coefficient, $r=-0.58$, $z=-0.66\pm0.24$, $p<0.006$, was obtained.

The data of the table were examined to learn whether the differences between alternate and adjacent r's differed significantly from random in their systematic drifts seen in figure 2. The r's were calculated for each of 4 selected time spans (10/29/74 to 7/21/75; 7/22/75 to 2/3/76; 2/4/76 to 1/14/77; and 1/17/77 to 4/12/77) and transformed to z's. For the unlined cages, all except the third of these spans of data showed statistically significant deviations from randomness ($z=-0.347$) independently for adjacents and alternates. p ranged from <0.05 to <0.002. The mumetal cages showed no such significant differences, even when the only 3 consecutive negative or positive values were selected. When the differences are positive the interactions are favoring alternate signs of the differences from means, $+-+-$ or $-+-+$, and when negative, the same signs, $++--$ or $--++$.

Definite interactions must be occurring to permit closely the same pattern, though now inverted, to exist between the 2 4-cage series. Also, interactions must account for the significant systematic upward trend of the differences over the period of this study. Both describe intra-group patterns of association.

Since the effectiveness of a mumetal shield essentially disappears for dynamic, or oscillating, magnetic fields, an oscillating biomagnetic field would pass the shield with relative ease. The results, therefore, suggest strongly that dynamic biomagnetic fields are involved in the interactions. Such low-energy fields not only can be generated readily by living systems but great specificity in their characteristics is theoretically obtainable.

Inversional tendencies between simultaneous studies have been reported between day by day rates of bean water uptake in the open laboratory and under the same conditions except in a ferric-metal sheathed room[17,18]. It seems probable, therefore, that differences in ambient magnetic field levels are responsible for the inverted fluctuations.

This study, together with the earlier[2], demonstrates that significant departures from randomness can occur as a consequence of interactions, with either the adjacents or alternates the more negative. Significant drifts in departure directions can occur systematically over many months. It is conceivable that biomagnetic fields and the mitogenetic radiation of Gurewitsch[19] may be the same. The inversional propensity demonstrated here could have effected the frequent failures to confirm the phenomenon which led ultimately to cessation of its study.

Should organismic responsiveness to dynamic magnetic fields prove widespread or universal, as seems probable, constant experimental conditions would be virtually unattainable. Current enigmas such as biological senses of time and space could gain new parameters of informational input.

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Different effects of D-glucose anomers for respiration of bacterial germinated spores

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Summary. Effects of α- or β-D-glucose on the respiration of germinated spores (only germinated spores not including swollen spores and elongated spores) of *Bacillus subtilis* and *B. megaterium* were studied. In our conditions, net amount of oxygen consumed by 10^{10} germinated spores of *B. subtilis* per min after addition of α- or β-D-glucose was 1.6 µg or 6.6 µg ($\beta/\alpha=4.13$), while that by *B. megaterium* was 4.5 µg or 6.8 µg ($\beta/\alpha=1.51$), respectively. However, the net amounts of oxygen consumed by 10^{10} vegetative cells per min after addition of α- or β-D-glucose were identical, for *B. subtilis* in both cases 443.0 µg and for *B. megaterium* in both cases 604.4 µg.

Hachisuka et al. reported in 1956 that dormant spores of *Bacillus subtilis* do not consume oxygen, but that germinated spores rapidly oxidize D-glucose added to a suspension culture of spores[1]. It is well-known that the dormant spores of *B. subtilis* and *B. megaterium* have respiratory enzymes for D-glucose oxidation, but the activities of these enzymes are generally not detected. The activities appear distinctly only after germination[2–7]. We were interested in finding out whether the germinated spores of *B. subtilis* strain NRRL B558 and *B. megaterium* strain QM B1551 can distinguish α- from β-anomers of D-glucose for respiration or not.

Materials and methods. B. subtilis strain NRRL B558 and *B. megaterium* strain QM B1551 were used and maintained on nutrient agar slant. Cultures on nutrient broth (liquid), inoculated with a loopfull from an overnight culture on nutrient agar slants, were incubated at 37 °C with vigorous shaking (120 strokes/min). Vegetative cells were harvested by centrifugation (9000 g × 30 min) at 4.5 h after inoculation and washed 3 times with chilled saline.

