Bacterium melaninogenicum—A Misnomer

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SUMMARY: The morphological and cultural characteristics of seven recently isolated strains of *Bacterium melaninogenicum* closely resembled those given by previous authors. Fermentation reactions were unsatisfactory, owing to the production of acid in basal media without added carbohydrate. The growth of two strains studied in detail was greatly facilitated by X factor and to a much smaller extent by V factor; it was best on chocolate agar. The strains were non-pathogenic in mice. Serologically, they behaved alike in precipitin and complement-fixation tests. There was insufficient information to classify them into subtypes.

The black pigment, extracted by a new technique which avoids the use at any stage of alkalis, proved to be haematin (not melanin), united in the cells with a bacterial protein to form a parahaematin. Crystalline pyridine haemochromogen and haemin have been prepared from the isolated pigment. The haemin when coupled with renatured ox globin gave reconstituted methaemoglobin, oxy- and carboxy-haemoglobins. Ultra-violet and visible spectral absorption data support the identification of the pigments. In particular, the possible reduction of the vinyl groups of the porphyrin nucleus by the organism, leading to meso- or deuterohaematin, has been excluded.

It is suggested that the organism be assigned to the genus *Fusiformis*, and the specific name be altered from *melaninogenicus* to *nigrescens*, making *Fusiformis nigrescens*.

In 1921 Oliver & Wherry isolated an anaerobic Gram-negative cocco-bacillus from the human throat, urine and faeces, and from an infected surgical wound. The organism grew on human blood agar slopes after 1–2 weeks' incubation, using up haemoglobin during growth and giving rise to a confluent, jet-black growth. Believing the pigment to be melanin, they named the organism *Bacterium melaninogenicum*. Since then several authors have mentioned the organism, and some have studied it in detail.

This paper records the properties of seven recently isolated strains of *Bact. melaninogenicum* and the identification of the characteristic brown-black pigment as haematin, not melanin.

The strains were as follows: J. S. from pyorrhoea alveolaris, associated with micrococci and non-haemolytic streptococci; no. 8680 from an abscess of the jaw, associated with an *Actinomyces bovis*; no. 3999 and no. 4814 from the throat in Vincent's angina; no. 1136 from an abscess of the neck, associated with an anaerobic streptococcus and aerobic and anaerobic Gram-negative bacilli; no. 1362 from an abscess of the nasal septum which also yielded a *Staphylococcus aureus* and a diphtheroid; 'M' from a case of 'black tongue', associated with anaerobic Gram-negative bacilli and anaerobic streptococci.

DESCRIPTION OF THE STRAINS

Morphology

Microscopically the organisms showed no remarkable variation either among the different strains or when the same strain was grown on solid or in fluid media. The cells were mostly ovoid, measuring $0.4-1.0 \times 0.3-0.4 \mu$. Occasional cells up to 8.0μ . long were encountered. Some cells showed bipolar staining; no capsules could be demonstrated. The organisms were non-motile, non-acid-fast and did not form spores.

Cultural characters

Two per cent horse blood agar plate. Five days at 37° : smooth, shiny, flat-convex, jet-black colonies, about 1 mm. in diameter, with an entire edge. Haemolysis was first evident, after 2-3 days, in the heavily inoculated part of the plate, and the plates were practically cleared of haemoglobin after 14 days (Pl. 1, fig. 1). The colonies were brown at first.

Gelatin enriched with Fildes's extract and 2% peptone (Evans). Four weeks at 37° : a semi-opaque, mucinous mass of growth with liquefaction of the gelatin.

Fluid media. Growth did not readily occur in simple broth or peptone water. In Fildes's broth (Fildes, 1920) a ropy, mucinous deposit was produced in 2-3 days.

Loeffler's serum slope. Two days at 37° : confluent, colourless, moist growth.

Metabolism. All the strains were strictly anaerobic, and required the presence of about 5% CO_2 in the gas phase. All cultures had a foul odour.

The effect on growth of various media was measured by the average size of ten discrete colonies attained under comparable conditions by the strains 1136 and 4814. On nutrient agar, strain 1136 grew only in minute colonies and strain 4814 failed to grow. When 2% Evans peptone (Evans' Medical Supplies, Ltd., London) was added to the nutrient agar, both strains gave small colonies. The largest colonies were obtained on chocolate agar, the mean diameters for the two strains were 1.80 and 2.45 mm. compared with 0.14 and 0.45 mm. on blood-saline agar.

