2 species were not detected under the same conditions when oxygen consumptions were measured.

It can therefore be presumed that the preferential oxygen consumption after the addition of β-D-glucose versus α-D-glucose in germinated spores of both species is due to the production of β-D-glucose specific dehydrogenase in the germinated spores.

By contrast, vegetative cells of both species in which the activity of β-D-glucose dehydrogenase was hardly detectable, do not show any preferential oxygen consumption by β-D-glucose to α-D-glucose. The reasons why the vegetative cells of both species oxidize α- and β-D-glucose equally well, however, should be studied in future.

Our studies on the different effects of anomers of D-glucose for the germination of spores of *B. megaterium*[15] in comparison to *B. subtilis*, will give some interesting suggestions on the metabolism of α- and β-anomers of D-glucose in other bacteria, plants and higher animals[16].

1 Y. Hachisuka, N. Asano, M. Kaneko and T. Tanabe, Science *124*, 174 (1956).
2 J.A. Bach and H.L. Sadoff, J. Bact. *83*, 699 (1962).
3 K. Tochikubo, J. Bact. *108*, 652 (1971).
4 Y. Fujita, R. Ramaley and E. Freese, J. Bact. *132*, 282 (1977).
5 L.K. Shay and J.C. Vary, Biochim. biophys. Acta *538*, 284 (1978).
6 C. Prasad, M. Diesterhaft and E. Freese, J. Bact. *110*, 321 (1972).
7 T. Ohkubo, J. Nagoya City Univ. Med. Ass. *28*, 486 (1977).
8 M. Kondo and J.W. Foster, J. gen. Microbiol. *47*, 257 (1967).
9 I. Miwa, J. Okuda, H. Niki and A. Niki, J. Biochem. *78*, 1109 (1975).
10 J. Okuda and I. Miwa, Analyt. Biochem. *43*, 312 (1971).
11 J. Okuda and I. Miwa, Meth. biochem. Analysis *21*, 155 (1973).
12 I. Miwa and J. Okuda, J. Biochem. *75*, 1177 (1974).
13 M. Goldman and H.J. Blumenthal, J. Bact. *87*, 377 (1964).
14 W.M. Waites, D. Kay, I.W. Dawes, D.A. Wood, S.C. Warren and J. Mandelstam, Biochem. J. *118*, 667 (1970).
15 I. Miwa, H. Ishihara, H. Fujii, H. Nagano and J. Okuda, J. Biochem. *85*, 315 (1979).
16 J. Okuda, Trends Biochem. Sci. *3*, 161 (1978).

Experimental studies of schistosomal pigment from *Schistosoma japonicum*

M. Sano and A. Ishii

Department of Parasitology, Hamamatsu University, School of Medicine, Hamamatsu (Japan), 31 May 1978

Summary. The schistosomal and malarial pigments were distinguishable before and after extraction from the host liver. Presence of iron in both pigments was ascertained by the elemental X-ray analysis. Histochemically, however, schistosomal pigment was similar to that of malarial pigment.

Adult schistosomes and malarial parasites both produce brownish-black pigments derived from haemoglobin, which accumulate in the liver of parasitized animals[1-3]. Malarial pigment has been extensively examined, but schistosomal pigment has been less well studied. Recently, it was reported that the purified malarial pigment contained about 1% iron oxidized to the ferric state[4].

A considerable number of different types of pigments in tissues have been characterized histochemically[5,6]. The haemoglobin derivatives can be demonstrated by a number of histochemical method[6,7], but the nature of schistosomal pigment is not well understood. The present publication deals with the purification and histochemical study of the schistosomal pigment.

Materials and methods. 1. Parasites. Male albino mice (ddY strain) were infected by i.p. injection with *Plasmodium berghei* (Yoeli-NK 65 strain), or with *Schistosoma japonicum* (Yamanashi strain). When not less than 70% of the erythrocytes were infected with malarial parasite, blood was collected, red cells were lysated in distilled water, and lysate was stored at −20 °C. Adult schistosomes were recovered 6–8 weeks later and fixed for electron microscopy. Pigment-rich livers of white mice infected with both parasites were also removed and stored until required.

