Dr. KRUKENBERG has added important details to those furnished by its discoverer with regard to turacin. And I may add in this place the remark that, if turacin be transformable in the way above described into turacoverdin, it is impossible that the latter can contain as essential elements either iron or manganese.

The numerous observations which have been made from time to time as to the occurrence of copper in certain Mollusks and Arthropods have finally resulted in the detection of a definite cupreous pigment in the blood of certain members of these two groups. This body exists in two states, and possesses, like hæmoglobin, respiratory functions: oxidized it is blue, but in the reduced condition colourless. Its spectrum has no definite absorption bands. It was named hæmocyanin by LÉON FREDERICQ,† to whom we owe some admirable researches on this important compound. It has been recognised in several genera of Crustacea, of Arachnida, of Gastropoda, and of Cephalopoda. It is a coagulable proteid belonging to the globulin group, and contains a very small proportion of copper—a proportion which may be just high enough to be comparable with the proportion of iron present in hæmoglobin.‡ Hæmocyanin is thus widely removed by its chemical and physical characters, as well as by its physiological *rôle*, from turacin. It is a far less stable body, and contains a comparatively insignificant percentage of the characteristic metallic element. It would, I think, be desirable to learn if there could be obtained from hæmocyanin a cupreous derivative bearing to its source a relation analogous with that borne by hæmatin to hæmoglobin. At present, hæmocyanin and turacin are the only definite animal pigments containing copper which have been recognised.

In this place reference may be made to a letter§ on the subject of copper in Birds

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* 'Nature,' December 19, 1889.

† 'Bulletin de l'Académie Royale de Belgique,' 2^{me} série, vol. 46 (1878).

‡ [F. HEIM has recently ('Comptes Rendus,' vol. 114, pp. 771–4) controverted several of the statements made by FREDERICQ as to the composition and properties of hæmocyanin; he denies that copper is an essential constituent of this compound.—Postscript, September 1, 1892.]

§ 'Chemical News,' vol. 28, p. 212.

—a letter written by Mr. SYDNEY LUPTON in 1873. Mr. LUPTON detected copper in the ash of seven or eight feathers of an Australian love-bird, *Melopsittacus undulatus*. He suggested that the plumage of this bird might contain a green cupreous pigment.

§ 2. *Occurrence of Turacin.*

In my previous paper I named twelve species of Touracos, in which I had recognised, by chemical or optical methods, the occurrence of turacin; from four of these species I had actually extracted this pigment. Since the publication of that memoir several new species have been discovered, and old species split, while the nomenclature of the whole family of the Musophagidæ has been revised. The order Picariæ, to which that family belongs, contains two sub-orders of equal rank, Scansores and Coccyges. The Coccyges comprise four families, namely, Indicatoridæ, Capitonidæ, Cuculidæ, and Musophagidæ. The Musophagidæ are arranged under five genera, and comprise twenty-five species. I am now able to state that turacin occurs in eighteen species, namely, in all the fourteen species of *Turacus*, in the two species of *Gallirex*, and in the two species of *Musophaga*. It is absent from the seven species comprised in the genera *Corythæola*, *Schizorhis*, and *Gymnoschizorhis*. It is of interest to note that the zoological classification of these Birds is now in accord with what may be called their "chemical" sequence. Formerly, a single Bird destitute of turacin, and now constituting the solitary species of *Corythæola*, was included in *Corythaix*, that is, *Turacus*; the anomaly of its presence among the turacin-bearers has now been removed. I ought to say that I have had the opportunity of examining specimens of 17 out of the 18 known species of *Turacus*, *Gallirex*, and *Musophaga* with the spectroscope, and have recognised the presence of turacin by means of its characteristic spectrum in them all. I have, moreover, actually separated the pigment from the wing feathers of eleven different species of Touraco; the species not available for this experiment were: *Turacus reichenowi*, *T. livingstoni*, *T. schuetti*, *T. fischeri*, *T. leucolophus*, *T. hartlaubi*, and *Musophaga rossæ*, most of which are very rare Birds.*

The following conspectus of the genera and species of Musophagidæ is taken from the account of this family recently written by Captain G. E. SHELLEY;† the seven African ornithological sub-regions, in which the plantain-eaters are distributed, are those which the same most competent authority has suggested to me in a private communication, dated 26th October, 1891; they may be thus defined:—

W. The whole coast from Senegal to the Quanza River, and inland to 30° E. long.
S.W. South of the Quanza to the Orange River, and inland to Lake Ngami.

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* [Turacin occurs not only in the wing-feathers of *Turacus meriani*, but in those of the crest, the tips of which are crimson; these tips yield up their pigment to dilute ammonia with some difficulty. I have extracted turacin also from the red head-feathers of *Musophaga violacea*. It should be stated that two species of *Schizorhis* (*S. africana* and *S. zonura*) show on their wing-feathers white patches destitute of pigment but corresponding in position with the red tracts of the vanes in the turacin-bearers.—Postscript, September 1, 1892.]

† 'Catalogue of the Birds in the British Museum,' vol. 19, pp. 435–456 (1891).

S. Cape Colony and north to the Orange River.
S.E. Natal, Transvaal, Zambesi, with the Shiré tributary, and north to 15° S. lat.
E. The coast from Mozambique (15° S. lat.) to 5° N. lat., and inland to 35° E. long.
C. The Central or Lakes sub-region, comprising Victoria Nyanza, Albert Nyanza, south-east portion of Niam-Niam country, and extending south to Tanjanyika Lake.
N.E. The northern half of Niam-Niam, the whole Nile district from the Albert Nyanza, east through Gala land and Somali, and north to the mouths of the Nile.