The effect of incorporating X and V factors (Thjøtta, 1921), supplied as yeast extract and haemin respectively, in nutrient agar, was as follows:

Strain	Agar with V (mm.)	Agar with X (mm.)	Agar with X and V (mm.)
1136	0.23	2.13	2.37
4814	0.61	1.48	2.42

Since strain no. 4814 failed to grow on nutrient agar and the colonies of both strains were largest when both X and V factors were present, it is reasonable to assume that both factors facilitated growth.

In confirmation of Slanetz & Rettger's (1933) results with fusiform organisms, potato extract stimulated the growth of strain no. 4814. Cysteine, which they also recommended, had some beneficial effect. As might be expected from

the foregoing, all strains grew readily in Fildes's broth. A fall in pH took place in all fluids in which there was growth.

Biochemical. Fermentation reactions were impracticable because acid was produced in the basal media commonly used for sugar reactions. Six strains, incubated for a week in peptone (Evans) water, were indole-positive. In Fildes's agar stabs, six strains produced hydrogen sulphide, as judged by the blackening of lead acetate contained in a layer of agar on top of the stab.

Sensitivity to sulphonamides was tested on a series of nutrient agar laked-horse-blood slopes (Harper & Cawston, 1945) containing 220 mg./100 ml. of sulphanilamide, 8.5 mg./100 ml. of sulphapyridine, 18 mg./100 ml. of sulphathiazole, 4 mg./100 ml. of sulphadiazine, and less than 30 mg./100 ml. of sulphamethazine. The concentration of the sulphonamides was in each case a half-saturated solution at room temperature. At these concentrations, growth was inhibited only by sulphanilamide.

The minimal bactericidal concentration of penicillin was estimated in Fildes's broth; for five strains it was 0.3 unit/ml. and for the sixth 0.6 unit/ml.

Serology

Shevky, Kohl & Marshall (1934) produced rabbit antisera with titres of up to 1:10,240; clumping was granular rather than flocculent, and their strains were serologically homogeneous. Weiss (1937), following the technique of Heidelberger & Kendall (1931), extracted from two strains bacterial proteins which were antigenic. The proteins from these two strains were immunologically distinct by precipitin reaction. Cross-reactions occurred with group A *Strep. haemolyticus* antiserum. Weiss related his findings to those of Heidelberger & Kendall (1936) who used haemolytic streptococci, and suggested the existence of a conjugated carbohydrate protein, analogous to the artificial pneumococcus type III polysaccharide protein synthesized by Avery & Goebel (1931).

Preparation of anti-sera. A rabbit anti-serum was prepared by intravenous inoculation with growth washed off a blood-agar plate and killed in 0.25% formol saline. After 6 weeks its agglutination titre was 1:320. Since the granular nature of the suspensions made direct agglutination tests difficult to interpret, the precipitin and complement-fixation reactions were used.

Precipitin tests. The precipitin tests were set up by extracting growth from blood plates in 0.1 N-HCl for 10 min. at 100°, neutralizing with 0.5 N-NaOH, centrifuging and layering the supernatant fluid on to neat serum in the stem of a Pasteur pipette. An opaque ring was clearly visible at the interface of the two liquids within 10 min. All our strains reacted with the serum. No strain reacted positively with four sera prepared against strains of non-pigmented Gram-negative anaerobes of the *Fusiformis* type, isolated from human material during the last year; nor with Lancefield's A, B, C and G grouping sera (cf. Weiss, 1937).

Saline extracts, prepared by boiling a suspension for 5 min. or holding it at 37° for 2 hr., also gave a positive precipitin test. Intravenous inoculation

into a rabbit of the saline extract, which by chemical test contained carbohydrate but not protein, failed to produce any antigenic response after 6 weeks.

Complement-fixation tests. The technique described by Price (1933) for the preparation of gonococcal antigen was used to prepare extracts for complement-fixation. Using the homologous antigen at 1:30 the anti-serum fixed complement to a titre of 1:480. When the test was carried out simultaneously on all seven strains, using each antigen at 1:30, titres of 1:160 in four cases and 1:320 in three resulted.

