Medicine. — *Degenerated cysts and black spores in Anopheles infected with benign tertian malaria.* By A. DE BUCK. (From SWELLENGREBEL's Laboratory in the Institute of Tropical Hygiene [Director Prof. Dr. W. A. P. SCHÜFFNER] of the Royal Colonial Institute at Amsterdam). (Communicated by Prof. W. A. P. SCHÜFFNER).

(Communicated at the meeting of March 28, 1936).

In another paper [1]), recording some experiments on the influence of cold on the development of the malaria parasite in the mosquito, I referred to the degeneration of oocysts at various stages of their growth. This may be caused by cold, but it may likewise occur under favourable conditions of temperature and humidity, especially if the number of zygotes is excessively large. In the case of cold being the cause, the visible signs of degeneration do not as a rule make their appearance till after the mosquitoes have again been returned to an environment with a more favourable temperature. Often the only symptom of degeneration is a white or yellowish opaqueness of the oocyst, the cytoplasm having a finely granular appearance. Other cysts have coarser granules and the coarser these granules, the darker the yellow colour. Often in this granular substance are seen a number of larger grains and globules, of various sizes, which mostly have a light-coloured centre (Fig. 5). All these types can be found before any of the cysts have reached maturity; I therefore conclude that these are cysts which have degenerated at an early stage.

In one experiment I met with a peculiar type of degenerated cysts: clear cysts with a large brown or yellow centre which in its turn may have a darker inner part (Fig. 8). These cysts showed an apparently normal development until the day on which the first sporozoites were found in the glands. Then a mosquito was dissected with as many as 70 cysts of this type. Some of these showed a few "small black spores" adhering to the brown centre (Fig. 9). From these facts I infer that these cysts have degenerated after they had already proceeded to the formation of the sporozoites. At which stage of this process degeneration sets in I leave undecided.

In the "degenerated" cysts I do not include the "black spore cysts". By the name of black spores I only mean the typical sausage- or banana-shaped bodies. They do not always occur as definite black spore cysts. Mostly there is nothing like a cyst wall visible, often the black spores form only a loose swarm with or without a denser centre, often a few

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1) This paper will be published in The American Journal of Hygiene of July 1936.

single black spores are scattered over the stomach (Figs. 1, 2, 3). Exactly the same type of black spores often occur on the salivary glands (Fig. 6). The shape and the light inner part of these bodies is very suggestive of the idea that they are chitinized sporozoites. Already GRASSI was of this opinion. Their formation seems to proceed so rapidly that as yet no one has been able to trace this hypothetical development. Still I feel strongly inclined to accept this view. In other words, typical black spores are only found, when the cysts have normally developed and reached maturity (see also BRUG, 1916).

Perhaps I had better not use the word "chitinized", since the chitinous nature of these bodies is as yet not absolutely certain. The same applies to the brown corpuscles and granules in the degenerated cysts and also to the brown centre of the above-described peculiar kind of degenerated cysts which, as my own observations have shown, is indissoluble in caustic potash.

KNOWLES and BASU (1933) speak of "degeneration and pigmentation" instead of chitinization and they are convinced that the pigment is derived from the parasite and not from the mosquito. This may be true, though I cannot agree with them that this "appears clear" from their Plates XXIX and XXX.

As I said before, I feel strongly inclined to accept GRASSI's view, and in this connection I think we may see in the small black spores adhering to the brown centre of some degenerated cysts the missing link in the development of the sporozoites into the typical black spores. The question, whether this is a process of exogenic chitinization or endogenic pigmentation is connected with the question, whether the sporozoites are transformed into black spores while they themselves and the oocyst are still living or after their death. KNOWLES and BASU believe black spores lying free in the coelomic cavity or elsewhere to be the result of rupture of the oocysts in dissection or in nature. Now anybody who has examined black spore cysts knows that these structures are very resistent and can only be crushed by pressing hard on the cover glass, but then the black spores themselves are crumbled into irregular pieces; indeed they do not seem apt to rupture and scatter their black spores all over the body of the mosquito. In my opinion, therefore, the black spores lying free outside oocysts have arisen from sporozoites which were transformed into black spores after their being expelled from ripe and ruptured cysts.

It is surprising how few observers have mentioned the presence of black spores on the salivary glands. Yet these black spores, which always have the typical banana-shape, are as frequent as those occurring on the stomach; they are never found in the cells of the salivary glands, but only on the coelomic surface of the glands (see also WALCH, 1921). These loose and scattered black spores are perhaps a support to the opinion that they are not the result of a process of endogenic pigmentation, but of exogenic chitinization, since it might seem inexplicable how these sporo-

zoites are able to produce the sometimes exceedingly thick layer of "pigment" they are enclosed by. Moreover, if chitinization by the insect host gives rise to the black spores, it might be supposed that only dead or weakened sporozoites are chitinized, which would account for the fact that black spores are relatively scarce.