The countries north of the Sahara desert to the Mediterranean coast belong, not to the Ethiopic, but to the European region.

It must be noted that our acquaintance with the African avi-fauna is too imperfect to admit of the construction of a distribution-chart of the Musophagidæ which shall be exhaustive and final.

TABLE Showing Distribution of the Touracos in West, South-West, South, South-East, East, Central, and North-East Africa.

	Genera and species.	W.	S.W.	S.	S.E.	E.	C.	N.E.
1	*Turacus leucotis* (RÜPP.)							—
2	*T. persa* (L.)	—						
3	*T. buffoni* (V.)	—						
4	*T. schalowi* (REICHEN.)		—					
5	*T. livingstoni* (GRAY)				—			
6	*T. reichenowi* (FISCHER)					—		
7	*T. corythaix* (WAGL.)			—				
8	*T. schuetti* (CAB.)	—					—	
9	*T. macrorhynchus* (FRASER)	—						
10	*T. meriani* (RÜPP.)	—						
11	*T. fischeri* (REICHEN.)					—		
12	*T. erythrolophus* (V.)	—						
13	*T. leucolophus* (HENGL.)							—
14	*T. hartlaubi* (FISCHER and REICHEN.)					—		
15	*Gallirex porphyreolophus* (VIG.)			—	—			
16	*G. chlorochlamys* (SHELLEY)					—		
17	*Musophaga violacea* (ISERT.)	—						
18	*M. rossae* (GOULD)	—					—	
. . .	. . . .							
19	*Corythæola cristata* (V.)	—					—	
20	*Schizorhis africana* (LATH.)	—						
21	*S. zonura* (RÜPP.)							—
22	*S. leucogaster* (RÜPP.)					—		—
23	*S. concolor* (SMITH)		—	—				
24	*Gymnoschizorhis personata* (RÜPP.)							—
25	*G. leopoldi* (SHELLEY)					—		
25		10	2	3	2	6	3	5

NOTE.—The 18 species above the dotted line constitute 3 genera, and may be considered to contain turacin, which is certainly absent from the remaining 7 species of the family.

With regard to the formation of turacin in the organism of these birds we have much to learn. That the pigment is not always present in the blood is certain. I could not detect a trace of it in a specimen of *Turacus persa*, which was examined immediately after death, although I did recognise the presence of a minute quantity of copper in the ash of its flesh. It is desirable that the blood of the birds should be tested for turacin during the moulting period.

§ 3. *Isolation of Turacin.*

The mode of extracting turacin from Touraco feathers has been slightly improved. The red parts of the vane are first thoroughly washed with distilled water, and then, after drying, treated successively with absolute alcohol and with ether. The material is allowed to dry before being extracted, not, as previously, with very dilute sodium hydrate solution, but with exceedingly weak aqueous ammonia. The crimson solution thus obtained is filtered, and then precipitated by pouring it into a large excess of pure strong hydrochloric acid diluted with twice its bulk of water. It is curious to observe the immense bulk assumed by freshly-precipitated turacin. Thus, in an operation in which about 4 grms. of this pigment were dealt with, it was necessary to increase the volume of the mixture of turacin, hydrochloric acid, and water to nearly one gallon before its thick consistency could be so reduced as to permit of its being brought upon the filter. Filters of the finest calico were found to be far preferable to those of paper; they were first thoroughly cleansed by means of boiling distilled water: the method of vacuum filtration was adopted. All the operations should be conducted quickly; exposure of the moist turacin to light should be avoided as far as possible.

§ 4. *Characters of Turacin.*

The account given in my previous paper of the properties of turacin and of its behaviour with several reagents needs one addition. When powdered turacin is dissolved in cold oil of vitriol* it is profoundly changed, a part of its copper being removed and a new colouring matter produced. On pouring the sulphuric acid solution into a considerable volume of water, a flocculent precipitate of a reddish-chocolate colour appears. Collected on a filter and thoroughly washed with hot water, this precipitate is found to be still rich in copper, although a part of the metal has been withdrawn by the action of the sulphuric acid, and is found in the filtrate. The precipitate dissolves in dilute ammonia-water, with a crimson hue.† Dr. C. A. MacMunn, of Wolverhampton, has kindly examined this solution for me, and states

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* Oil of vitriol (containing 96 per cent. of H_2SO_4) appears to exert the same action on turacin, and to yield the same derivative, at temperatures much higher than 14° C., at which the first experiment was tried.

† [The ammoniacal solution of this substance, which I have called turacoporphyrin, exhibits a bright red fluorescence which is not seen in a similar solution of turacin; the solution in dilute sulphuric acid also fluoresces red.—Postscript, September 1, 1892.]

that its spectrum bears a very close resemblance to that of alkaline hæmatoporphyrin. It has four well-marked absorption-bands (shown in fig. A), the wave-lengths of which, in millionths of a millimetre, are—1st band, λ 619 to 601; 2nd band, 577 to 550·5; 3rd band, 545 to 526; 4th band, 512·5 to 488. These bands differ in position and relative intensity and breadth from those of a similar solution of turacin (compare figs. 6 and 7). The resemblance of this new derivative of turacin to hæmatoporphyrin is strongly accentuated by the further observation, that the acid filtrate from the precipitate above described possesses a spectrum which Dr. MacMunn says has "a most remarkable resemblance to that of acid hæmatoporphyrin." It has a purple rather than a crimson hue; and, though its spectrum has four bands (shown in fig. B), they differ in position and in relative intensity from those of the alkaline solution just described, and from those of an alkaline solution of turacin. Their wave-lengths are—1st band, 601 to 587; 2nd band, 579 to 569; 3rd band, 562 to 535; 4th band, 523 to 499; the 2nd band is faint, the 4th a slight shading only. This acid solution, when very nearly neutralised with ammonia, becomes colourless, depositing a flocculent precipitate, which proves to be identical with that previously separated on pouring the oil of vitriol solution into cold water. It is, in fact, soluble in some dilute acids, but not in pure water nor in solutions of neutral salts.