In an attempt to relate the precipitinogen to the complement-fixing antigen, a suspension of the homologous strain was divided into three portions. From the first portion antigen was prepared as above. The second and third portions were extracted in normal saline for 10 min. at 100° and 2 hr. at 37°, respectively, centrifuged and precipitinogen demonstrated in the supernatant fluid. The supernatant fluid from the second extraction contained no demonstrable precipitinogen. The remaining deposits were then extracted by Price's technique. The extract from the first portion fixed complement at a serum titre of 1:320. The final extracts from the second and third portions partly fixed complement in the 1:20 dilution, thus showing that, when precipitinogen was removed from the bacterial cells, the latter were incapable of fixing complement in the usual way. It is therefore probable that the two serological activities, precipitation and complement-fixation, are referable to the same antigen. This is substantiated by the fact that saline extract fixed complement to titre.

Animal pathogenicity

Burdon (1932) described one strain which after subcutaneous inoculation into rabbits and guinea-pigs produced extensive cutaneous gangrene and death after 48 hr.; Weiss (1943) described similar severe effects in rabbits with recently isolated strains. Apart from these reports, claims for pathogenicity are somewhat unconvincing or based on indirect evidence.

Four tests were made of mouse pathogenicity. In the first intravenous, intraperitoneal and intramuscular injections were tried. In the second a recently isolated strain was inoculated intraperitoneally. In the third and fourth, another recently isolated strain was inoculated intraperitoneally, suspended in 2.5 and 5.0% 'biomucine' (Laboratoires Robert et Carrière). The results were uniformly negative.

Comparison with previously described strains

Reference to the literature shows that the organism has been recovered chiefly from the mouth, genitalia and sites of chronic suppuration. It is a strict anaerobe, grows slowly, and is difficult to separate from contaminants. Cultures have a foul smell. Morphologically it is Gram-negative, non-motile, non-sporing and non-acid-fast. Shevky *et al.* (1934) and Weiss (1943) claim to have seen a capsule in clinical material and in smears from animal autopsy respectively, but other authors do not describe capsule formation. There is

slight variation in the descriptions of the bacterial cell, but all agree that it is polymorphous and that bacillary and coccal forms coexist. Shevky *et al.* (1934) mention bipolar staining in the coccal forms.

The black pigment is formed slowly, and its formation is accompanied by the disappearance of haemoglobin from the medium. No pigment is formed in the absence of blood; Shevky *et al.* (1934) say that on serum agar there is late production of a brownish pigment.

These observations are in substantial agreement with those made in this paper. On the biochemical reactions of the organism there is wide disagreement, especially with regard to fermentation reactions. There is considerable variation of opinion on the capacity of this organism to liquefy gelatin; most authors give the indole reaction as positive.

There is, nevertheless, sufficient correspondence to leave no doubt that the organisms described in this paper are identical with those previously described as *Bact. melaninogenicum*.

THE PIGMENT

Hitherto it has been generally accepted that the black pigment produced by the organism is melanin or a melanin-like pigment. Considering that the pigment is formed only in the presence of blood, that its formation coincides with the disappearance of haemoglobin and that neither adrenalin nor ascitic fluid assist in its formation (Shevky *et al.* 1934), it seemed more likely to be a breakdown product of haemoglobin.

The evidence upon which the pigment was first described as a melanin by Oliver & Wherry (1921) is entirely inadequate. They noted its insolubility in several organic solvents and recorded that it occurred as extracellular amorphous masses; it was also found to dissolve slowly in sodium hydroxide solution, but apparently no spectroscopic investigations were made. Shevky *et al.* (1934) confirmed the insolubility of the pigment in solvents such as acetone, chloroform, etc., and its solution in weak alkalis, again without recording any spectroscopic examination.

Our own observations have shown that the pigment is readily dissolved from the bacterial growth by pyridine, in which it forms a greenish brown solution with maximum spectral absorption in the region of 533m μ . Addition of a small quantity of sodium hydrosulphite (Na₂S₂O₄) produced a rapid change in colour to brownish red, and at the same time the intense and characteristic absorption bands of pyridine haemochromogen (pyridine ferroprotoporphyrin) appeared (maxima 557·4 and 528·3 m μ). This behaviour is consistent with that of a haem pigment, but in view of the fact that the organisms had been removed from an agar plate originally containing blood, rigorous purification to remove all haemoglobin derived from the medium was essential before such a test could be considered unequivocal. It was, in addition, highly desirable that crystalline derivatives should be prepared in order to support any spectroscopic evidence. It is, however, well known that treatment with alkalis such as sodium hydroxide, the only good and easily manipulated solvent for

haematin, so alters this pigment that it is frequently impossible to prepare crystalline derivatives from it after such extraction (Fischer, 1924; Keilin, 1943). Moreover, solution of haematin in sodium hydroxide and reprecipitation at the isoelectric point failed to eliminate accompanying protein, which largely reprecipitated with the pigment. A new extraction and purification technique was required which would avoid the use at any stage of sodium hydroxide. Such a technique was found in the use of 90% phenol as described below.

