not recorded as asbestos. Figure 1a shows a portion of a chrysotile asbestos fiber found by optical microscopy.

To confirm the presence of asbestos, 6 of the 16 samples taken in 1969 and a duplicate of one of the positive samples were scanned by electron microscopy. In five of the seven cases, including all those in which asbestos fibers were identified optically, concentrations of asbestos several times those in background controls were found. Examples of asbestos found in two samples by means of transmission electron microscopy are shown in Fig. 1, b and c.

During fall 1970 and spring 1971, a second set of drug samples was investigated to ascertain whether asbestos might still be found in typical parenteral drug preparations. In this second study, only ultramicroscopic analysis was undertaken of the residue from filtered drugs. The results shown in Table 1 indicate that contamination of parenteral drugs by asbestos was still a common occurrence. Here, the quality of asbestos in 6 of 17 samples significantly exceeded background levels.

The amounts of asbestos estimated in some of these samples exceeded a microgram and were much higher than those reported for other environmental circumstances. Asbestos concentrations measured in ambient air, for example, are typically in the range of nanograms per cubic meter. Contamination with nanograms or micrograms of asbestos may be evaluated with the knowledge that 10^{-9} g of asbestos might represent 10^6 fibrils of a size typically seen in drugs (400 Å in diameter by 1000 Å in length). It should be noted that negative or indefinite results for a particular sample do not guarantee the absence of asbestos in the drug lot from which the sample was taken. A single vial is an inadequate sample of a large production run. Moreover, the drugs sampled represent only a small fraction of those on the market. On the other hand, the finding of asbestos in one third of the single vials that were examined in this investigation over a 1-year period indicates significant asbestos contamination of some parenteral drugs at this time.

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Crustacean Color-Change Hormone: Amino Acid Sequence and Chemical Synthesis

Abstract. *The blanching hormone of the prawn,* Pandalus borealis, *is pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2. Its structure was settled by a combination of mass spectrometry and Edman-dansyl analysis of a thermolysin fragment. Confirmation of the structure was obtained by chemical synthesis from amino acids. This neurosecreted hormone is active in picogram amounts when tested in shrimps.*

Many crustaceans have the ability to change their body color in order to match their background. These color changes are brought about by hypodermal chromatophores, that is, specialized cells containing movable pigment granules and having richly ramified cell processes. Crustacean chromatophores are controlled via neurosecreted hormones, which are released from nerve endings in the sinus gland, a neurohemal organ located in the eyestalks of most decapod crustaceans (*1*).

One color-change hormone, the blanching (red-pigment-concentrating) hormone, has been isolated from eyestalks of the prawn, *Pandalus borealis* (*2*). This hormone, which is active in shrimps in picogram amounts (*3*), is present in very minute amounts in the crustacean eyestalks (*3*) and, therefore, only about 90 μg of the pure blanching hormone has been available for our studies of its structure. The hormone was found to be a small peptide with a blocked NH_2-terminus (*2*), and we now report the deduction of its complete structure, which we have confirmed by chemical synthesis.

Quantitative amino acid analysis (*4*) on 8 μg of the isolated hormone (after hydrolysis in 6*M* HCl at 110°C for 24 hours at reduced pressure) and determination of its tryptophan content by ultraviolet spectroscopy (*2*) gave the following composition: $Asp_{1.23}Glu_{0.99}Gly_{0.98}Leu_{1.14}Phe_{0.96}Pro_{1.01}Ser_{1.18}Trp_{1.00}$ (*5*), which accounts for 89 percent of the weight of the hormone.

The hormone is electrophoretically immobile at acid, neutral, and alkaline *p*H (*6*), in accordance with its blocked NH_2-terminus, and proving that none of its carboxyl groups is free.

