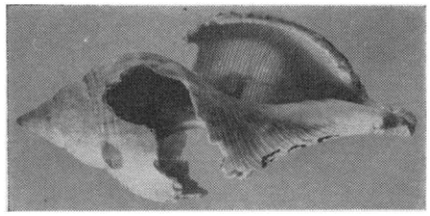

Fig. 3. Shell of *Fasciolaria tulipa* (length, 66.6 mm), containing a hermit crab (*Clibanarius vittatus*), that was opened by *C. flammea*.

removed by the chela or maxillipeds for eating.

An opened tulip shell, *Fasciolaria tulipa* Linn., appears in Fig. 3; similarly broken shells are common in the field. While opening a shell, the crab reacts immediately with quick grasps with the ambulatory legs and chelae to movements of escape by the prey.

This previously unrecognized mechanism, with the associated behavior pattern, enables these crabs to open shells that they could not otherwise open and thus expose shell-protected animals. Certainly this is the most refined shell-opening mechanism yet reported for the Crustacea.

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Agent of Disease Contracted from Green Monkeys

Abstract. *An infectious agent obtained from patients who became ill after exposure to tissues of African green monkeys is viral in character. By electron microscopy, the agent appeared cylindrical, 90 to 100 nanometers in diameter, and 130 to 2600 nanometers in length. Cross-striations at 5-nanometer intervals and a core diameter of 45 nanometers were observed. The agent was completely resistant to the effects of the metabolic inhibitor 5-bromodeoxyuridine, which may mean that RNA is the genetic material. It was sensitive to ether and relatively sensitive to destruction by heat.*

In August and September 1967, an outbreak of disease occurred in Germany and Yugoslavia among laboratory workers engaged in removal and processing of kidneys from African green monkeys for cell culture production; additional cases involved medical personnel attending the patients. At least 30 cases with seven deaths were observed. Gordon Smith *et al.* (*1*) attempted to define the etiology of this disease.

Efforts to culture bacteria or leptospires were unsuccessful; its filtration characteristics indicated that the agent was larger than most viruses. The apparent size of the organism and its failure to propagate on artificial media led to the consideration that the agent might be a rickettsia. Accumulated evidence indicates that it is a true virus. Using guinea pigs, Gordon Smith and co-workers produced complement-fixing (CF) antigens and antiserums, but these reagents did not react with any of a variety of viral reference antigens and antiserums. However, patients developed antibody to the experimental antigen.

A Porton isolate was provided by Simpson (*2*); it had been passaged nine times through guinea pigs, twice through monkeys, and once again through a guinea pig. Frozen blood from a nonfatal case ("Flak"), collected on the 6th day of illness, and liver from the first guinea-pig passage of liver from a fatal case ("Popp") were provided by May (*3*).

Each specimen was inoculated into guinea pigs (350 g, male, Hartley strain) by the intraperitoneal route. The Porton agent produced a rapidly progressing disease, resulting in death 6 to 7 days after inoculation. Clinical signs in the guinea pigs consisted of fever (to 40.3°C), inappetence, conjunctivitis, dyspnea, and progressive weakness. The pathological changes have been described (*1*). The Popp isolate killed guinea pigs on the 11th and 12th days after inoculation. The clinical signs and pathological changes were similar to those observed with the Porton specimen.

The Flak specimen initially produced a more slowly progressing disease in guinea pigs and was not uniformly fatal until the fourth passage. The lesions observed in these animals were similar to those previously described (*1*), and guinea pigs that survived the disease developed complement-fixing antibodies to an antigenic extract produced with the Porton agent.

Crude complement-fixing antigens for the Porton agent prepared from liver from infected guinea pig (20 percent suspension) in barbital-buffered saline had titers of 1 : 16 to 1 : 32, by the Laboratory Branch complement-fixation micromethod (*4*). Treatment of the antigens with 1 : 2000 β-propiolactone reduced the titer to 1 : 8, whereas heat (56°C for 30 minutes) lowered titers to 1 : 4.