MAYNE (1919, p. 129) has said that "true chitin is of ectodermal origin and that the tracheae are the only ectodermal tissue present inside the mosquito's body", as a support of his theory that black spores are of tracheal origin. But it has as yet not been proved that black spores consist of true chitin and, moreover, an abnormal process of chitinization may as well abnormally originate from other than ectodermal cells. I for my part have never in a single instance been able to establish the alleged connection between black spores and the tracheal system [1]), not even in the specimen of Fig. 2 which showed many very long and curved black spores in which the layer of "chitin" was only very thin or even partly absent. The minute terminal tubes of the tracheae, which could be traced into the immediate vicinity of the swarm of black spores (though they are no longer visible in the photograph), had the same calibre and were curved in exactly the same manner as the black spores of this specimen. Indeed the resemblance was highly suggestive of a close relationship of these black spores to the tracheal system. Yet, even in this instance no direct connection between the two structures could be ascertained, nor was it possible to speak with MAYNE of a "snarl of tracheae" (p. 129), for the whole was really a "swarm of black spores", there being no connection between the single bodies.

From all this it may be gathered that I am convinced that the "typical black spores" are associated with sporozoites and that I do by no means agree with MAYNE, who speaks of the error of associating black spores with malaria parasites and infectivity. In some 1700 specimens dissected before the date on which ripe sporozoites could be expected, I only once observed typical black spores, on the seventh day following infective bite, but this was in a batch of wild mosquitoes caught in a stable in the neighbourhood of Amsterdam; so in this case it cannot be excluded that the black spores were the result of an old infection contracted in the wild state. In laboratory-bred uninfected mosquitoes I have never found typical black spores. It would be interesting if MAYNE had given photomicrographs of the black spores which he has observed in unfed laboratory-bred females and males of *Anopheles* and *Culex* and in house-flies. Now we can only accept the view of KNOWLES and BASU that all these apparently non-

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[1]) The only finding which might seem to contradict this was a structure of exactly the same appearance as that represented by MAYNE (Plate XII, Fig. 18). It was found in a wild uninfected female of A. maculipennis. The spiral markings of the trachea were even visible within the dark-brown body. If this, however, were to be the normal mode of origin of typical black spores, I think it would be strange that similar findings are not more common.

malarial black spores recorded by MAYNE are not typical black spores, but must belong to the other structures which they separate from the true black spores as "chitin corpuscles" and "fungus infections of the tracheal system" (p. 773 l.c.). Of these latter structures I have seen a few remarkable examples in which the greater part of the stomach was wrapped in by what might be interpreted as hyper-chitinized tracheae or hyphomycetes or both.

Of course, it should be admitted that it is quite possible that these "pseudo-black spores" sometimes develop in such a way as closely to resemble the typical black spores and, therefore, we may in a sense agree with MAYNE "that one of the practical points to consider is to guard against applying the presence of black spores as a test of any pathogenic relationship to malaria, especially in the absence of parasites" (p. 130). Great care should be taken, whenever no parasites are found, to conclude from the presence of a few black spore-like structures to previous malarial infection of the mosquito.

It is interesting in this connection to compare the above-described specimen of Fig. 2 with that of Fig. 4. This photograph represents the stomach of a wild female which did not harbour oocysts on the stomach nor sporozoites in the glands. As it was caught in a house where a number of infected females were found on the same day, one might be inclined to regard this stomach as covered with black spores and consequently as a sign of heavy infection. Yet, this female was registered as negative and the structures represented here were considered as "pseudo-black spores". They covered half of the stomach, their colour was light-brown, and in several places they could be traced as long uninterrupted threads which in the denser parts formed an entangled mass; some of the threads have the appearance of a string of beads, as is seen in the figure, but the single beads have no resemblance to typical black spores. These structures are apparently very rare; they were observed only twice on some thousands of dissected stomachs.

Special caution is required in the case of another group of pseudo-black spores which I am inclined to consider as chitinized bacteria and which we are wont to call "coffee-beans" at our institute, because of their peculiar shape (Fig. 7). They may occur in dense clusters; sometimes even what may look like a cyst-wall is present and then, in consequence of the dark-brown colour of the whole, it is not always easy to ascertain the characteristic coffee-bean-shape of the single parts. These cyst-like structures might be taken for true black spore cysts on a too superficial examination.

But apart from a few exceptional cases, a trained observer will have no difficulty in making sure, whether the structures he is examining are true or pseudo-black spores. This statement, however, is only meant to be valid with regard to benign tertian malaria and *Anopheles maculipennis*. I am well aware that the species both of mosquito and malaria parasite

A. DE BUCK: DEGENERATED CYSTS AND BLACK SPORES IN ANOPHELES INFECTED WITH BENIGN TERTIAN MALARIA.

PLATE I.

