contaminants of the isolated hormone, for example, from slight dissolution of the LH-Sephadex used in the last purification step (*2*).

These comparisons have thus shown no real differences in chemical or biological properties between the synthetic peptide and the isolated hormone. Therefore, we think it has been proved that the given chemical structure is that of the isolated blanching hormone of *Pandalus borealis*. Like at least two of the neurosecreted hormones of vertebrates, the thyroid stimulating hormone-releasing hormone (*17*) and the luteinizing hormone-, follicle stimulating hormone-releasing hormone (*18*), this nonvertebrate neurosecreted hormone has an NH_2-terminal pyroglutamic acid and a COOH-terminal amide.

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5. The abbreviations used are as follows: Asn, asparagine; Asp, aspartic acid; Asx, aspartic acid or asparagine; DNS (dansyl), 1-dimethylaminonaphthalene-5-sulfonyl; Glu, glutamic acid; Glx, glutamic acid or glutamine; Gly, glycine; Leu, leucine; pGlu, pyroglutamic acid; Phe, phenylalanine; Pro, proline; Ser, serine; Trp, tryptophan; Trp-NH_2, tryptophan amide.
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24 April 1972

Pitting Function of the Spleen in Malaria: Ultrastructural Observations

Abstract. *Ultrastructural studies of spleens from monkeys infected with* Plasmodium knowlesi *suggest that the spleen removes or "pits" malaria parasites from red cells. This function may explain the presence of nonparasitized spherocytic erythrocytes in the peripheral blood and may in part account for the discrepancy between the excessive hemolysis and the number of parasitized erythrocytes in animals with experimentally induced malaria.*

After splenectomy of animals with experimentally induced malaria, parasitemia in the peripheral blood is frequently increased (*1*). Hemolysis in malaria occurs in excess of that expected from the number of red cells that are parasitized in the peripheral blood (*2*). This excessive hemolysis has been explained on the basis of immune mechanisms (*3*) directed at the malaria parasite or the red cell or both, as well as on the basis of a direct toxic effect of circulating antigens (*4*) on the red cell and the action of the spleen (*5*) upon parasitized and nonparasitized (*6*) red cells. In this report, we present morphologic evidence that the spleen in monkeys infected with *Plasmodium knowlesi* is capable of "pitting" the parasite from the red cell. Pitted red cells may in part explain the discrepancy between the degree of hemolysis and the number of parasitized red cells and may also explain the presence of spherocytes in the peripheral blood. In addition, the entire parasitized red cells are phagocytized by cordal macrophages, and red cells may be hemolyzed extracellularly in the splenic microvasculature.

A rhesus monkey (*Macaca mulatta*) was infected with 4.5×10^5 parasites of *P. knowlesi*, H strain. Six days after inoculation the parasite count reached 360,000/mm^3, and the monkey was splenectomized (*7*). The splenic tissue was fixed for 5½ hours in a solution containing 3 percent glutaraldehyde, 4 percent sucrose, and phosphate buffer at *p*H 7.3. The tissue was then washed in phosphate buffer and treated for 1 hour with 1 percent osmium tetroxide, buffered with phosphate. The tissue was embedded in Epon 812, and sections were cut with a diamond knife on a Porter-Blum MT-2B ultramicrotome. Thin sections were stained with uranyl acetate and lead citrate (*8*) and examined with an RCA EMU-3H electron microscope.

The spleen can sequester red cells or parts of red cells because of the peculiar microanatomy of its red pulp (*9*). Red cells that enter the cords of Billroth by way of the terminal arterioles and arterial capillaries must be readily deformable in order to traverse the narrow, circuitous, macrophage-lined cords and squeeze through the small fenestrations of the basement membrane between cords and sinuses. Although the normal biconcave red cell is very plastic and deformable, red cells with rigid inclusions, or red cells with damaged and rigid cell membranes that have lost much of their plasticity, may not be able to squeeze through the narrow fenestrations between cords and sinuses. The portion of the red cell which contains inclusions is then trapped on the cordal side of the basement membrane and severed from the rest of the cell; the part of the red cell which bears no inclusions remains free to continue in the circulation.