Digestion of the hormone (23 μg) with thermolysin and fractionation of the digest on a column of Sephadex G-25 gave two major peptide fragments. Upon acid hydrolysis, one of these gave aspartic acid, glutamic acid, and leucine in about equimolar proportions. Since no free NH_2-terminal group was obtained by the DNS-Cl method (*7*), this peptide was an NH_2-terminal fragment of the hormone. Acid hydrolysis of the other peptide yielded about equimolar amounts of glycine, phenylalanine, proline, and serine. In addition, it contained tryptophan, which was determined by ultraviolet spectroscopy. It had a free NH_2-terminus, and analysis by the Edman-dansyl method, essentially as described by Gray and Smith (*8*), established its sequence as Phe-Ser-Pro-Gly-Trp-NH_2. The COOH-terminus was identified by omitting the hydrolysis after the DNS-Cl treatment which followed a four-cycle Edman degradation. The DNS product so obtained was identical to a reference DNS-tryptophan amide when compared in polyamide thin-layer chromatography

Table 1. Thin-layer chromatography of isolated and synthetic *Pandalus borealis* blanching hormone. Chromatography was carried out on silica gel thin-layer chromatography plates without fluorescence indicator (Merck, Darmstadt). Approximately 1 μg each of the isolated hormone and the synthetic peptide were spotted on each plate. After chromatography, peptide material was visualized by the chlorine-starch method (*19*).

Solvent system	Proportion (by volume)	R_F*
Butan-2-one–pyridine–acetic acid–water	80 : 10 : 2 : 10	0.39
Ethyl acetate–ethanol–pyridine–acetic acid–water	60 : 30 : 2 : 1 : 8	0.40
Chloroform–methanol–acetic acid	40 : 50 : 10	0.68
Propan-2-ol–ammonia†–water	80 : 10 : 10	0.71
Chloroform–methanol–ammonia†	45 : 45 : 10	0.85

* R_F values were identical for the isolated hormone and the synthetic product. † Concentrated.

(*9*) in which several different solvent systems were used.

Thermolysin thus cleaved the hormone into two parts: one NH_2-terminal, the blocked tripeptide (Asx,Glx,Leu) and one COOH-terminal, the pentapeptide Phe-Ser-Pro-Gly-Trp-NH_2. Such a cleavage is in agreement with the specificity of thermolysin (*10*).

The blocked NH_2-terminus of the hormone prevented a sequence determination of the NH_2-terminal part by the Edman-dansyl method. Therefore, since the hormone is rather nonpolar (*6*), mass spectrometry was used (Fig. 1). The most abundant peak in the spectrum is at *m/e* 84, indicating an NH_2-terminal pyroglutamic acid (*11*). The "sequence ions" at *m/e* 197 and 225 show the hormone to have the NH_2-terminal sequence pGlu-Leu-, which is consistent with the results from the analysis of the thermolysin fragments. There were no interpretable fragments at *m/e* values higher than 225; the largest recorded ion had an *m/e* value of 408. The peak at *m/e* 186 confirms the presence of a COOH-terminal tryptophan amide according to the fragmentation pattern observed for peptides containing tryptophan (*12*). Most of the other strong peaks are fragments from the various amino acids present in the hormone, for example, *m/e* 130 is derived from tryptophan (*12*).

The results are consistent with the following structure for the hormone:

pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2

The presence of the aspartic acid residue as its amide, that is, asparagine, was inferred from the electrophoretic immobility of the hormone (*6*), and its position in the peptide was obtained by exclusion.

A peptide having the deduced amino acid sequence was synthesized from amino acids in two main steps: The heptapeptide, pGlu-Leu-Asn-Phe-Ser-Pro-Gly, was first synthesized by the Merrifield solid-phase technique (*13*), as described by Stewart and Young (*14*). It was purified, characterized, and then coupled with tryptophan amide in solution. This coupling, which was performed with the dicyclohexylcarbodiimide method (*15*) in dimethylformamide, required that *N*-hydroxysuccinimide was included in the reaction mixture (*16*) in order to give a satisfactory yield. The final product was purified by adsorption chromatography on a column of Sephadex G-25 in water—the synthetic product showed the same retention on the column as the isolated hormone (*2*).

