diameter. Humified cell wall material, which can be identified by its characteristic ultrastructure, also contains some carbohydrate (*W*). Small deposits of amorphous carbohydrate fill pores less than 0.03 μm in diameter in the clay fabric, as shown in Fig. 6 (arrow), and silver-reactive material coats many of the clay platelets as in the silver methenamine–treated soils.

The use of polysaccharide-specific stains on ultrathin sections provides new information on the morphology and distribution of organic matter in natural soil fabrics. As expected, carbohydrates are seen to be associated with roots and soil microorganisms and with dead tissue fragments and humified materials, but some is associated with the clay fraction. The precise morphology and location of polysaccharides in clay fabrics explains in part why some carbohydrates are protected from microbial attack and why small quantities of added polysaccharide have so profound an effect on soil stability. Some carbohydrates may be immune to decay simply because they are physically separated from soil microorganisms.

Although bacterial populations in soils are large (10^9 cells per cubic centimeter), they are mainly aggregated in rhizospheres and in colonies near cell wall fragments (*5*, *6*). Very few bacteria lie in the clay fabric as in Fig. 1, and only the carbohydrate in their immediate vicinity will be available to them. Since most bacteria are ≧ 0.3 μm in diameter, they are unable to enter the finer crevices and lyse the more substantial deposits of polysaccharide inside. Bacteria isolated in clay fabrics are therefore frequently devoid of stored material (Fig. 1), in marked contrast to those in adjacent rhizospheres (*6*). Much carbohydrate associated with clays will remain inaccessible to the microflora until the crevices are opened by the mechanical action of soil animals or until the bacteria are brought in contact with carbohydrate deposits by the effects of experimental manipulations (*13*). It is unlikely that normal agricultural practices open such small pores.

As soil dehydrates, surface tension effects will tend to concentrate polysaccharides in crevices between adjacent clay platelets and these will be bonded strongly where their edges overlap. In this manner a small amount of carbohydrate may give rise to strong boxlike cardhouse structures (*14*), which will be resistant to dispersion or collapse, especially if the polysaccharides become irreversibly denatured on dehydration. Since the individual threads of the fibrous polysaccharides produced by microorganisms are so small in cross-sectional area, a small mass will contain a large length of fiber and so will enmesh large volumes of fabric. This explains why quantities as small as 0.02 to 0.2 percent of added microbial carbohydrate markedly stabilize clay aggregates (*3*).

Because they are electron-dense, Ru/Os and PA/Ag deposits produce bright backscatter electron images, and they may therefore be used to demonstrate carbohydrates in uncoated fabrics in the scanning electron microscope as well as in the transmission electron microscope mode. Treatment with hydrolytic enzymes specific for particular carbohydrate linkages in advance of Ru/Os or PA/Ag treatment allows individual types of carbohydrate to be located and identified in soil fabrics (*15*).

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Hemin Lyses Malaria Parasites

Abstract. *Malaria parasites isolated from mouse erythrocytes are lysed by ferriprotoporphyrin IX chloride (hemin) or by a chloroquine-hemin complex in amounts that could be produced by release of less than 0.1 percent of the heme in erythrocytic hemoglobin. This effect of hemin may explain the protection against malaria provided by thalassemia and other conditions causing intracellular denaturation of hemoglobin. The toxicity of the chloroquine-hemin complex may explain the selective antimalarial action of chloroquine.*

Recently it was found that ferriprotoporphyrin IX chloride (hemin) lyses *Trypanosoma brucei* (*1*) and normal erythrocytes (*2*) and that the formation of a chloroquine-hemin complex in erythrocytes does not inhibit hemolysis (*2*). These observations have important implications for the biology and chemotherapy of malaria. Erythrocytic malaria parasites exist in an environment rich in hemoglobin and could be exposed to toxic amounts of heme when hemoglobin undergoes denaturation, as occurs spontaneously in hemolytic anemias associated with the production of Heinz bodies (*3*). Malaria parasites also degrade hemoglobin and produce a large amount of heme, which normally is sequestered in