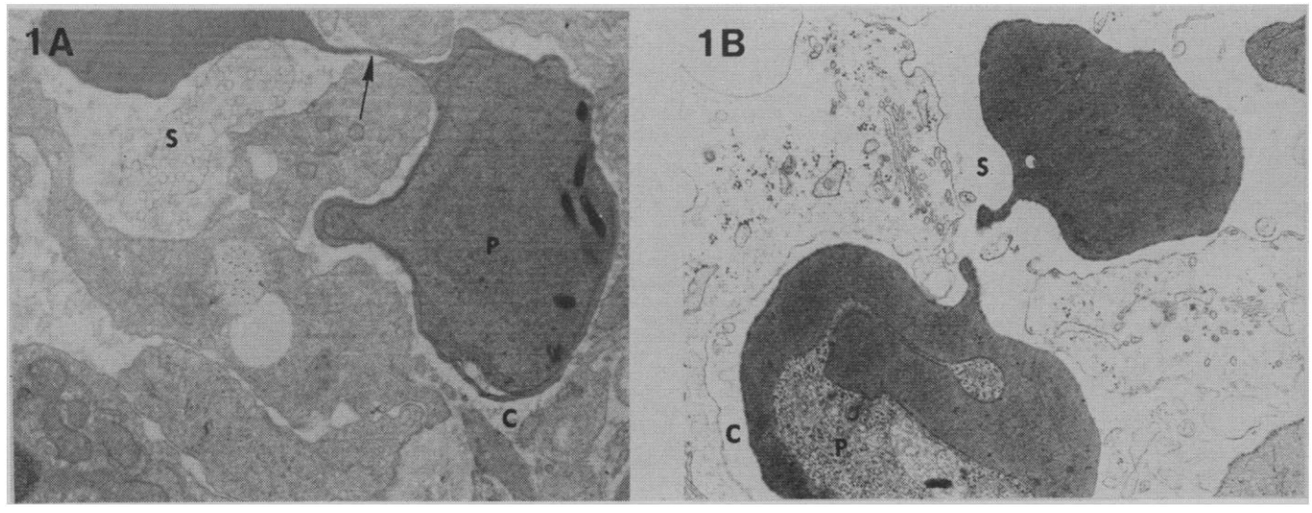
This pitting of inclusions, or fragmentation of red cells containing inclusions such as Heinz bodies, has been demonstrated morphologically by electron microscopy (*10*). The ability of the spleen to remove inclusions such as iron particles from red cells and to return the pitted red cell to the circulation has been demonstrated by Crosby (*11*) by means of transfused red cells labeled with chromium-51. Using a similar experimental method, Conrad

and Dennis (*12*) suggested that in monkeys infected with malaria, the spleen removes parasites from the red cells and returns the deparasitized red cells to the peripheral blood.

The results of our studies of ultrastructural morphology lend support to these functional studies. Figure 1A shows a red blood cell in transit from cord to sinus. The nonparasitized part of the cell is in the lumen of the sinus, while the parasitized portion of it is still in the cord and is unable to squeeze through the fenestration of the basement membrane and between the endothelial cells of the sinus. The two parts of the red cell are connected by a tenuous stalk of hemoglobin, which will eventually be severed. In Fig. 1B, a red cell that appears to have been split in two is seen. The nonparasitized part is in the sinus, while the parasitized portion remains in the cord. Such trapping or pitting of the parasitized part of the red cell was seen at least once at almost every junction of sinus and cord.

In addition to the pitting or fragmentation of red cells between cord and sinus, pitting of parasites from red cells by macrophages was also occasionally observed in the cords. In Fig. 2, the large parasitized portion of the red cell is seen within the cytoplasm of a macrophage, while the nonparasitized part appears to be in the process of being pinched off by the enveloping pseudopods of the macrophage. Such pitted red cells lose a greater amount of surface than of volume, become spherocytic, and are therefore more susceptible to hemolysis. Spherocyte formation has been demonstrated by George *et al.* (*13*), who observed two populations of red cells in animals with malaria; one type of red cell was a small, nonparasitized spherocytic cell that was easily lysed in hypotonic saline solution. Subsequent hemolysis of such nonparasitized spherocytes may be one of the causes of hemolysis in excess of that anticipated from the number of red cells parasitized.