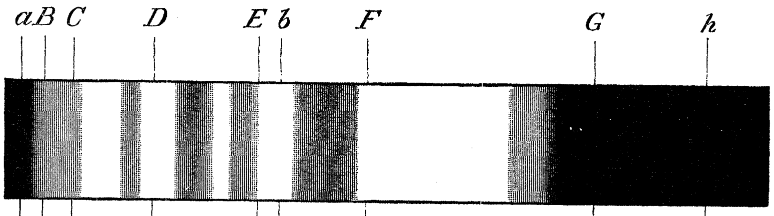

Fig. A

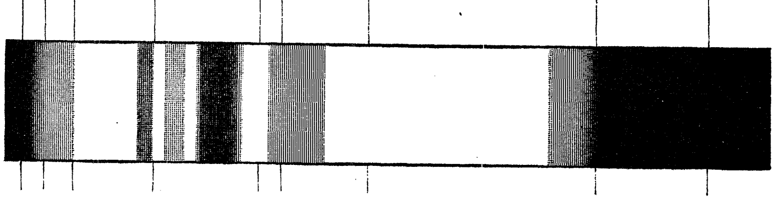

Fig. B

From the analyses which will be presently given, it seemed likely that the atomic ratio of metal to nitrogen in turacin might prove to be 1 : 4, as in hæmatin; and it was, indeed, the resemblance between these two pigments which induced me to try and ascertain whether turacin would yield, by solution in oil of vitriol, a coloured metal-free derivative similar to that which hæmatin produces under the same circumstances. My expectation has, it will be seen, been partially realised; but the new coloured derivative of turacin still retains much copper. Its solubility in dilute sulphuric acid and the spectra of its alkaline and acid solutions afford, however, strong corroborative evidence in favour of the view that the new derivative of turacin is nearly related to hæmatoporphyrin, and that turacin itself, though nearly related to hæmatin, has a somewhat more complex constitution.

My former description of the effects of heat upon turacin needs one important addition. The violet, or rather purple vapour given off when dry turacin is strongly and suddenly heated, consists of, or contains, an organic compound of which copper is an essential constituent. This vapour condenses on cooling into a partly crystalline sublimate, which, unlike turacin, is soluble in ether and in alcohol, but insoluble in dilute ammonia. It is of a reddish colour, and is obtained in the crystalline state by the spontaneous evaporation, in dry air, of its etherial solution. It well deserves further investigation, for the complete analysis of this derivative could hardly fail to throw light upon the constitution of turacin itself. For the same reason, the derived pigment obtained by means of sulphuric acid merits extended study; for this latter body I propose the name of turacoporphyrin.

If the very limited amount of turacin at my command has prevented me from making with it certain chemical and physical experiments which might have helped to elucidate its nature, and to determine its molecular weight, yet the study of its characteristic spectra has afforded invaluable aid in its recognition, especially in the case of the rarer species of *Turacus*. The chief features of these spectra were given in my former paper. The two well-marked absorption bands in the spectrum of turacin as it exists in the feather, closely resemble those of oxyhæmoglobin; the two chief bands in an alkaline turacin solution are not unlike those of CO-hæmoglobin. But it was desirable to investigate the spectroscopy of this unique pigment more thoroughly by examining solutions of different strengths, and prepared in different ways.

For the series of drawings of turacin-spectra reproduced on p. 519, I am indebted to the skill and kindness of Dr. C. A. MacMunn, of Wolverhampton. This able experimenter, whose acquaintance with the spectroscopy of animal pigments is unrivalled, gives the following explanatory notes concerning the chart of spectra.

The spectra were mapped by means of a one-prism chemical spectroscope made by Mr. Adam Hilger. The solutions were examined in a Preyer's hæmatinometer, which is provided with plane parallel glass sides one centimetre apart; the layer of solution examined was thus one centimetre thick.

Fig. 1. Solar spectrum with some of the Fraunhofer lines, the scale at the top being the arbitrary one of the instrument.

Fig. 2. Spectrum of the crimson web of a feather of *Musophaga violacea* with transmitted light. The feather was mounted in front of the slit, the light of a so-called "Sun" Argand gas-burner being condensed upon it it by means of a bull's-eye lens. All the remaining spectra figured were observed by means of the same light source. The following are the approximate measurements, in millionths of a millimetre, of the wave-lengths of the absorption-bands in the spectrum of this feather:—1st band, named α: shading begins at λ 599, is dark at 597, extends dark to 571, shades off to 567·5; centre is at 585 or 583. 2nd band, named β: is feebly shaded at 557, becomes dark at 553·5, extends dark to 529, is shaded off to 521·5; centre is at 538. The shading at the violet end begins about 510.

Fig. 3. Spectrum of a freshly-prepared aqueous and faintly ammoniacal solution, obtained directly from a feather. The measurements, in wave-lengths, of the bands in this spectrum were:—1st band, or α : shading begins at 579, is black at 575, extends back to 550·5, is shaded off to 548. 2nd band, or β : is shaded feebly at 540, is dark at 536·5, extends dark to 511, is shaded off to 505 ; centre of band $\alpha = 562$; centre of band $\beta = 523$. Note, also, the faintly-shaded band δ on either side of F ; this extends from 496 to 475.