To prepare dormant spores, growth cultures of the 2 species were inoculated as described above, and *B. subtilis* was grown and sporulated on nutrient agar plates at 37 °C for

Oxygen consumptions of *B.subtilis* and *B.megaterium* after addition of α- or β-D-glucose

			Oxygen consumption (μg)* Non glucose	α-D-glucose	β-D-glucose	β-D-glucose** / α-D-glucose**
B.subtilis	Dormant spores		0.1	0.1 (0.0**)	0.1 (0.0**)	-
NRRL B558	Germinated spores	a)	0.6	2.2 (1.6**)	7.2 (6.6**)	4.13
	Vegetative cells		221.5	664.5 (443.0**)	664.5 (443.0**)	1.0
B.megaterium	Dormant spores		0.1	0.1 (0.0**)	0.1 (0.0**)	-
QM B1551		a)	1.2	5.3 (4.1**)	7.5 (6.3**)	1.54
	Germinated spores	b)	1.2	5.7 (4.5**)	8.0 (6.8**)	1.51
		c)	1.2	5.3 (4.1**)	7.3 (6.1**)	1.49
	Vegetative cells		120.8	725.2 (604.4**)	725.2 (604.4**)	1.0

* Oxygen consumption shows oxygen (μg) consumed/10^{10} spores or cells/min. The experiments of oxygen consumption were carried out with 10^{10} dormant spores, 5×10^9 germinated spores, 3×10^8 vegetative cells of *B.subtilis* and 10^{10} dormant spores, 2.5×10^9 germinated spores, 2×10^8 vegetative cells of *B.megaterium*, respectively. a) Spores germinated with 1 mM L-alanine in 50 mM tris buffer (pH 7.8). b) Spores germinated with 1 mM D-glucose in 50 mM tris buffer (pH 7.8). c) Spores germinated with 10 mM KNO_3 in 50 mM tris buffer (pH 7.8). ** Net oxygen consumption.

10 days, while *B.megaterium* on plates with sporulation agar[8] at 37 °C for 4 days. Spores were harvested, washed repeatedly with cold deionized water until no sporangial debris could be detected by light microscopy, then lyophilized to dryness and stored.

To prepare germinated spores, dormant spores were heat-activated at 65 °C for 15 min and incubated in 0.05 M tris-HCl buffer, pH 7.8, at a final concentration of 0.6 OD at 660 nm (OD_{660}, 10 mm cell). The spores of *B.subtilis* were germinated at 37 °C for 90 min by addition of L-alanine (1 mM) and of *B.megaterium* by addition of L-alanine (1 mM), equilibrated D-glucose (1 mM), or KNO_3 (10 mM). Germination was estimated by a decrease in OD_{660} and confirmed by observation under a phase contrast microscope. After germination, the OD_{660} for *B.subtilis* or *B.megaterium* was decreased to 53% or 66% respectively, of that before germination.

To count germinated spores, dormant spores were suspended (OD_{660} 0.6) and germinated as described above. The suspension of germinated spores was heated at 70 °C for 30 min and diluted 10^5-fold. The diluted suspension (0.1 ml) was inoculated on nutrient agar plate. After cultivating at 37 °C for 24 h, viable cells were determined by colony counting.

Pure α-D-glucose was prepared by crystallization from equilibrated D-glucose in 80% acetic acid and β-D-glucose was made by crystallization from equilibrated D-glucose in anhydrous pyridine as described before[9]. The anomeric purities of the α- and β-anomers of D-glucose thus prepared were established to be better than 98% by our method for anomer determination[10,11] using β-D-glucose oxidase [EC 1.1.3.4], hog kidney aldose 1-epimerase [mutarotase, EC 5.1.3.3] and polarographic oxygen electrode (model 777, Beckman Instr. Inc., Irvine, Cal, USA). Oxygen consumptions by dormant, germinated spores and vegetative cells were measured using an apparatus equipped with an oxygen electrode (model 777, Beckman Instr. Inc.), which was depicted in our previous paper[12]. Spores or cells of the 2 species (1×10^{10} dormant spores of both species, 5×10^9 germinated spores and 3×10^8 vegetative cells of *B.subtilis*, 2.5×10^9 germinated spores and 2×10^8 vegetative cells of *B.megaterium*) suspended in 1 ml of 0.9% NaCl containing 10 mM tris-HCl, pH 7.2, were kept in the vial of the oxygen electrode at 37 °C for 3 min. After recording the rate A of oxygen consumption without D-glucose, 20 μl of 500 mM α- or β-D-glucose prepared just before use was added to give a final concentration of 10 mM, and the rate B of oxygen consumption in the presence of each anomer of D-glucose was recorded. The difference B-A of both rates was regarded as a rate of oxygen consumption caused by addition of each anomer of D-glucose. Mutarotase activities of suspensions of dormant and germinated spores or vegetative cells of the 2 species were estimated by our method[12].