The cordal macrophages of the spleen also phagocytize entire parasitized red cells. Various stages of breakdown of phagocytized red cells and parasites were seen in the cytoplasm of the phagocytic cells. Ghosts of hemolyzed red cells were also noted in cords and sinuses. Some of the red cell ghosts contained parasites, while others consisted only of empty, mem-



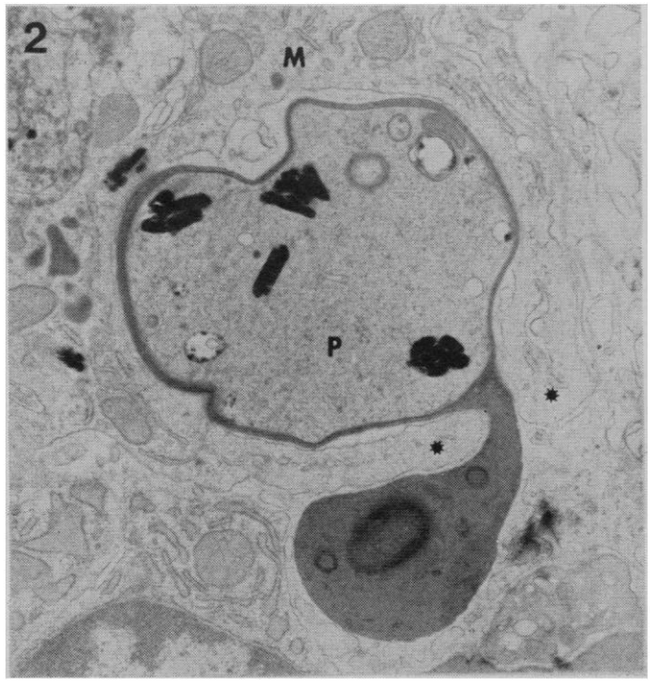


Fig. 1. Electron micrographs of the spleen. (A) A red blood cell is seen in transit from cord (*C*) to sinus (*S*). The nonparasitized part of the red cell is in the sinus and is attached to the parasitized (*P*) part of the cell, which is in the cord, by a thin tenuous stalk (arrow) (×8,000). (B) The nonparasitized part of a red cell is in the sinus (*S*), while the parasitized part remains in the cord (*C*). Thus, it appears that the red cell has been broken into two parts. Note the rounded ends where the break occurred (×12,000). Fig. 2. A parasitized red cell being pitted of its parasite-containing part by a cordal macrophage (*M*). The parasite (*P*) is surrounded by a rim of hemoglobin. Two pseudopods (*) of the macrophage appear to be in the process of separating the nonparasitized part of the red cell from the phagocytized part. Malaria pigment, which has a crystal-like appearance, is present in the parasite (×12,400).

brane-bound sacs. The latter cells may represent ghosts of spherocytes of previously pitted red cells that, because of their rigid cell membranes or because of damage to their cell membranes during the pitting process (*12*), are unable to traverse the splenic vasculature and that therefore undergo hemolysis.

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23 December 1971; revised 7 February 1972

Sex Pheromone of the Codling Moth: Structure and Synthesis

Abstract. *The structure of a sex pheromone of the codling moth,* Laspeyresia pomonella (*L.*), *has been determined by spectrometric and chemical degradative techniques and by synthesis to be* (2*Z*, 6*E*)*-7-methyl-3-propyl-2,6-decadien-1-ol. In field cage tests the synthesized sex pheromone was as attractive as the natural, but neither was as attractive as ten virgin females per trap.*

The codling moth is a serious, worldwide pest of most pome and stone fruits. We have studied this insect with the purpose of synthesizing a sex pheromone which can be used to control it. We have reported a laboratory bioassay and a method of isolation of a sex pheromone from females, and chemical tests and gas chromatographic data indicated the pheromone was an unsaturated alcohol (*1*). Further, the results of the laboratory bioassays were correlated with tests for attractancy in apple orchards when extracts of females at various stages of purification were tested (*2*). Pure sex pheromone was also attractive in the orchard test, but less so than ten females per trap.

Prior to structural studies, the purity of isolated pheromone was established by demonstrating that a single peak was obtained and the biologically potent fraction was coincident with the peak on gas-chromatographic columns of Apiezon L, SE-30, Zonyl E7, Carbowax 20 M, and diethylene glycol succinate.

