PHOTOELECTRIC PROCEDURES TO CHASE THE DAILY EXCRETION OF URINARY MELANOGEN

SUSUMU SHIBATA AND HIROSHI TAKAHASHI

Department of Clinical Pathology, Yamaguchi Medical School, Ube

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Patients with melanosarcoma excrete melanogen, a colorless precursor of melanin, in urine. Two tests, the ferric chloride test and Thormählen's reaction, have been known as the measures for the detection of melanogen. An attempt was made in our laboratory in the hope that these qualitative test might be adapted to a photoelectric colorimeter for the purpose of tracing the vicissitude of daily urinary excretion of melanogen in a case of melanosarcoma whom we encountered last year.

METHOD

I. Ferric chloride test

(1) Reagent 10 g./dl. ferric chloride solution: Dissolve 10g. of the lump of ferric chloride $FeCl_3$ in 100ml. of N/10 hydrochloric acid.

(2) Procedure: Into test tubes A, B, C and D introduce the urine of a patient with melanosarcoma and the reagent as listed below, mix and filter through two sheets of filter paper Tôyôroshi No. 7 to obtain clear filtrates.

	Urine	distilled water	ferric chloride reagent	
A	0	18.0	0.3	
B	0.5	17.5	0.3	
C	1.0	17.0	0.3	
D	1.5	16.5	0.3	(ml.)

Measure the absorbance of the solutions B, C and D in Erma's photoelectric spectrophotometer which is set (test tube cell, diameter 20mm.) to zero with solution A over the whole range of visual spectrum.

II. Thormählens reaction

(1) Reagent

a) 1g./dl. aqueous solution of sodium nitroprusside

b) 10g./dl. sodium hydroxide solution

c) 30 per cent acetic acid: Dilute 30 ml. of glacial acetic acid to 100 ml. with distilled water.

(2) Procedure

Transfer the urine of melanosarcoma patient and the reagents into test tubes A, B, C and D as follows, in the order from left to right

	Urine	sod. nitroprusside	sod. hydroxide	acetic acid	distilled water
A	0	0.5	2.0	2.0	9.5
B	0.5	0.5	2.0	2.0	9.0
C	1.0	0.5	2.0	2.0	8.5
D	1.5	0.5	2.0	2.0	8.0

Mix by inversion (with stoppers at the orifice of the test tubes), and measure immediately thereafter the absorbance of the solutions B, C and D with solution A as blank over the whole range of visual spectrum in the same way as described in I.

Results and Discussion

The absorption spectrums of the ferric chloride test and Thormählen's reaction are depicted in Figures 1 and 2. The scrutiny of these figures reveals that the absorbance of the former increases monotonously with the decrease in the wave length of the light, whereas the latter exhibits two peaks of absorbance, at 625 mμ and in the range shorter than 500 mμ, respectively.

Figures 3 and 4 which illustrate the correlation of absorbance (A) to the concentration of melanogen (C) disclose that the ferric chloride test follows Beer's law (A=KC; K is a constant) over a relatively wide range of spectrum, i. e., 450 to 510 mμ, although the law holds, in Thormählen's reaction, only for a narrow range of wave lengths around 625 mμ.

Ferric chloride test gives a dark brown color which is stable at the ordinary room temperature but gradually darkens when it is warmed at 50 C. for an hour. The coloration is so intense that it outshadows the light absorption of the proper urinary pigments and renders its interference in absorbance negligible as clearly visualized in Figure 1 where the absorption spectrum of concentrated urine of a healthy person (urine 1.5 ml. + distilled water 16.8 ml; distilled water

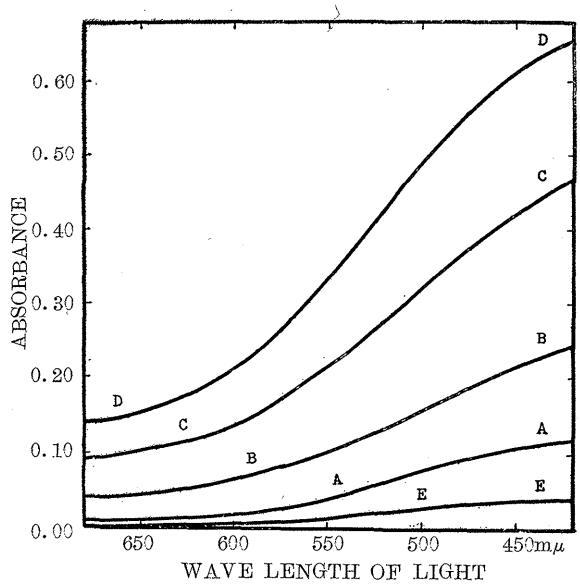

Fig. 1 Absorption spectrum of the ferric chloride test.
(As for the notations A, B, C, D and E see the description given in the Procedure as well as in the Results and Discussion)