The synthetic peptide was obtained in an overall yield of about 60 percent (from the original resin-bound glycine). Upon acid hydrolysis, it gave (*4*): $Asp_{1.01}Glu_{1.02}Gly_{0.98}Leu_{1.04}Phe_{1.00}Pro_{0.99}Ser_{0.86}$ (uncorrected) and, according to its ultraviolet spectrum, its tryptophan content was 93 percent of the theoretical. The peptide was ninhydrin negative and electrophoretically immobile. Further, it was homogeneous in all tested thin-layer chromatography systems, some of which are given in Table 1.

The synthetic peptide was compared with the isolated hormone by several means. In adsorption chromatography on Sephadex G-25, as mentioned, they both had the same retention, and in thin-layer chromatography (Table 1) no differences at all were observed between them. Furthermore, a mass spectrum of the synthetic peptide differed from that of the isolated hormone (Fig. 1) only in having much less abundant peaks at *m/e* values 58, 59, 60, 89, and 218. These peaks are probably derived from impurities in the hormone preparation.

The synthetic peptide caused blanching of *Pandalus borealis* as well as of the shrimp, *Palaemon adspersus*. In quantitative bioassays, with the use of the latter species (*3*), log dose-response curves for the synthetic peptide and the isolated hormone were parallel. The specific activity of the synthetic peptide was 1.30×10^6 unit/mg (95 percent confidence limits: 1.13×10^6 and 1.50×10^6) to be compared with that of the isolated hormone, 0.97×10^6 and 1.08×10^6 unit/mg, respectively, for two different hormone preparations (*2*). The difference is probably explained by the presence of nonpeptide

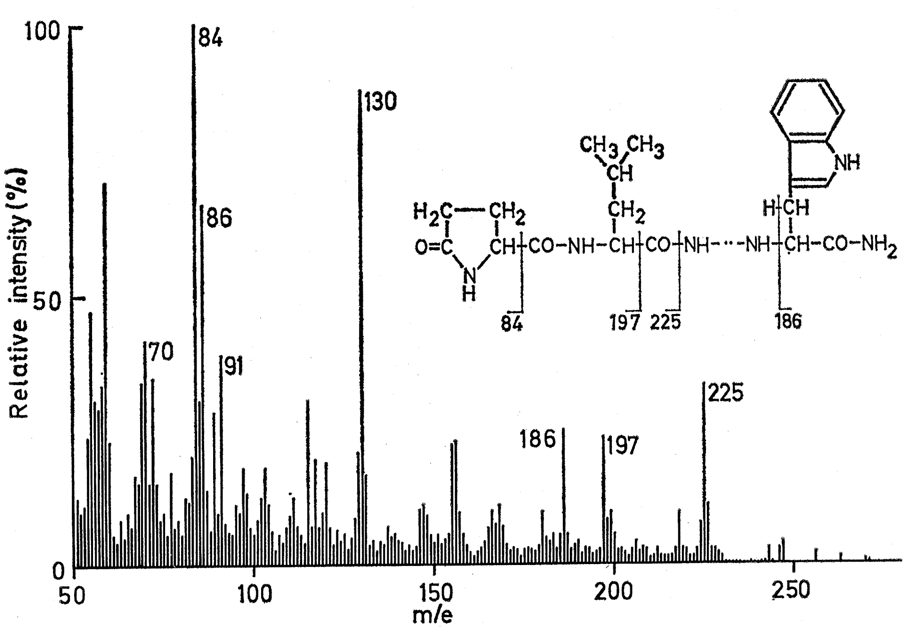

Fig. 1. Mass spectrum of *Pandalus borealis* blanching hormone. The spectrum was obtained from 6 μg of the hormone on an AEI MS 902 instrument with a direct inlet system.

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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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