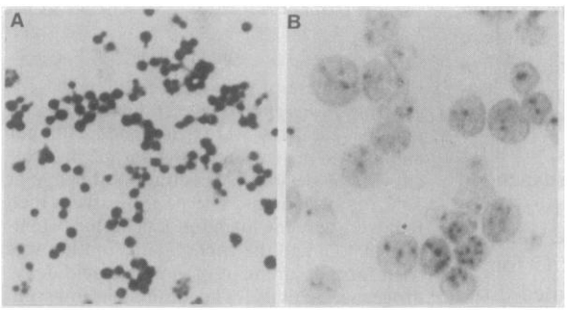

Fig. 1. Microscopic appearance of isolated *P. berghei* (×1100). (A) Control view. The parasites were suspended (10^7 per milliliter) in the standard medium and incubated. At the end of the incubation period glutaraldehyde was added to achieve a final concentration of 2 percent and the mixture was left at room temperature for 20 minutes. The mixture was centrifuged and the pellet was washed once with standard medium. Smears of the washed pellet were made on albumin-coated glass slides, air-dried, and stained. (B) Parasites from a portion of the same suspension used in (A), incubated in the presence of 20 μ*M* hemin. Treatment of the parasites with a complex formed from 20 μ*M* hemin and 5 μ*M* chloroquine produced results similar to those seen in (B); chloroquine alone caused no change.

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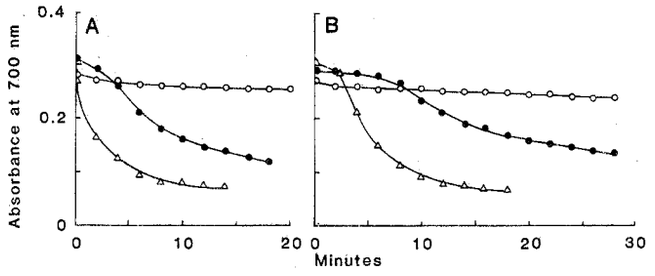

Fig. 2. Time course of the effect of hemin on the turbidity of *P. berghei* suspensions. Isolated parasites were suspended (10^7 per milliliter) in the standard medium and incubated at 37°C with no hemin (○) or with 10 μM (●) or 20 μM (Δ) hemin. (A) The results obtained when no chloroquine was present in the incubation medium; (B) the results when 5μM chloroquine was present. In the experiments in which both hemin and chloroquine were present, the two compounds were mixed before being added to the suspension of parasites. Approximately 30 seconds elapsed between the addition of hemin or the chloroquine-hemin complex and the first measurement of absorbance shown in the figure. The absorbance at 700 nm was measured with a Beckman model 25 spectrophotometer.

malaria pigment (*4*), presumably in an innocuous form. On exposure to chloroquine, however, erythrocytes infected with malaria parasites accumulate a chloroquine-heme complex (*5*) that could be toxic. In this report we provide evidence that hemin and a chloroquine-hemin complex are indeed toxic to malaria parasites.

The NYU-2 strain of *Plasmodium berghei* was studied. Two lines (*6*) of this strain, one chloroquine-susceptible and the other chloroquine-resistant, are available in our laboratory. The former produces abundant pigment and the latter no detectable pigment in mouse erythrocytes. To minimize confounding of the data by the parasites' own heme, the chloroquine-resistant line was chosen for the present study. (Preliminary experiments with the chloroquine-susceptible line have shown that it responds to hemin and the chloroquine-hemin complex in the same way as the chloroquine-resistant line.)

Parasites were obtained from the blood of heavily infected male Swiss-Webster mice (parasitemia in excess of 30 percent). The blood from several mice was pooled and mixed with an equal volume of a standard isotonic medium (*p*H 7.4) (*7*) containing 1 mg of heparin per milliliter. White blood cells were removed by passing this mixture through a column of Whatman CF 11 cellulose powder. The erythrocytes were then recovered by centrifugation, suspended in 10 volumes of standard medium, and centrifuged again. To lyse the erythrocytes, the washed pellet was suspended in 20 volumes of 0.015 percent (weight to volume) saponin (Sigma) in the standard medium and incubated at 37°C for 30 minutes. Parasites were isolated from the lysate by centrifugation and washed three times by suspension in 40 volumes of cold standard medium and centrifugation at 4°C. All centrifugations were conducted at 500*g* for 15 minutes.

Figure 1 shows photomicrographs of the isolated parasites, fixed with glutaraldehyde and stained with Giemsa. There was minimal contamination by intact erythrocytes. In the absence of hemin the parasites appeared as small blue cells with barely visible red nuclei; at 2°C, they retained their normal appearance for several hours (Fig. 1A). After exposure to hemin from equine blood (Sigma) or the chloroquine-hemin complex for 10 minutes, the parasites became swollen, and there was a decrease in cytoplasmic staining (Fig. 1B). The swelling produced by hemin (20 μM or higher) was maximal within 10 minutes (*8*).