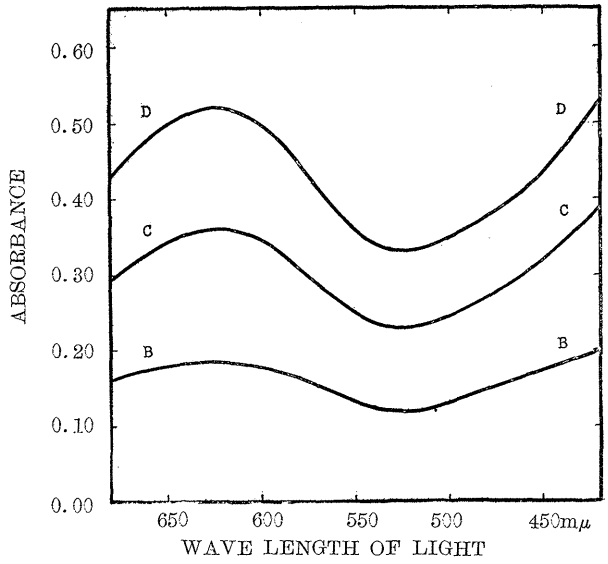

Fig. 2 Absosption spectrum of Thormählen's test.
(As for the notations B, C and D see the description given in the Procedure)

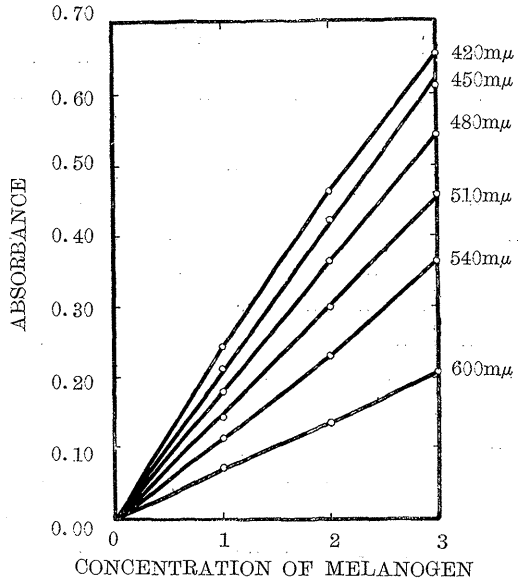

Fig. 3 Correlation of absorbance to the concentration of melanogen in the ferric chloride test.

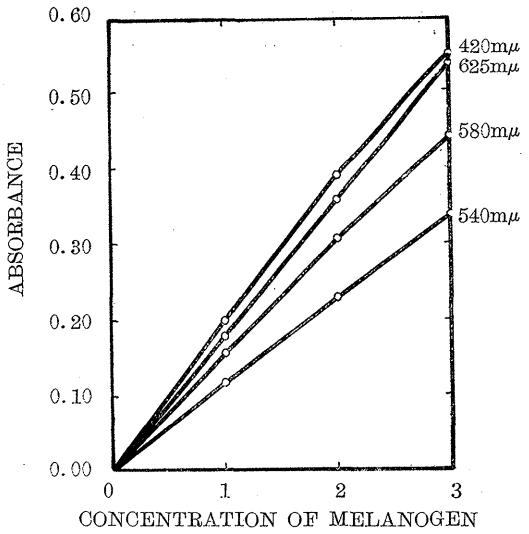

Fig. 4 Correlation of absorbance to the concentration of melanogen in Thormählen's test.

as blank), denoted by E, is contrasted to that of the blank A (ferric chloride solution 0.3 ml. + distilled water 18.0 ml.; distilled water as blank)

Beautiful green coloration is yielded by Thormählen's reaction. It reaches the maximum intensity one minute after mixing, remains stable for about two minutes, and fades out rapidly. Speedy handling is, therefore, required in colorimetry, although this is performed at 625 mμ without any interference of urinary pigment, because urine exhibits eventually no absorbance in this region of spectrum.

Inasmuch as the ferric chloride test and Thormählen's reaction obey Beer's law at 450–510 mμ and at 625μ, respectively, the quantitative determination of urinary melanogen would be possible, if melanogen solution were available for the construction of calibration curve. Emerson[1] is of the opinion that pyrocatechol is identical with melanogen. If pyrocathechol is the same substance as pyrocatechin, Emerson's concept seems to be hardly maintained, because ferric chloride test and Thormählen's reaction for the aqueous pyrocatechin solution (100 mg./dl.) are quite different from those of the melanosarcoma urine. The points of distinction are listed as below.

1) Ferric chloride test is green in color shortly after mixing, turns to yellow within a few minutes, returns to green after an hour, and finally becomes dark green about ten hours thereafter. (Melanosarcoma urine remains dark brown all the time). Warmed pyrochatechin solution yields green ferric chloride test, but it turns to green while it is allowed to room temperature. The greenish hue lasts a long time, if sodium hydrosulphite is added.