Histochemical characteristics of pigments

Reaction	Pigments Melanin	Malarial	Schisto-somal	For-malin
Perls	−	−	−	−
Schmorl	+	−	−	−
Masson-Fontana	+	−	−	−
Method I*	−	+	+	+
Method II**	+	−	−	−
Hydrogen peroxide	+	+	+	+
Formic acid	−	+	+	+
Acetic acid	−	±	±	+
Hydrochloric acid	−	−	−	−
Sodium hydroxide	−	+	+	+
Alcoholic picric acid	−	+	+	+
Alcoholic sulfuric acid	−	+	+	+

* Extraction method for malarial and formalin pigment. ** Extraction method for melanin.

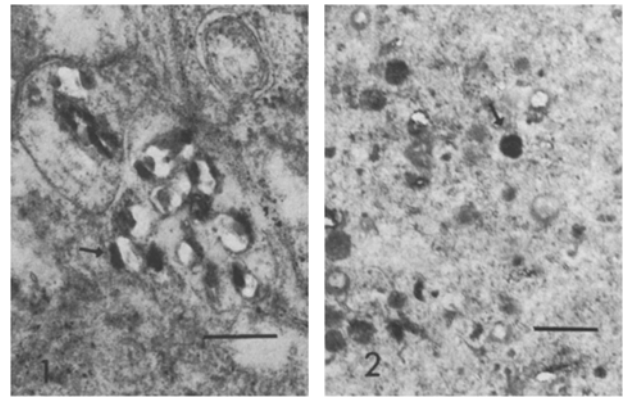

Fig. 1. Pigment (arrow) within parasite. 1. Malarial pigment. 2. Schistosomal pigment. Scale: 1 μm.

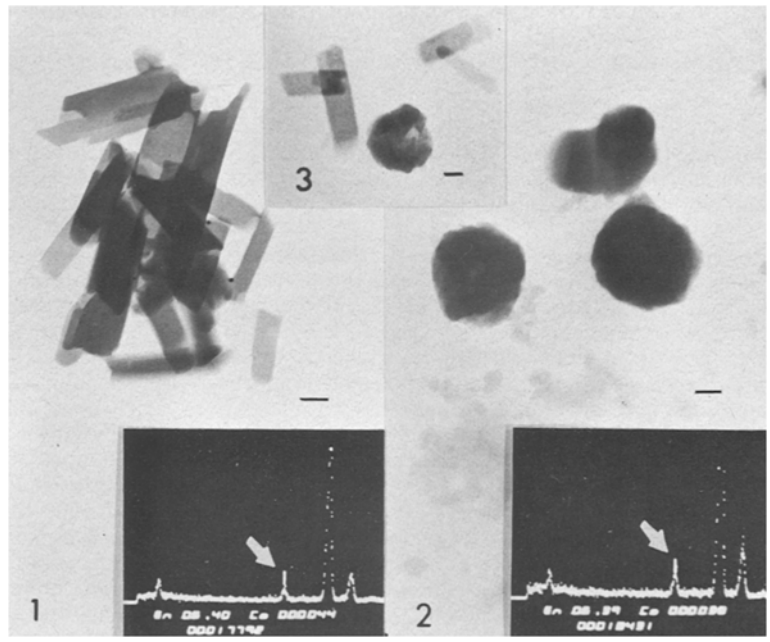

Fig. 2. 1. Extract of malarial pigment. 2. Extract of schistosomal pigment. 3. Extract of mixed pigments from liver infected with *S. japonicum* and *P. berghei*. Scale: 0.1 µm. Insets: Elemental X-ray analysis. Arrow indicates the peak of Fe.

2. Preparation of pigment. Both pigments were prepared as described by Homewood et al.[4,8]. a) Schistosomal pigment: Pigment was obtained from the livers of white mice infected with *S. japonicum*. Livers were homogenized with distilled water, washed with 0.1% SDS by centrifugation at 40,000 × g for 30 min at 10 °C, and finally suspended in 0.1 M Tris-HCl buffer pH 7.5, containing 1 mg/ml pancreatin and 20 µg/ml chloramphenicol. The suspension was incubated at 37 °C for at least 2 days and then pigment was prepared for electron microscopy. b) Malarial pigment: Pigment was obtained from the lysate of infected red cells and livers. The procedure was essentially the same as described above.