The time course of swelling was evaluated by measuring the turbidity of parasite suspensions. On exposure to hemin there was a rapid decrease in absorbance at 700 nm (Fig. 2). Concomitant with the swelling, glutamic acid dehydrogenase (E.C. 1.4.1.4) was released from the parasites (Fig. 3) (*9*), indicating loss of intracellular contents. These findings point to lysis of the parasites.

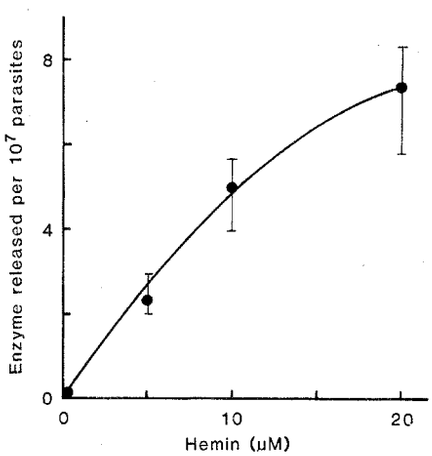

Fig. 3. Release of glutamic acid dehydrogenase from *P. berghei* in the presence of hemin. Isolated parasites were suspended (10^7 per milliliter) in the standard medium and incubated with the desired concentration of hemin at 37°C for 10 minutes. The parasites were then removed by centrifugation (3000*g* for 5 minutes at 4°C) and the supernatant solution was used for measurement of glutamic acid dehydrogenase activity. The ordinate shows enzyme activity expressed as nanomoles of α-ketoglutarate per minute. The means and ranges for three experiments are shown. In control studies without hemin, which were included in each experiment, no enzyme release was detectable.

Addition of chloroquine diphosphate (Sigma) to hemin to form a complex delayed but did not prevent lysis (Fig. 2). Only the effect of 5 μM chloroquine is shown in Fig. 2, but the responses to 1 and 10 μM chloroquine were also studied. The delay was less with 1 μM chloroquine and somewhat greater with 10 μM chloroquine. In equilibrium dialysis experiments, it has been found that a maximum of one molecule of chloroquine can be bound by two molecules of hemin (*5*). In agreement with the erythrocyte model of Chou and Fitch (*10*), chloroquine alone did not cause malaria parasites to swell or lyse.

Considering that the hemoglobin concentration in erythrocytes is approximately 34 g per 100 ml of packed cells and that the molecular weight of hemoglobin is approximately 64,000, release of less than 0.1 percent of the heme in erythrocytic hemoglobin would be sufficient to produce intracellular heme concentrations as high as 20 μM. The amount of heme released when hemoglobin undergoes denaturation intracellularly in such conditions as thalassemia and sickle cell anemia has not been measured, although denaturation is known to accelerate heme release (*3*). Since the release of only a small proportion of erythrocytic heme would be enough to lyse malaria parasites, however, heme toxicity may explain the protection against malaria provided by thalassemia and other conditions associated with intracellular denaturation of hemoglobin.

Chloroquine-susceptible malaria parasites may also be exposed to heme from within, since they degrade hemoglobin and sequester large amounts of heme in the form of malaria pigment (*4*). This sequestered heme is not available to bind chloroquine (*5*) and apparently causes little or no toxicity. By contrast, when susceptible parasites are exposed to chloroquine, heme accumulates in a different form that is available to bind the drug (*5*). The biochemical explanation for accumulation of heme as a drug complex rather than as malaria pigment remains unknown. Nevertheless, erythrocytes infected with the chloroquine-susceptible line of *P. berghei* accumulate 20 μmole of chloroquine per kilogram or more as the chloroquine-heme complex

(*5*, *6*). Since the present results demonstrate that this amount is more than enough to lyse isolated malaria parasites, we propose that the selective antimalarial action of chloroquine is due to the accumulation of a toxic chloroquine-heme complex. Although the mechanism underlying this toxicity has not yet been studied, it may be similar to that observed in the erythrocyte model (*10*).