Extraction and purification of the pigment

Plates were used that showed a good growth of organisms and from which the haemoglobin had almost entirely disappeared. The growth was removed with as little adhering agar as possible, stirred up with water at 50–60° and centrifuged, the supernatant being discarded. Washing in this manner was repeated until all traces of agar and haemoglobin were removed. The pigmented organisms were then stirred with about 20 times their volume of 90% phenol (10 ml. of water + 90 g. phenol). Extraction of the colouring matter proceeded slowly, and it was found convenient to remove each lot of phenol solution by centrifugation after 2–4 hr. contact (first extraction 12 hr.) with the bacterial mass. When the solvent no longer extracted spectroscopically detectable pigment (i.e. a well-defined broad band with centre 627·5 m μ .) from the cell debris, the extracts were combined, and to the dark brown solution twice its volume of absolute ethanol was added. This served to precipitate a small quantity of dissolved protein which was removed by a no. 4 sintered glass filter.

The solution was then dialyzed in a collodion bag (Visking) against tap water. Separation of two liquid phases soon occurred, and it was necessary to mix the contents of the bag frequently. Finally, when all but a trace of phenol was removed, the dark pigmented bottom layer showed signs of turbidity due to precipitating pigment. It was found convenient to remove it at this stage into a centrifuge tube, add plenty of distilled water, stir, centrifuge down the granular pigment and repeat the water washing two or three times.

We believe that by this process we obtained the pigment with as little chemical alteration as possible. That it no longer contained or was associated with protein was demonstrated by the fact that a solution in dilute NaOH, when reduced by Na₂S₂O₄, gave only the bands of reduced haem and no trace of a haemochromogen. Dissolution in alkali was, however, rather slow, a phenomenon frequently encountered with preparations of haematin isolated from natural sources.

Preparation of crystalline derivatives

When a small portion of the isolated pigment was mixed on a glass slide with a drop of Takayama's reagent (Harrison, 1944) it dissolved. A cover-slip was applied, and after a few minutes the colour changed to the red of pyridine haemochromogen and crystal masses were visible under the microscope. Even

when prepared from pure haematin the shape of these crystals was variable, ranging from prismatic needles grouped in stellate clusters to flat overlapping plate-like structures. We observed that the type of crystal produced depended somewhat upon the maturity of the Takayama reagent and hence on the velocity of reduction, and also that the needles first formed tended to become transformed on the slide into plates. Control experiments were therefore always done at the same time with pure crystalline haemin. Pl. 1, figs. 2-7, record some of the preparations obtained.

The haemin (Pl. 1, fig. 8) was similarly obtained from the bacterial pigment by warming on a slide with acetic acid-KCl mixture, as in preparing Teichmann's crystals from blood.

Properties and nature of the pigment

That the pigment isolated by the phenol method was a haem pigment was readily demonstrated by the spectroscopic examination of its solutions in different solvents, by the formation of a pyridine haemochromogen and, lastly, of a porphyrin when the pigment was treated with concentrated sulphuric acid. These results are presented in Table 1, where the data for haemin similarly treated are given for comparison. The specimen of haemin was a sample recrystallized as described by Rimington (1942), and all measurements were made with the Hartridge reversion spectroscope. It will be seen from the spectroscopic data that in all respects the bacterial pigment very closely resembled haematin (ferriprotoporphyrin). The possibility, however, had to be borne in mind that a reduction of the two vinyl groups $-\text{CH}=\text{CH}_2$ of the protoporphyrin nucleus might have been brought about by the bacterial cell, leading to mesohaematin, the Fe complex of mesoporphyrin, or even that the vinyl side chains might have been completely removed, resulting in deuterohaematin, the Fe complex of deuteroporphyrin. Both these porphyrins have been found in faeces, where they are undoubtedly derived from protoporphyrin by bacterial action (Zeile & Rau, 1937).