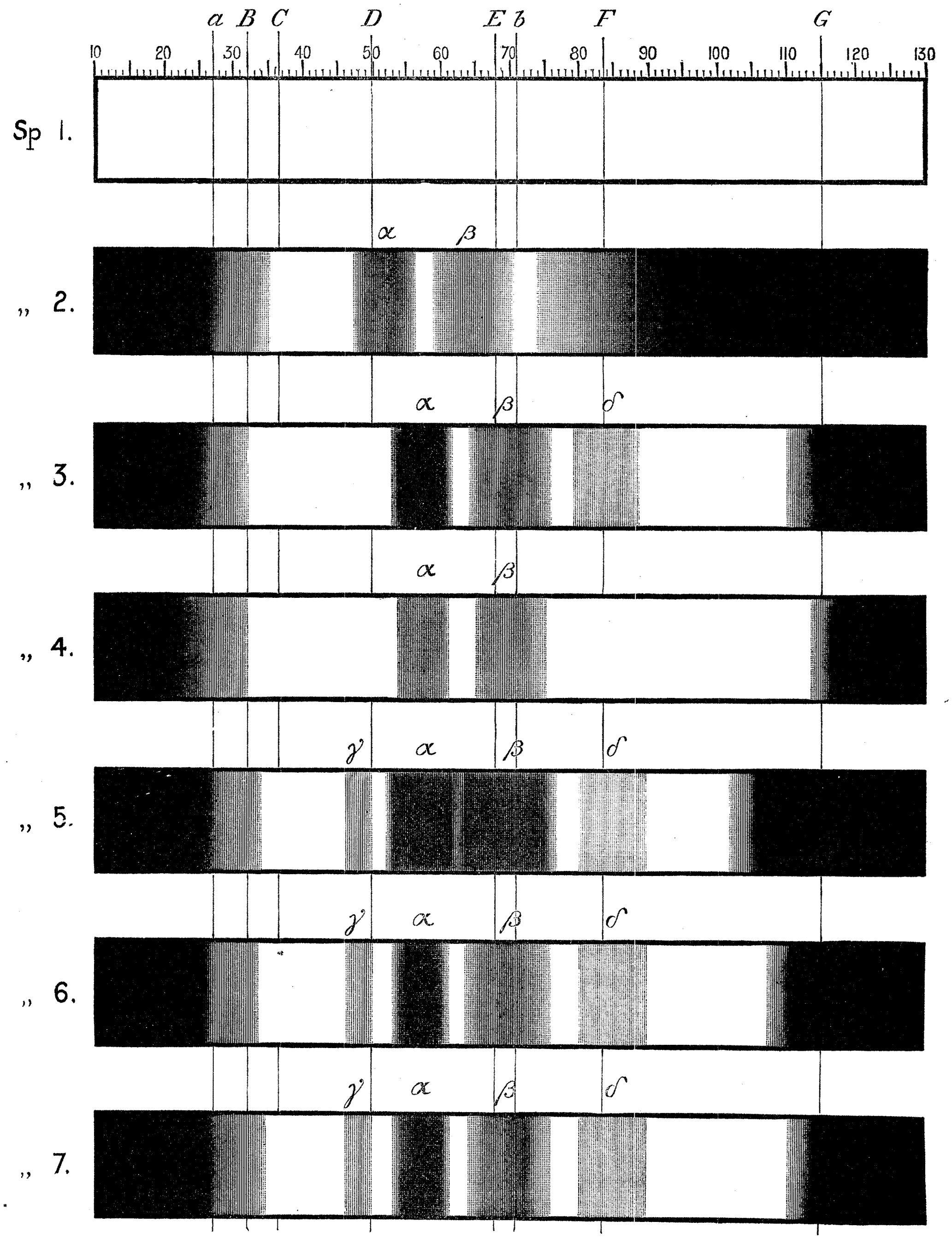

Fig. 4. Spectrum of a weaker solution prepared as above. 1st band, or α: shading begins at 577, is dark at 573, extends dark to 553·5, is shaded off to 549; centre of band $\alpha = 562$. 2nd band, or β: begins to be shaded at 538, is dark at 533, extends dark to 514, is shaded of to 506; centre of band $\beta = 523$.

Fig. 5. Spectrum of a strong solution of pure isolated turacin in faintly ammoniacal water. Note the occurrence, beside the band δ already described, of a fourth and stronger, though narrower, band γ near the line D. Measurements:—Band γ: 605 to 589: centre 597. Band α: shading begins at 581, is black 577, extends black to 547; there is a shaded space from 547 to 540. Band β; extends from 540 to 506, is shaded to 503. Band δ extends from 494 to 473.

Fig. 6. Spectrum of a weaker solution of turacin than that shown in fig. 5, but prepared in the same way. Measurements:—Band γ: 605 to 589. Band α: begins to be shaded at 577, is black at 573, extends black to 552, is shaded off to 547; centre at 562. Band β: begins to be shaded at 540, is dark at 533·5, extends dark to 511, is shaded off to 506; centre at 523. Band δ extends from 494 to 473.

Fig. 7. Spectrum of a solution, prepared in the year 1868, of turacin in weak ammonia water. Measurements: Band γ, 605 to 589. Band α: begins to be shaded at 579, is black at 573, extends black to 552, is shaded off to 549; centre at 562. Band β: begins to be shaded at 540, is dark at 535, extends dark to 514, is shaded off to 506; centre at 523. Band δ extends from 494 to 473.

[The bands γ and δ in the spectra are shown too dark in the figures—δ is, in fact, barely perceptible.]