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Thymosin Stimulates Secretion of Luteinizing Hormone–Releasing Factor

Abstract. *Partially purified thymosin fraction 5 and one of its synthetic peptide components, thymosin β_4, but not thymosin α_1, stimulated secretion of luteinizing hormone–releasing factor from superfused medial basal hypothalami from random cycling female rats. In addition, luteinizing hormone was released from pituitary glands superfused in sequence with hypothalami. No release of luteinizing hormone in response to thymosin was observed from pituitaries superfused alone. These data provide the first evidence of a direct effect of the endocrine thymus on the hypothalamus and suggest a potentially important role for thymic peptides in reproductive function.*

Experimental evidence supports the concept that the thymus gland participates in the development of the neuroendocrine system in mammals (*1*). With regard to reproductive function, we have previously documented that congenitally athymic nude mice have reduced pituitary concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and that these hormones can be restored to normal by thymic transplantation on the first day of life (*2*). Furthermore, hypothalamic concentrations of luteinizing hormone–releasing factor (LRF) appear reduced in athymic animals whereas gonadal function in vitro seems intact (*3*). In the experiments reported here, we attempted to determine if thymosin fraction 5, a partially purified thymic preparation known to play an important regulatory role in the function of the thymus-dependent lymphoid system (*4*), and two of its component peptides, thymosin α_1 and β_4 (*5*), might also be important in reproductive function. To investigate the role of thymic peptides in the regulation of gonadotropin secretion, we used medial basal hypothalami (MBH) or pituitary glands that were obtained from randomly cycling female rats and were superfused in a sequential double chamber system.

Sprague-Dawley rats were decapitated at 0900 hours and the MBH and pituitary gland were removed. In some studies, the MBH was placed into the first 0.1-ml plastic chamber of a double chamber superfusion system, and the pituitary was placed into the second chamber. In other studies, either the MBH or pituitary was perfused singly in the corresponding chamber of the series. The superfusion system, including the anatomical boundaries of the dissected MBH tissue, have been described (*6*). The sequential chambers were perfused with Medium 199 (Gibco) saturated with 95 percent O_2 and 5 percent CO_2 at 37°C at a flow rate of 3 ml per hour. Bacitracin (5 μl, 2 m*M*; Sigma) was added to the collection tubes to prevent the enzymic degradation of LRF. The dissected tissue was allowed to equilibrate for 2 hours, and then the collection of 0.5-ml fractions of media was initiated. After fractions were collected for 1 hour, 20 μg of thymosin fraction 5, or $4 \times 10^{-11} M$ synthetic thymosin α_1 or β_4 in a volume of 10 μl in Medium 199, or Medium 199 alone as a control, was injected into the first chamber and samples were collected for an additional 2 hours. The fractions were stored at − 20°C until assay. The LH or LRF in these effluent samples was measured by radioimmunoassay as described (*2*, *7*). All samples from a given study were measured in the same assay. For LH the intra-assay coefficient of variation was 6.4 percent when approximately 50 percent of the hormone was bound and for LRF it was 3.8 percent. The sensitivities of these assay systems were 10 ng/ml for LH (with NIH RP-1 being used as the reference preparation) and 2.5 pg/ml for LRF (with a synthetic preparation being used as standard). Thymosin fraction 5, thymosin α_1, thymosin β_4, and Medium 199 did not displace iodinated hormone in either assay system. Statistical significance of the changes in the hormone concentrations was determined by the Student's *t*-test for unpaired data.

Figure 1A shows that the injection of either thymosin fraction 5 or Medium 199 caused no change in LH when the pituitaries from female rats were superfused without MBH. In contrast, when the MBH and the pituitary from individual female rats were superfused in sequence, the LH released from the pituitaries into the efflux increased in response to thymosin in comparison to the LH concentrations in the effluent from those receiving only Medium 199 ($P < .05$). The final effluent concentrations of LH from thymosin-treated pituitaries superfused with MBH's were 200 percent increased over basal levels. The administration of thymosin fraction 5 produced significant increases in mean LRF concentrations in the effluents from MBH's in comparison to control groups that received only Medium 199 (Fig. 1B).

In other experiments (Fig. 1C), injection of thymosin β_4 but not thymosin α_1 or Medium 199 alone elicited release of LH from pituitaries superfused together with MBH. Furthermore, thymosin β_4 stimulated a greater than 100 percent increase in secreted LRF over basal levels (Fig. 1D).

To our knowledge this is the first time that thymosin fraction 5 and at least one of its component peptides, thymosin β_4, have been shown to directly affect the reproductive system by inducing release

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