Fig. 1.

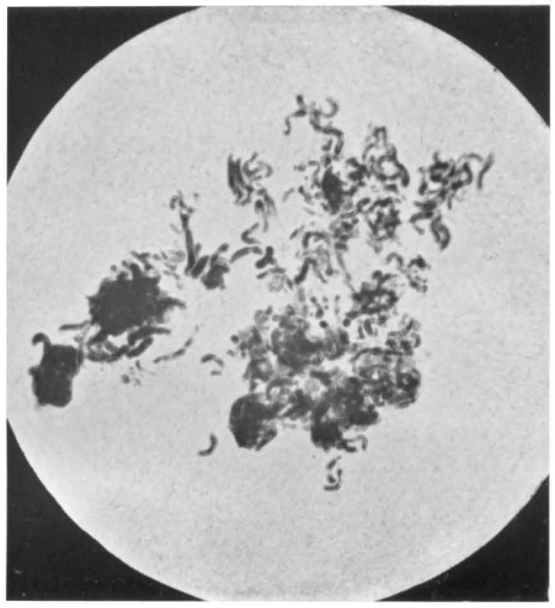

Fig. 2.

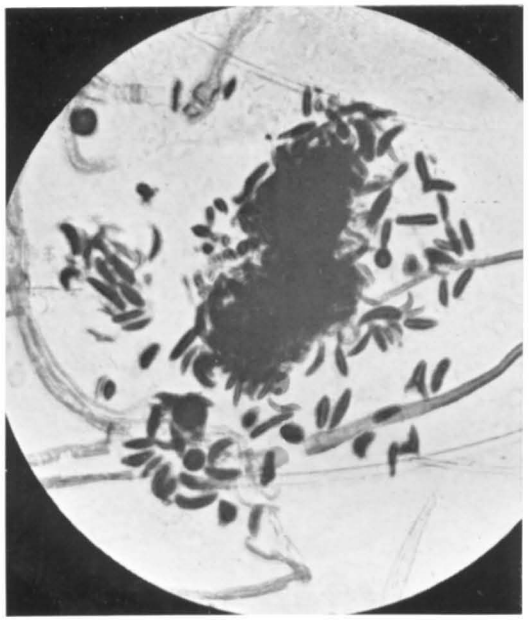

Fig. 3.

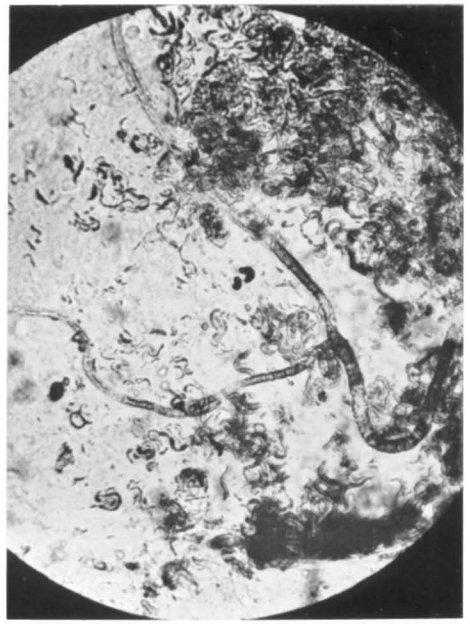

Fig. 4.

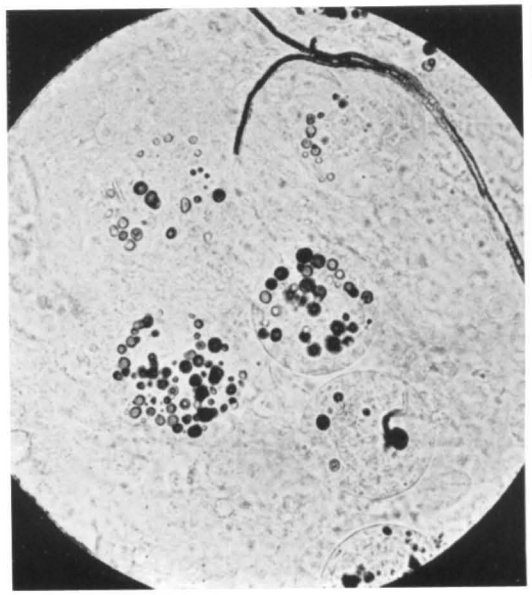

Fig. 5.