The general results of these spectrum observations may be summarised thus:—Turacin, as it exists in the feathers, possesses two absorption bands, one of which, α, is rather darker and less wide than the other, and is situated about the line D, extending some distance on its more refrangible side; the other band, β, lies between the first-named and the line *b*. Turacin in alkaline solution always shows the same two bands, the first being, as before, darker and narrower than the second, but both bands are shifted towards the more refrangible end of the spectrum. When an alkaline solution of turacin is weak, it shows the above-named pair of bands only; when its strength is increased, a third band, δ, faint but broad, appears near the line F. But if some isolated and dried turacin be dissolved in weak ammonia water, a liquid is obtained which, if strong enough to show the band δ, will also show a fourth band, γ, darker and much narrower than δ, and situated on the less refrangible side of D. The recognition of the bands δ and γ is due to Dr. MACMUNN; the remaining features of these spectra were figured in my previous memoir, but with less completeness and exactitude. A question arises as to whether the band γ—which is not seen in the spectra of turacin solutions freshly and directly prepared from the feathers, but only in the spectra of solutions of such turacin as had been previously isolated and dried—belongs to the unaltered pigment, or whether its occurrence is a sign of the presence of a decomposition or oxidation-product of turacin. The spectral position of the band is, I think, identical with that of the characteristic band (as figured in my previous paper) of "altered turacin," and with the band of KRUKENBERG'S turacoverdin. But the band γ is so much fainter and so much narrower than the bands in question, that it can betoken the existence of no more than a trace of an alteration-product in the carefully-prepared sample of turacin with which the majority of the analyses here recorded were performed; the presence of such a trace can scarcely have affected in an appreciable degree the figures obtained. In connection with the probable presence of traces of an alteration-product of turacin in solutions made from the isolated pigment it is important to compare the spectra 6 and 7 in the cut, p. 519, they are virtually identical. There is no increase in the prominence of the suspicious γ band in the spectrum of a solution of isolated turacin in ammonia water after the lapse of twenty-three years. The particular specimen of turacin solution examined had been kept in a white glass bottle nearly full, but from which the stopper had been several times withdrawn; it had been exposed to moderately strong diffused daylight during fifteen years, but had been preserved in the dark for the eight years which immediately preceded its spectroscopic examination. Such a result is in striking contrast with that which is obtained when turacin, precipitated in flocculi by an acid from an alkaline solution, is allowed to remain for some time in contact with distilled water and atmospheric air. The precipitate loses its red hue, becoming dull and dark at first, and then acquiring a decided green colour. The same changes occur when a red Touraco feather is repeatedly wetted with pure water and dried, or when a piece of paper, stained with an ammoniacal solution of turacin, is kept for some time under the varying conditions of ordinary atmospheric moisture. The green turacin derivative produced in these experiments shows the spectral band γ with great distinctness, but it is always accompanied by the three bands proper to pure turacin in alkaline solution; this observation is true, even when every endeavour is made to complete the process of change.

Although the two spectra of turacoporphyrin described at the beginning of this section closely resemble the spectra of hæmatoporphyrin (obtained from hæmatin by the same treatment), yet the presence of a metallic constituent in the former derivative, and its absence from the latter is sufficient to prove that the two bodies are not exact analogues.

§ 5. *Nitrogen in Turacin.*

The production of the volatile derivative of turacin, when that pigment is strongly heated, is probably the cause of the difficulty experienced in determining correctly its percentage of nitrogen, this volatile product being formed in varying proportions, and being very difficult of combustion. The mean percentage of nitrogen given in my former paper was 6·38, a figure which, on a careful recalculation of the analytic data, comes out rather lower, and which was founded on three determinations by

Dumas' method. A fourth determination by the same process had given me 7·01 per cent., a figure which I rejected at the time as not being in accord with the three other analyses, which agreed well with one another. Subsequently I made a large number of other "absolute" nitrogen determinations, with more discrepant results, some yielding figures a good deal higher, and others, again, a good deal lower than those previously obtained. Many of these analyses were performed with the same sample of turacin; in one instance only did the percentage obtained, 6·75, show a fair agreement with the earlier numbers. I then secured the aid of two expert analysts, accustomed to the use of all the modern refinements in apparatus and processes for the determination of nitrogen by the absolute method, and constantly performing analyses of the most varied and complex nitrogenous bodies. But their results, six in number, though all obtained with the same sample of turacin, agreed neither with one another nor with any of my own previous determinations, the highest figure in the whole set being only 6·15 per cent. I had previously tried once more the soda-lime combustion method, making two analyses, in a current of dry hydrogen, with the following results:—

	i.	ii.
	grm.	grm.
Turacin taken, corrected for ash	·2448	·478
$(NH_4)_2PtCl_6$ obtained	·2713	·5265
Platinum obtained from above salt	·1222	·233
Nitrogen percentage, calculated from platinum found (Pt = 194·8)	7·17	7·01

These results, it will be observed, tended to confirm the higher figures obtained in some of the Dumas' analyses. I therefore made two more determinations by the soda-lime method, conducting the combustion, as before, in a current of hydrogen, but receiving the evolved ammonia in a standard sulphuric acid. On subsequent titration of the contents of the bulbs, the percentages of nitrogen obtained were 7·23 and 7·38. This volumetric estimation was difficult to complete, owing to the hue of the indicator being interefered with by that of some coloured distillation-products in the bulbs, and I do not attach much importance to these very high figures, the mean of which is no less than 7·3. It seemed, however, worth while to make further experiments with the modified soda-lime method, and Dr. F. E. Matthews, of Cooper's Hill College, very kindly undertook to determine the nitrogen by this process, which he had brought to great perfection, and had practised, with undoubted success, in the case of substances very difficult to analyse satisfactorily. Dr. Matthews purposely employed very small quantities of turacin, and he used the Nessler method of estimating the evolved ammonia, but I have every reason to believe that his results are the most exact obtainable under the conditions named. He used part of the same sample of turacin (the whole weighed 4 grms.)