Mass spectra (*3*) of the pheromone and its acetate were nearly identical. Both showed strong peaks at 192, 149, and 121 *m/e* units. Long-chain alcohols and acetates give weak or undetectable molecular ions, because they readily lose water or acetic acid in the mass spectrometer. This fact and the similarity of the two spectra indicated that the molecular weight of the pheromone was 210. A high-resolution mass spectrum indicated a formula of $C_{14}H_{24}$ for the 192 peak (calculated, 192.1878; found, 192.1886) and therefore the molecular formula is $C_{14}H_{26}O$. The ultraviolet spectrum showed no absorbance between 200 and 320 nm (*4*).

The infrared spectrum was remarkably similar to that of geraniol (*5*). The following diagnostic peaks were found (KBr pellet; μ): 3.00 (OH), 3.32 (olefin C–H), 3.40 to 3.52 (saturated C–H), 6.05 (C = C), 6.88 ($CH_2 + CH_3$), 7.29 (CH_3), 10.0 (C–O), 12.1 (trisubstituted olefin). The nuclear magnetic resonance spectrum (*6*) was (CCl; δ): 5.37 (1.00 proton; triplet; $J \sim 7$ hz; $C = CHCH_2$), 5.05 (0.84 proton; broad, unresolved multiplet; C = CH), 4.00 (2.04 protons; doublet; *J*, 6.7 hz; $C = CHCH_2OH$ coupled to the proton at 5.37 according to decoupling experiment), 2.02 (6.81 protons; distorted quartet; $C = CCH_2$), 1.66 [3.12 protons; singlet; $C(CH_3)C = C$], 1.27 (4.8 protons; singlet; CH_2 connected to saturated carbon), 0.91 (6.4 protons; triplet; CH_3 connected to saturated carbon). The number of protons assigned to each value of δ was the whole number nearest to the integrated value except for the absorptions at 2.02 and 1.27 ppm where 8 and 4 protons, respectively, were assigned. These were the only possible values consistent with the structural data.

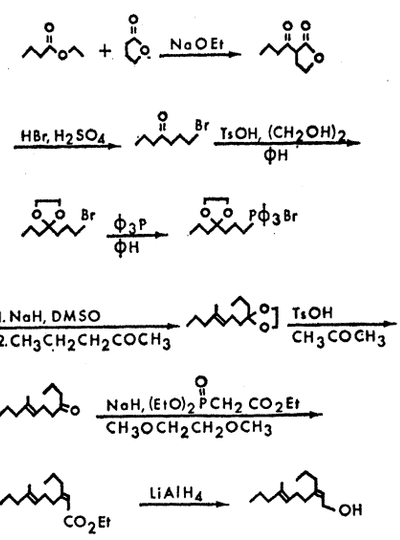

Fig. 1. Synthesis of mixed *Z*, *E* isomers of 7-methyl-3-propyl-2,6-decadien-1-ol. Abbreviations: Et, ethyl; Ts, tosyl; ϕ, phenyl; DMSO, dimethyl sulfoxide.

The structural deductions possible from the spectrometric data could be summarized as follows:

$$CH_3(CH_2)_l\underbrace{C(H)=C(H)}_{H\text{-}}(CH_2)_nC=CHCH_2OH$$

$$\overbrace{\qquad\qquad}^{CH_3(CH_2)_m\text{-},\ CH_3\text{-}}$$

where $l \geqq 1$; $m \geqq 1$; $n \geqq 2$; and $l + m + n = 6$. Apart from *Z*, *E* isomers, 24 structural formulas satisfied the given conditions.

Further information was obtained by hydrogenolysis (*7*). Upon hydrogenolysis, primary alcohols lose the carbon atom attached to the OH (*8*). Consequently, we expected a branched tridecane from the pheromone. The product obtained had a gas-chromatographic retention index (*9*) of 1223 on SE-30, which suggested a doubly branched tridecane. A mass spectrum also indicated a tridecane (M^+ 184) and showed prominent peaks corresponding to loss of propyl and methyl groups and exceptionally weak peaks corresponding to loss of ethyl or butyl groups. By excluding structures containing ethyl or butyl groups, we reduced the possibilities from 24 to 4.

Upon ozonolysis of the pheromone, only one product was detected by gas chromatography. Presumably other products were contained in the solvent front. Its retention index was 1605 on Carbowax 20 M and 1017 on SE-30. This suggested an ozonolysis product with seven carbon atoms and two carbonyl groups. The mass spectrum