Immune serum was produced in guinea pigs by intraperitoneal inoculation of liver from infected guinea pig (0.7 ml of a 20 percent suspension) (Porton) previously treated for 24 hours with 1 : 2000 β-propiolactone. Thirteen days after this injection 0.1 ml of infectious liver suspension was injected intraperitoneally. Serums were collected 12 days later. Complement-fixing antibody titers ranged from 1 : 64 to 1 : 512. These serums did not react in complement-fixation tests against antigens prepared from vesicular stomatitis (New Jersey and Indiana serotypes), Cocal, Kern Canyon, or Hart Park viruses, or against a wide variety of arboviruses.

We also attempted to establish the agent in cell cultures. The Porton agent was inoculated into a variety of cell cultures including VERO and GMK AH-1 stable lines of kidney of vervet monkey, the WI2 and CCL10 clones of BHK21, and diploid strains of fibroblasts from guinea pig heart and human foreskin. The growth and maintenance media have been described (*5*). After three passages in BHK21-CCL10 and AH-1 cells, a similar complete cyto-

pathic effect was evident. With low dilutions of infected cell culture fluids, the first pathologic changes appeared on the 2nd day after inoculation and consisted of focal areas of spindling and retraction of the cells from one another. The foci became confluent by the 4th to 5th day after inoculation. The cells eventually became rounded and pyknotic, and the majority sloughed. Progression of the cytopathic effect was more rapid in the BHK21 cells, but titrations were difficult because the control cultures remained in a satisfactory condition for only 7 to 8 days. The AH-1 cell cultures were maintained well for at least 2 weeks and were therefore used to perform infectivity titrations. During the first few serial passages in BHK21 cells, the infectivity titer ranged from $10^{3.0}$ to $10^{4.5}$ $TCID_{50}$ (tissue culture infectious doses, 50 percent effective) per 0.1 ml. By the seventh passage, the observed titer was $10^{6.5}$ $TCID_{50}$.

Initially, the cell cultures were not as sensitive to the agent as guinea pigs were, in that the same starting preparation produced a titer of only $10^{3.0}$ in cell cultures, whereas guinea pigs inoculated with a $10^{-5.0}$ dilution succumbed rapidly. A guinea pig inoculated with fluid from the fourth cell culture passage of the agent developed a rapidly fatal disease with characteristic pathological changes. No cytopathic effect was produced in guinea pig heart cells. Although apparent cytopathic effect was produced upon initial inoculation of the foreskin fibroblasts, no cytopathic effect was evident upon serial passage. An incomplete cytopathic effect was produced in VERO and BHK21-WI2 cells.

Cultures of BHK21-CCL10 were treated with medium from which thymidine was deleted and to which 5-bromodeoxyuridine (40 μg/ml) was added. After 24 hours, the medium on half of the cultures was replaced with the usual medium which contains thymidine (8 μg/ml), and the other half was changed to fresh medium containing the 5-bromodeoxyuridine. The African-green-monkey–associated agent (10^{-2} dilution of sixth BH21 passage) was inoculated onto cells maintained with each medium. Coxsackie B-1 virus and herpesvirus hominis were inoculated into similar sets of cultures as known examples of RNA and DNA viruses. When the cytopathic effect was advanced in tubes with the medium containing thymidine, both sets were harvested and titrated for infectivity. The titer of the agent associated with African green monkey was $10^{6.5}$ $TCID_{50}$ per 0.1 ml from cultures treated with 5-bromodeoxyuridine and thymidine. The yield of the Coxsackie B-1 virus containing RNA was similar ($10^{4.5}$ $TCID_{50}$) from cells maintained in either medium, but no infectious virus could be recovered from the 5-bromodeoxyuridine cultures inoculated with the DNA-containing herpesvirus. The cultures inoculated with herpesvirus, which were maintained in a medium containing thymidine, yielded $10^{1.5}$ $TCID_{50}$ infectious virus per 0.1 ml. The failure of the 5-bromodeoxyuridine to interfere with the replication of agent associated with the African green monkey disease would indicate that DNA is not involved, and therefore that its genetic material is probably RNA. This is also evidence that the agent is viral in character.