The close correspondence of the absorption spectra of the pigment of *Bact. melaninogenicum* with haematin, and of the porphyrin derived from it with haematoporphyrin rather than mesoporphyrin or deuteroporphyrin (see Table 1), strongly suggests that the vinyl groups remain intact. Drabkin (1942) has shown that the presence of free vinyl groups in haem pigments displaces the absorption maxima about 11 m μ . towards the red, the α band of pyridine haemochromogen, for example, lying at 558 m μ . and that of pyridine mesohaemochromogen at 547 m μ . Further evidence was obtained, however, by coupling the bacterial pigment with native globin to form methaemoglobin, reducing this to oxyhaemoglobin with Stokes's reagent, and measuring the absorption bands of these pigments and the CO haemoglobin obtainable by saturating the solution of the oxy compound with coal gas. Measurements were also made of the Soret bands in the near ultra-violet, and a complete set of data was simultaneously obtained upon an authentic specimen of reconstituted haemoglobin for which the starting point was pure crystalline haemin.

Preparation of reconstituted haemoglobins

Globin hydrochloride was prepared from ox blood (ox globin being relatively more stable than horse or human globin) and partially renatured by dialysis for 24 hr. against running tap water according to the method of Anson & Mirsky (1929-30). Dialysis was completed against $M/30\text{-K}_2\text{HPO}_4$ solution at

Table 1. *Spectroscopic data obtained from the bacterial pigment and from crystalline haemin*

Solvent, treatment, etc.	Pigment from <i>Bact.</i> <i>melaninogenicum</i> (wave-length, m μ .)	Haemin (wave-length, m μ .)	Remarks
0.1 N-NaOH extract of growth	613.2	—	—
Do. + Na ₂ S ₂ O ₄	558.3	—	{ Haemochromogen type spectrum
	527.9	—	
Pyridine extract of growth	532.8	—	—
0.1 N-NaOH solution of isolated pigment	613.2	615.7	Broad band
Do. + Na ₂ S ₂ O ₄	573.8	575.0	Broad band
	535.5	540.0	Broad band
0.1 N-NaOH solution + pyridine + Na ₂ S ₂ O ₄	557.2	557.2	Very sharp
	527.9	529.8	—
90 % phenol	627.1	627.6	—
	540.3	540.0	—
Acetic acid	629.1	633.1	Broad band
	538.2	541.4	Broad band
Ether-HCl	637.6	637.6	—
	543.6	543.7	—
Conc. H ₂ SO ₄	601.9	601.7	Very sharp
	557.2	556.6	—
Porphyrin from above	625.6	625.8	Very sharp
in pyridine	579.6	579.3	—
	540.0	540.3	—
	507.5	507.8	—
<i>Haematoporphyrin</i> in H ₂ SO ₄	—	601.6	Very sharp
	—	554.3	—
<i>Mesoporphyrin</i> in H ₂ SO ₄	—	594.5	—
	—	551.9	—

the pH of which denatured globin is insoluble. After centrifugation the supernatant liquid, containing the renatured protein, was carefully adjusted by KH₂PO₄ solution to a pH of about 6.0. To separate portions of this solution were added solutions of bacterial pigment or crystalline haemin, respectively, in dilute NaOH, until the band of methaemoglobin at about 630 m μ . was clearly visible. After this measurement, freshly prepared Stokes's reagent (Harrison, 1944) was added drop by drop until the colour changed from brown to red and the methaemoglobin spectrum had given place to that of oxy-

haemoglobin. Over-reduction led to the single, broad band of reduced haemoglobin (purple-coloured solution), but shaking with air restored the two-banded HbO₂ spectrum. This demonstrated incidentally the capacity of the synthetic globin-pigment complex made from the bacterial pigment to function, like haemoglobin, as an oxygen carrier.

It was observed that during the addition of the Stokes's reagent some unavoidable denaturation occurred, so the solutions were centrifuged before proceeding further from this stage. For the measurement of the Soret bands, the reconstituted haemoglobins were equilibrated to pH 7·2 by dialysis against M/80-phosphate buffer, since the exact position of this maximum is influenced