which had been employed in the six estimations of nitrogen by DUMAS' method, to which reference has already been made, as discrepant and low. The results obtained by Dr. MATTHEWS were as follows :—1 centigrm. of turacin, dried for 15 hours at 100° C., was mixed with soda-lime and burnt in a stream of hydrogen. The contents of the nitrogen-bulbs (previously faintly acidulated with hydrochloric acid), were diluted to 250 c.c., and then Nesslerized.

i. 50 c.c. required 13·5 c.c. of a solution of NH_4Cl, 1 c.c. of which = ·00001 gram of nitrogen ; result = 6·75 per cent. N.
ii. 10 c.c. required 2·75 c.c. ; result = 6·825 per cent. N.

A second combustion was made of the same amount of turacin, the same solutions being employed.
iii. 50 c.c. required 13·55 c.c. = 6·775 per cent. N.
iv. 10 c.c. required 2·75 c.c. = 6·87 per cent. N.

The mean of these four percentages is 6·8, or, when corrected for ash, 6·87. Before considering the value of this number it will be advisable to give some results obtained by means of KJELDAHL'S process. My friend, Professor KINCH, of Cirencester, having had great experience with this method, was good enough to make two estimations of nitrogen in turacin by its means, using the identical sample which had been employed by Dr. MATTHEWS. Here are the results :—

i. ·1718 grm. turacin gave ·01335015 grm. NH_3 = 6·399 per cent. N.
ii. ·3075 grm. turacin gave ·0236034 grm. NH_3 = 6·321 per cent. N.

The mean of these two results, when corrected for ash, becomes 6·419. In the face of the higher percentages obtained by other methods, and of the known difficulty in oxidising turacin completely, I incline to regard this figure as too low.

We are now in possession of five pairs of nitrogen determinations, all made with great care and by methods differing more or less in character. The percentages obtained, duly corrected for ash, are arranged in this table.

Series A.	Series B.	Series C.	Series D.	Series E.
By DUMAS' method.	By soda-lime and platinum.	By soda-lime and standard acid.	By soda-lime and Nessler.	By KJELDAHL'S method.
i. 7·01 ii. 6·82	i. 7·17 ii. 7·01	i. 7·23 ii. 7·38	i. 6·855 ii. 6·895	i. 6·453 ii. 6·384
Mean 6·92	7·09	7·30	6·87	6·419

The mean percentage deduced from the five series is 6·92. If Series C be omitted the percentage becomes 6·82; if Series E, it is 7·05; if both these series be excluded the percentage stands at 6·96. These three numbers are so near together that it seems hardly material which we accept, but after having given due weight to the various circumstances likely to affect the validity of the several results obtained, I incline to adopt the figure 6·96. I may here mention that this percentage of nitrogen corresponds to 7·7 per cent. of copper, if the ratio $N_4 : Cu$ be assumed, or to 7 per cent. of copper in the case of the ratio $N_9 : Cu_2$.

§ 6. *Copper in Turacin.*

For the copper-determinations recorded in my first paper (*loc. cit.*, p. 632) I was not able, through scarcity of material, to employ adequate amounts of turacin. But the differences between my earlier results and those which will be given presently arose mainly from a strange and unsuspected peculiarity of turacin. When the pure dry pigment is quickly and strongly heated it gives off, as I mentioned in my previous memoir, "a violet vapour resembling that of iodine." Now this vapour contains an organic copper compound, capable of being condensed into a red crystalline solid, which is soluble in ether and insoluble in alkalies—unlike the original body which yields it. If the turacin be slowly heated it gives off no visible or coloured vapours, even when the temperature is subsequently raised to a dull red heat. Here, then, is to be found the cause of the discrepancy between the six per cent. of copper found in my earlier determinations and the seven per cent. recorded below. In all the older analyses, the turacin, after having been dried at 100° C., was heated alone in a crucible, whereby it lost about one-sixth or one-seventh of its metallic constituent in the form of the violet (or rather, purple) vapour mentioned above. When it was found that turacin, if first submitted for ten minutes to the temperature of boiling mercury, gave off no visible vapours when afterwards more strongly heated or even incinerated, an easy method for estimating both the copper and the accidental ash-constituents of this pigment seemed available; accordingly, two quantitative experiments were carried out in the following way. The turacin was dried at 100° C. until constant in weight; it was then heated for one hour in a bath of boiling mercury, and finally incinerated over a Bunsen burner until the weight of the residue became constant. The total ash was weighed, and then the copper present in it was determined, first, by titration with potassium iodide and sodium thiosulphate, according to Brown's method; and, secondly, by a gravimetric process, the iodine and the organic matter from the starch-indicator having been removed. The copper was precipitated as sulphide, and afterwards converted into and weighed as oxide. The total ash in the sample having been first of all ascertained in these analyses, an opportunity was afforded for the estimation of the fixed matter other than copper oxide left on the incineration of turacin. Here are the results:—

	i.	ii.
	grm.	grm.
Turacin taken, dried at 100° C.	·4627	·6832
Total ash obtained	·0454	·066
Turacin taken, corrected for ash	·4581	·6764
Copper, volumetrically determined	·0322088	·0464738
Copper oxide, gravimetrically determined	·0413	·0588
Copper, gravimetrically determined	·03297	·046936

These results correspond to the following percentages :—

	i.	ii.
Total ash	9·81	9·66
Ash other than CuO	1·0	1·09
Copper, volumetrically determined	7·03	6·87
Copper, gravimetrically determined	7·19	6·94

In the above calculations it has been assumed that the proportion of adventitious ash in turacin amounts to 1 per cent. This figure has been amply confirmed by other determinations, and has been adopted throughout the present paper as a correction.