3. Electron microscopy. An aqueous suspension of pigment was spread on a supporting grid covered with formval coated with carbon[9] and examined with JEM-100C electron microscope. The elemental X-ray analysis of pigments was also performed[10]. The other specimens were fixed at 4 °C for 1 h in 2% glutaraldehyde and postfixed for 1 h in 2% osmium tetroxide, both of which were buffered with Sorensen's phosphate, pH 7.0. After dehydration, they were embedded in Epon 812. The ultrathin sections were stained with uranyl acetate and lead for examination.

4. Histochemistry. Freezing method was used in this study. Tissues were frozen by Fleon 22 and sections were prepared with COLDTOME CM-41 microtome. The pigments examined histochemically[6] included melanin, formalin, malarial and schistosomal pigment.

Result and discussion. The schistosomal pigment in schistosome was obviously quite different in appearance and in size from the apparantly crystalline malarial pigment in malarial parasite (figure 1). As Moore et al.[11] reported, these differences are supposed to be due to the differences in the mode of formation of pigment by the 2 parasites.

Schistosomal and malarial pigment were clearly distinguishable before and after extraction from livers of white mice infected with *S. japonicum* and *P. berghei* respectively, or infected with both parasites (figure 2). Also, malarial pigments from liver and from lysate were the same in appearance and in size. Elemental X-ray analysis clearly showed the presence of iron in the pigment located in the parasite as well as in the purified pigment (figure 2, insets).

The result of histochemical study of melanin, formalin, malarial and schistosomal pigment was summarized in the table. Different views have been presented on the histochemical characteristics of schistosomal pigment. Johnson et al.[1] found that the histochemical reactions of pigment of schistosomiasis were identical with those of malarial pigment. On the other hand, Sawada et al.[12] indicated that the pigment produced in mouse by *S. japonicum* was analogous to melanin. According to our present study, the schistosomal pigment showed negative Schmorl's and negative Masson-Fontana's reaction, and was not extracted by method II, the extraction method for melanin. Therefore, the schistosomal pigment was dissimilar to melanin. However, the schistosomal pigment had similar characteristics to malarial and formalin pigment. In addition, by the negative Perls' reaction, it was evident that the schistosomal pigment was not haemosiderin. In this study, the schistosomal pigment had not the characteristics of melanin, but that of malarial and formalin pigment.

1 F.B. Johnson, P.K. Hamilton and M.F. Gridley, J. Histochem. Cytochem. *2*, 481 (1954).
2 J.D. Ostrow and K.S. Warren, J. clin. Invest. *44*, 1083 (1965).
3 T. Deegan and B.G. Maegraith, Ann. trop. Med. Parasit. *50*, 208 (1956).
4 C.A. Homewood, G.A. Moore, D.C. Warhurst and E.M. Atkinson, Ann. trop. Med. Parasit. *69*, 286 (1975).
5 A.G.E. Pearse, in: Histochemistry, vol. 2, p. 1090. Churchill Livingstone, Edinburgh and London 1972.
6 J.D. Bancroft, in: Histochemical Techniques, p. 182. Butterworths, London and Boston.
7 A.G.E. Pearse, in: Histochemistry, vol. 2, p. 1075. Churchill Livingstone, Edinburgh and London 1972.
8 C.A. Homewood, J.M. Jewsbury and M.L. Chance, Comp. Biochem. Physiol. *43B*, 518 (1972).
9 D.C. Pease, in: Histological Techniques for Electron Microscopy, p. 206. Academic Press, New York and London 1966.
10 Acknowledgment. The authors would like to express the thanks to JEOL Co., Ltd for the elemental X-ray analysis of pigments.
11 G.A. Moore, C.A. Homewood and H.M. Gilles, Ann. trop. Med. Parasit. *69*, 374 (1975).
12 T. Sawada, K. Hara, K. Takagi, Y. Nagazawa and S. Oka, Am. J. trop. Med. Hyg. *5*, 858 (1956).