2) Thormählen's reaction produces light brown color which gradually darkens as time passes. (Melanosarcoma urine gives beautiful green hue.)

Since it is apparent, as has been stated above, that pyrocatechin cannot be substituted for melanogen in order to construct a calibration curve, and melanogen has not as yet been purified with success, relative estimation is the best that is expected for the determination of melanogen, and its procedure will be designed as follows.

Ferric chloride test: Dilute 1.0 ml. of urine with 17.0 ml. of distilled water, add 0.3 ml. of ferric chloride solution, and measure the absorbance at 450-510 mμ in a photoelectric colorimeter which is set to zero by the mixture of distilled water (18.0 ml.) and ferric chloride solution (0.3 ml.) with a cuvette of known optical path (test tube cell of 20 mm diameter for instance).

Thormählen's reaction: To 1.0 ml. of urine add 0.5 ml. of sodium nitroprusside solution, 2.0 ml. of sodium hydroxide solution, 2.0 ml. of acetic acid and 8.5 ml. of distilled water in the order given, mix, and subject to photoelectric colorimetry at 625 mμ, employing, as blank, the mixture which has the compostion quite similar to that of the afore-mentioned except that 1.0 ml. of urine is substituted by 1.0 ml. of distilled water. A cuvette of known optical path is used throughout the procedure in the same manner as in the ferric chloride test.

Then compute the relative amount of daily excretion by the following equation.

$$\text{Daily excretion} = \text{Absorbance} \times \text{Daily Output of Urine.}$$

TABLE I

Ferric chloride test and Thormählen's reaction in neoplastic diseases

	Ferric chloride test (absorbance*)	Thormählen's test (absorbance*)
Melanosarcoma	0.406	0.360
Cancer of the skin	0.209	0.110
Cancer of the skin	0.135	0.020
Cancer of the liver	0.188	0.040
Cancer of the pancreas	0.138	0.025
Cancer of the uterus	0.114	0.010
Healthy person	0.075	0.003

*Test tube cell of 20 mm. diameter was used.

Table I presents the data of the ferric chloride test and Thormählen's reaction which were carried out in the way described above on some patient with neoplastic diseases. The table discloses that the urine of melanosarcoma is, of course, unequivocally abundant in melanogen, but other neoplastic diseases also excrete an appreciable amount, and even a healthy person appears to excrete a trace of melanogen. The result that more excretion of melanogen is encountered in neoplastic diseases than in healthy persons suggests that the ferric chloride test and Thormählen's test should be performed also on non-neoplastic patients

in order to verify whether or not increase in the urinary excretion of melanogen would be a phenomenon peculiar to neoplastic cases.

Figure 5 shows the vicissitude of the daily excretion of melanogen in a patient with melanosarcoma who received nitromine treatment. It is of interest that an irregular fluctuation which was independent of the injection of nitromine was observed.

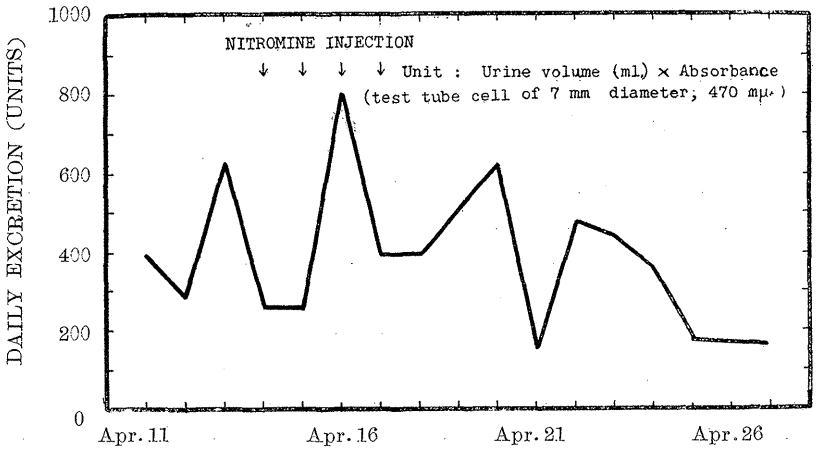

Fig. 5 Vicissitude of the daily excretion of melanogen in a patient with melanosarcoma who received nitromine treatment.

Summary and Conclusion

Ferric chloride test and Thormählen's reaction for the detection of melanogen was adapted to photoelectric colorimetry in order to measure the relative amount of this substance in urine. The procedure was given in detail in association with the results of observation carried out on a case of melanosarcoma and some other neoplastic diseases.

Reference

1) Emerson, K.: *Disorders of pigment metabolism,* Harrison, T.R.: Principles of Internal Medicin, Blakiston (New Yowk, Philadelphia, Tronto), 1951, (pp. 718–720).