The mean corrected percentage of copper in turacin, as deduced from the four determinations just given, is 7·01.* This figure, which corresponds to 8·79 per cent. of CuO, though higher than that obtained in my early experiments, is lower than that found by Mr. H. Bassett,† who by incinerating turacin with nitre and sodium carbonate obtained, in two analyses, oxide of copper equal to 7·6 and 8·0 per cent. of the metal. As no correction was made for ash these numbers are probably 1 per cent. below the truth, and may be safely taken to correspond to a mean percentage of 7·78. Now this number is confirmed by a determination which I made so long ago as September 14, 1874. The turacin employed was prepared from *Turacus corythaix*, and was purified and dried in the usual way. It was oxidised by long warming with concentrated nitric acid in a flask. The solution became at last of a clear green colour; it was cautiously evaporated to dryness and the residue incinerated. The residue was treated with nitric acid, and the solution filtered to remove a trace of silica. Finally the copper was precipitated by caustic potash and weighed as oxide. The figure obtained was :—

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* In another experiment ·2318 grm. turacin, oxidized by repeated treatments with fuming nitric acid, gave, by Brown's thiosulphate method, 6·87 per cent. of copper—a figure which when corrected for ash becomes 6·94 per cent. During the progress of this analysis and of another determination made in the same way, it was observed that turacin, if treated at first with an insufficient amount of nitric acid, yields an intermediate product having a "beetle-wing" lustre, and very difficult to oxidise by subsequent treatment with fresh portions of acid.

† 'Chemical News,' vol. 28, p. 201 (1873).

Turacin taken, corrected for ash	·3178 grm.
CuO obtained	·0323 ,,

This result corresponds to 8·11 per cent. of metallic copper. But on testing the black oxide of copper it was found to contain calcium phosphate and traces of iron and manganese.

A sample of turacin from *Musophaga violacea* was examined for copper by Professor KINCH, in November, 1891, the weighed substance being incinerated with every precaution in a crucible with sodium carbonate and potassium nitrate. The cupric oxide formed was dissolved in nitric acid, and precipitated by sodium hydrate, the precipitate being again dissolved, to remove silica, and re-precipitated.

The figures in this analysis were—

Turacin taken, corrected for ash	·1635 grm.
CuO obtained	·0168 ,,

This result corresponds to 8·2 per cent. of copper.

The mean percentage of copper in turacin, as deduced from the four determinations last recorded is 8·03. But there is a very good reason for not accepting this figure, for we have no guarantee that the precipitate produced by a fixed alkali in the solutions of turacin ash was pure. I conclude, indeed, that in Mr. BASSETT's two analyses and in the pair just recorded, the apparent percentage of copper has been raised, because the substance weighed really included the calcium phosphate and the traces of oxides of manganese and iron known to be present in turacin ash, and regarded as accidental or adventitious constituents thereof. This conclusion is strengthened by the following argument :—If turacin contains 8 per cent. of copper it must yield, when so incinerated that no loss occurs, quite 10 per cent. of CuO, which, added to the 1 per cent. of extraneous ash ascertained to be present, amounts to 11 per cent. But the true percentage of total ash is just under 10—a figure based on numerous determinations, and confirmed by a recent result of Professor KINCH, who, by oxidising ·1522 grm. of turacin with nitric acid, evaporating the solution to dryness and incinerating the residue, obtained ·0149 grm. of total ash, equal to 9·79 per cent. If, then, turacin be a definite compound possessing but one essential metallic constituent, there cannot be more than about 7 per cent. of copper in it. It might perhaps be argued that the iron and manganese are not accidental impurities, and that turacin may, after all, contain 8 per cent. of metal, namely, about 7 parts of copper and 1 part of iron and manganese. This assumption is, however, untenable, for the oxides of iron and manganese found in the ash of all preparations of turacin constitute but a small part of the 1 per cent. of so-called *accidental* ash, and are accompanied by other bodies such as calcium phosphate. I conclude, therefore, that the volumetric determinations of copper are to be accepted in preference, and that the higher figures obtained by precipitation with sodium hydrate arose from the presence of impurities.

It may be well to state here that there is no evidence, chemical or spectroscopic, that turacin suffers any loss of copper when treated with the somewhat diluted hydrochloric acid used in its preparation, or even when boiled with a mixture of one volume of the fuming acid with one volume of water.

§ 7. *Carbon and Hydrogen in Turacin.*

The percentages of these elements, as deduced from my previously published analyses, were, respectively, 54·63 and 5·22. The results of combustions of the very carefully prepared sample of turacin with which the greater number of the analyses recorded in the present paper were made gave somewhat lower figures. The smaller proportion of carbon found may be due to the precautions taken to remove the last traces of fatty matter from the sample, and to decompose any oxides of nitrogen formed in the combustion; the great care exercised in excluding accidental moisture may have reduced the percentage of hydrogen. These are the analytical numbers :—

Combustions of Turacin.