Table 2. *Spectroscopic data from reconstituted haem-ox globin pigments*

Pigment type	Bacterial pigment-ox globin (wave-length, mμ.)	Haematin-ox globin (wave-length, mμ.)
Met Hb (alk.)	596·7 538·9	— —
Met Hb (acid)	631·4	631·8
HbO ₂	577·7 539·6	577·7 539·4
HbCO	574·5 537·2	572·1 537·8
Pyridine haemo- chromogen	557·1 528·3	557·2 529·8
Soret bands		
HbO ₂	405·5	405·8
HbCO	416·3	416·0

by the pH of the solution. Good agreement between the reconstituted bacterial pigment and that from haemin was again found for both the oxy and carboxy derivatives (Table 2). The instrument used was a Hilger medium quartz spectrograph, and the intensity of the Soret bands was found to be of the order of 10 times that of the visible bands, as is the case with normal native oxy-haemoglobin. The actual maxima of the Soret bands were in each case at a wave-length shorter than that usually observed in the case of native HbO₂ and HbCO: 405·5 mμ. as compared with the usual 414·5 mμ. for HbO₂ and 416·3 mμ. as compared with the usual 420·0 mμ. for HbCO.

These shorter wave-lengths are those observed for the Soret bands on solutions of normal native HbO₂ and HbCO respectively which have been kept for some time and have apparently undergone some preliminary form of denaturation, which does not, however, appear to affect the visible band wave-lengths. In the case of the reconstituted bacterial pigments, the visible bands agreed closely with those of normal native HbO₂ and HbCO. The wave-lengths observed for the Soret band in these reduced reconstituted pigments and in partially denatured haemoglobins almost coincided with that of methaemoglobin. The presence of a Soret band is in itself strong evidence for the existence of the intact porphyrin ring.

State of the pigment in the bacterial growth

From all the evidence assembled above there remains no reasonable doubt that the pigment extracted from a culture of the organisms is identical with haematin (ferriprotoporphyrin). The next question to be considered was whether it was present in the bacterial mass as such or combined with bacterial protein; extractants such as pyridine and phenol might easily free haematin from a weak chemical union. The fact that solutions of the purified pigments in dilute NaOH gave no haemochromogen on reduction but that a simple extract made by stirring the growth with the same solvent afforded an intense haemochromogen spectrum on addition of $\text{Na}_2\text{S}_2\text{O}_4$, leads us to believe that in the cultures the haematin is united to a protein of the bacterial protoplasm, forming a parahaematin. This, indeed, might be anticipated from the known ease with which labile compounds are formed between proteins and the haem pigments.

DISCUSSION

The medical importance of *Bacterium melaninogenicum* is difficult to assess, and the observations in this paper have no direct bearing on it. The frequent association of the organism with pyorrhoea alveolaris, as noted by Liebetrueth (1934) and Burdon (1928), is perhaps worthy of mention. By breaking down extravasated blood the organism might well be responsible for the black flakes which form in the gum pockets of pyorrhoea.

Failure to isolate the organism in routine cultural work is no doubt related to its slow growth and carbon dioxide requirements. The carbon dioxide requirement is the probable explanation of the difficulty previous workers have found in maintaining the organism in pure culture. The biochemical reactions reported in the literature are not sufficiently reliable to attempt classification into biochemical types, and there is insufficient information on serological work to deny or affirm the existence of serological types.

The organism is classified by Wilson & Miles (1945) in the group *Fusiformis*, and its morphological and cultural characteristics are quite in accordance with their general definition of the group.

The name *melaninogenicum* is a misnomer, indicating a biochemical activity of which the organism is incapable. By analogy the name should now be *haematinogenicum*; this, however, is long and ugly, and we suggest in place of the inaccurate *melaninogenicum*, the species name *nigrescens*, indicating accurately the progressive production of a black pigment.

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EXPLANATION OF PLATE

- Fig. 1. 6-day-old culture of *Bact. melaninogenicum* showing clearing of medium by the organism; original size.
- Fig. 2. Pyridine haemochromogen from pigment of *Bact. melaninogenicum*. Prismatic needle forms with some plates in aggregates; $\times 760$.
- Fig. 3. Pyridine haemochromogen from pigment of *Bact. melaninogenicum*. Transition into flat plates; $\times 570$.
- Fig. 4. Pyridine haemochromogen from pigment of *Bact. melaninogenicum*. Overlapping thin plates, frequently with upturned edges; $\times 760$.
- Fig. 5. Pyridine haemochromogen from haemin. Needle forms in aggregates; $\times 200$.
- Fig. 6. Pyridine haemochromogen from haemin. Overlapping thin plates (note upturned edges); $\times 870$.
- Fig. 7. Pyridine haemochromogen from haemin. Final form; $\times 300$.
- Fig. 8. Haemin from pigment of *Bact. melaninogenicum*; $\times 570$.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8