Results and discussion. After adding α- or β-D-glucose to the suspensions of dormant spores of both *B.subtilis* and *B.megaterium*, oxygen consumptions were below 0.1 μg/10^{10} spores/min within the first 3 min as shown in the table. These results support previous data showing that the respiratory enzymes for glucose oxidation were present in the dormant spores, but that the activities of these enzymes were scarcely detectable[13].

When each anomer of D-glucose was added to the suspensions of germinated spores of *B.subtilis*, oxygen consumption/10^{10} spores/min after addition of α-D-glucose was found to be 1.6 μg and that after addition of β-D-glucose was 6.6 μg, therefore,

$$\frac{\beta\text{-D-glucose}}{\alpha\text{-D-glucose}} = \frac{6.6}{1.6} = 4.13.$$

In the spore suspensions of *B.megaterium*, germinated with D-glucose-tris buffer as germinants, oxygen consumption/10^{10} spores/min after addition of α-D-glucose was 4.5 μg and that after addition of β-D-glucose was 6.8 μg, therefore,

$$\frac{\beta\text{-D-glucose}}{\alpha\text{-D-glucose}} = \frac{6.8}{4.5} = 1.51.$$

The ratio of oxygen consumptions of β-D-glucose to α-D-glucose was 1.54 or 1.49 in the spores germinated with L-alanine-tris buffer or KNO_3-tris buffer. It can therefore be presumed that the difference of the β-/α-D-glucose ratio between *B.subtilis* (4.13) and *B.megaterium* (1.51) is due to strain and possibly species differences.

In both germinated spores of *B.subtilis* and *B.megaterium*, a higher oxygen consumption was observed after the addition of β-D-glucose than of α-D-glucose. It is suggested from the above data that the enzymes for metabolism of D-glucose in the germinated spores prefer β-D-glucose to α-D-glucose.

It is reported by other investigators that β-D-glucose dehydrogenase [EC 1.1.1.47] is not produced by vegetative cells but increases in sporulating cells about 1 h before the appearance of refractile spores in *B.subtilis*[14]. The dormant and germinated spores of *B.subtilis* contain this enzyme, while no activity is observed in vegetative cells[6]. Mutarotase activities of suspensions of spores or cells of the

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].

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Experimental studies of schistosomal pigment from *Schistosoma japonicum*

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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.

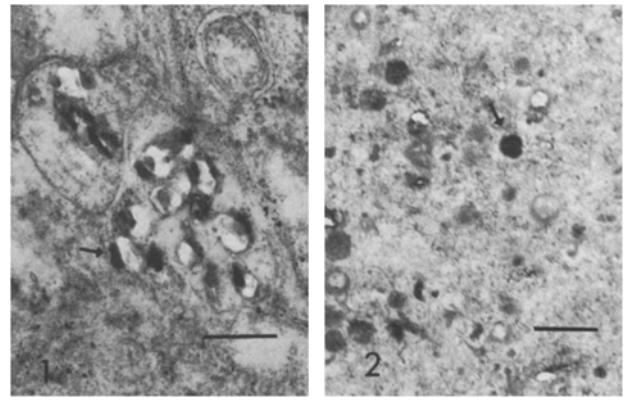

Fig. 1. Pigment (arrow) within parasite. 1. Malarial pigment. 2. Schistosomal pigment. Scale: 1 μm.