	Substance taken.	H_2O obtained.	CO_2 obtained.
	grm.	grm.	grm.
i.	·1362	·055	·2653
ii.	·2085	·0834	·4076
iii.	·1435	·0611	·2792

These numbers correspond to the following percentages :—

	Hydrogen.	Hydrogen corrected for ash.	Carbon.	Carbon corrected for ash.
i.	4·48	4·53	53·12	53·65
ii.	4·44	4·48	53·31	53·84
iii.	4·73	4·78	53·06	53·59

The mean corrected percentages are :—

Carbon	53·69
Hydrogen	4·60

§ 8. *Summary of Analytical Results.*

On the assumption that the only metallic constituent of turacin is copper its centesimal composition may be given as :—

Carbon	53·69
Hydrogen	4·60
Copper	7·01
Nitrogen	6·96
Oxygen	27·74

It is somewhat rash to suggest an empirical formula for a body which can neither be crystallised nor distilled, and to which few of the ordinary criteria of purity can be applied. The experimental percentages do not agree closely with any formula in which the ratio $Cu : N_4$ occurs—a ratio which analogy with hæmatin (which has $Fe : N_4$) would lead one to prefer, but they do correspond very nearly with the following more complex expression:—

Theory, $C_{82}H_{81}Cu_2N_9O_{32}$.			Experiment.
		Per cent.	Per cent.
C_{82}	= 984	= 53·78	53·69
H_{81}	= 81	= 4·43	4·60
Cu_2	= 126·6	= 6·92	7 01
N_9	= 126	= 6·89	6·96
O_{32}	= 512	= 27·98	27·74

The agreement of these theoretical and experimental numbers is satisfactory. Although I should have preferred a simpler empirical formula, and the atomic ratio between metal and nitrogen of 1 : 4 rather than the ratio 2 : 9, yet there exist two arguments in favour of a more complex expression. One of these arguments is based on the *partial* loss of copper which turacin suffers when strongly heated—a loss which seems to indicate that the whole of the copper present does not exist in the same state of combination. The other argument is founded on the *partial* retention of copper by turacoporphyrin. This shows that its formation does not proceed upon exactly the same lines as those of hæmatoporphyrin, which retains none of the metallic constituent present in its parent-substance, hæmatin.

I wish it to be understood that I lay no stress upon the formula which I have suggested, regarding it merely as a mode of expressing the results of analysing a substance which I believe to be constant in composition and very nearly pure. And, in the absence of sufficient data of control, I do not think it worth while to compare the experimental percentages with those demanded by such simpler formulæ as— $C_{40}H_{40}CuN_4O_{16}$; $C_{40}H_{40}CuN_4O_{15}$; $C_{40}H_{38}CuN_4O_{16}$; and $C_{40}H_{38}CuN_4O_{15}$.

§ 9. *Summary and Conclusions.*

The more important positions established by the present research are these:—

I. The constant occurrence, in 18 out of 25 known species of Musophagidæ, of a definite organic pigment containing as an essential constituent about 7 per cent. of metallic copper.

II. The "turacin-bearers" comprise all the known species of the three genera, *Turacus*, *Gallirex*, and *Musophaga*, while from all the species of the three remaining genera of the family Musophagidæ, namely *Corythæola*, *Schizorhis*, and *Gymnoschizorhis*, turacin is absent. Furthermore, the zoological arrangement of the genera constituting this family is in accord with that founded on the presence of turacin.

III. The spectrum of turacin in alkaline solution shows, besides the two dark absorption bands previously figured, a faint, broad band on either side of line F, and extending from λ 496 to λ 475.

IV. The spectrum of *isolated* turacin in ammoniacal solution shows, besides the three bands already named, a narrow fourth band, lying on the less refrangible side of line D, and extending from λ 605 to λ 589. It probably arises from the presence of traces of the green alteration-product of turacin formed during the preparation of that pigment in the isolated condition, an alteration-product which is likely to prove identical with KRUKENBERG's turacoverdin.

V. Turacin in ammoniacal solution remains unchanged after the lapse of 23 years.

VI. Turacin in the dry state, when suddenly and strongly heated, yields a volatile, copper-containing red derivative, which, though undissolved by weak ammonia-water, is not only soluble in, but may be crystallised from ether.

VII. Turacin in the dry state, when heated in a tube surrounded by the vapour of boiling mercury, becomes black, gives off no visible vapour, is rendered insoluble in alkaline liquids, and is so profoundly changed that it evolves no visible vapour when afterwards strongly heated.

VIII. The percentage composition of turacin is probably carbon 53·69, hydrogen 4·6, copper 7·01, nitrogen 6·96, and oxygen 27·74; turacin may possibly be represented by the empirical formula, $C_{82}H_{81}Cu_2N_9O_{32}$.

IX. Turacin presents some analogies with hæmatin, and yields by solution in oil of vitriol a coloured derivative having a spectrum much resembling that of hæmatoporphyrin, the corresponding derivative of hæmatin, but retaining, unlike hæmatoporphyrin, part of the metallic constituent of the parent-substance.

The rarity of turacin and the singular difficulty experienced in burning it completely may, I hope, be regarded as furnishing a legitimate apology for offering to the Society a long discussion of analytical results, for the lack of absolute decisiveness in the evidence brought forward as to the centesimal composition of the pigment, for the limited information obtained as to the behaviour of turacin with reagents, and as to its relationships and derivatives. When one looks back upon the steps by which the true formula of so definite and abundant a crystalline pigment as alizarin was finally established; when one recalls the mystery still shrouding hæmoglobin and chlorophyll, some excuse may perhaps be allowed for my failure to accomplish more towards the elucidation of a colouring matter so anomalous and costly as turacin. It is, however, certain that the chemical and physiological interest belonging to this pigment amply warrant its further and more thorough investigation.

In conclusion I have to express my thanks to the Royal Society for a handsome money grant in aid of the prosecution of this inquiry. My acknowledgments are also due to the several friends whose names have been recorded in the present paper, especially to Dr. McMunn, whose help in the spectroscopy of turacin has been invaluable.

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