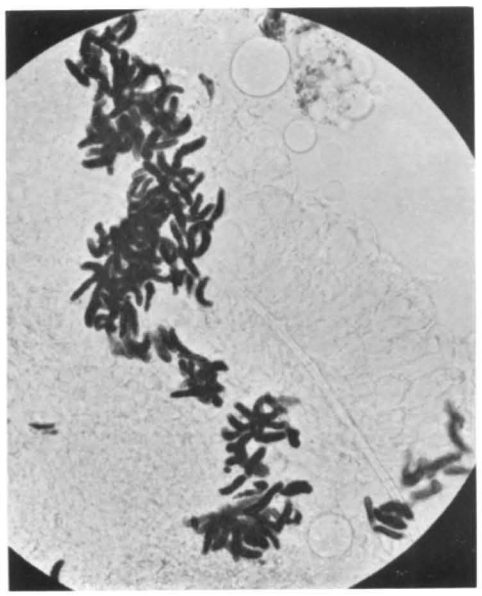

Fig. 6.

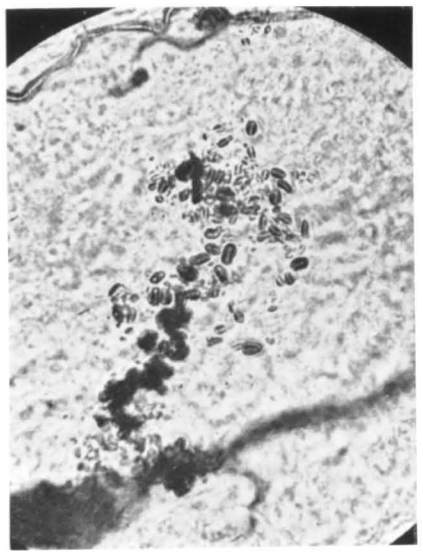

Fig. 7.

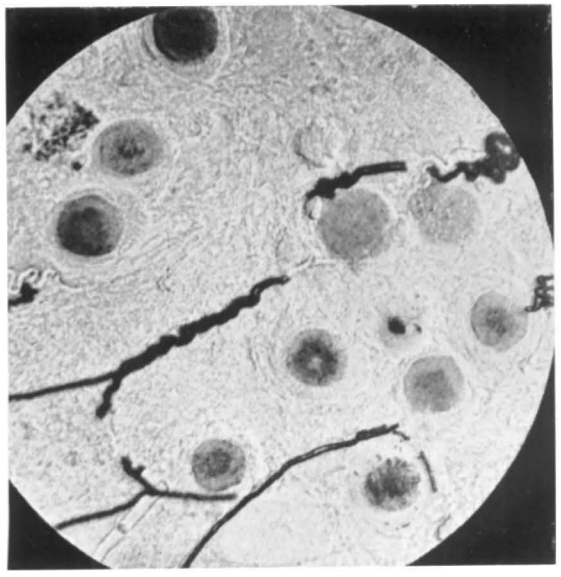

Fig. 8.

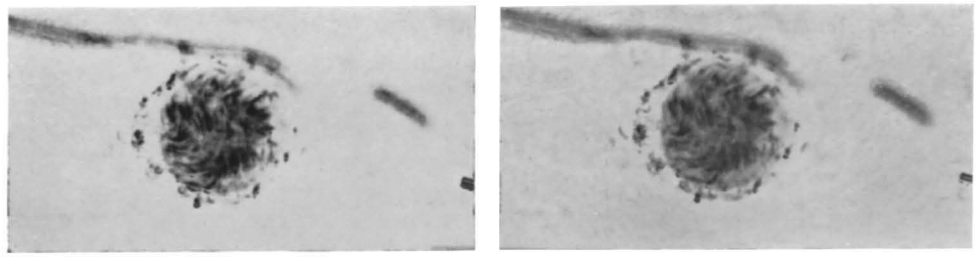

Fig. 9.

may have some influence on the occurrence and shape of all these structures.

Summary.

1. Typical banana-shaped black spores are not found before the cysts have reached maturity.

2. Typical black spores are never found in uninfected Anopheles.

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EXPLANATION OF THE PLATES.

Figs. 1, 2 and 3. True black spores on the stomach of infected mosquito. Magnified 325 ×.

Fig. 4. Pseudo-black spores on the stomach of a wild anopheles. Magnified 200 ×.

Fig. 5. Degenerated cysts. Magnified 370 ×.

Fig. 6. Black spores on salivary gland. Magnified 325 ×.

Fig. 7. Pseudo-black spores: "coffee-beans". Magnified 450 ×.

Fig. 8. Degenerated cysts on the stomach of a mosquito which was kept at room-temperature for one month after the infecting meal. Magnified 275 ×.

Fig. 9. Degenerated cyst of the specimen of Fig. 8, magnified 580 ×. Small black spores adhering to the brown centre. Two different reprints of the same negative.