Material harvested from the fifth passage of the agent in BHK21-CCL10 cells was mixed with an equal quantity of diethyl ether and allowed to stand for 20 hours at 5°C. After the ether was removed, no infectivity could be detected in the sample. A sample that was not treated with ether yielded $10^{4.5}$

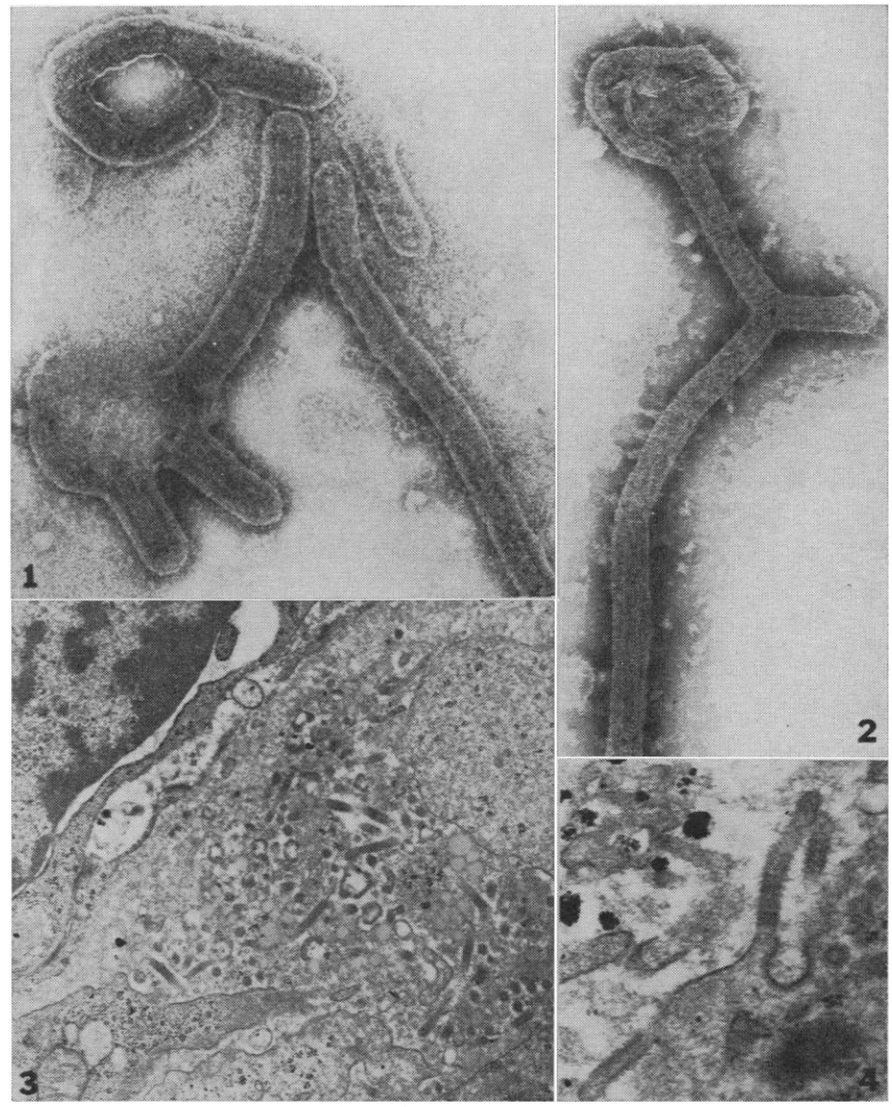

Fig. 1. Agent associated with African green monkey. Negative-contrast preparation from BHK-21 cell culture illustrating cylindrical particles continuous with membrane. Cross-striations and cores are evident (× 69,800). Fig. 2. Particle from infected BHK-21 cells. Membranous bleb at top is considered a result of avulsion of cytoplasmic membrane at time of particle release. Lower branch extended 2000 nm from the point of branching (× 53,800). Fig. 3. Ultrathin section of liver from infected guinea pig. Cylindrical particles are in a sinusoid filled debris (× 17,300). Fig. 4. Ultrathin section of liver showing cylindrical particles budding from marginal membrane of a hepatocyte (× 41,000).

$TCID_{50}$ per 0.1 ml. The infectivity titer was reduced from $10^{3.5}$ to $10^{0.05}$ $TCID_{50}$ per 0.1 ml after the cell-cultured agent was heated at 56°C for 30 minutes.

For electron microscopy, specimens obtained by ultracentrifugation of supernatants of BHK-21 cell cultures infected with sixth cell-culture passage of the Porton agent associated with African Green monkey were inactivated with osmium tetroxide, mixed with sodium phosphotungstate stain, and sprayed onto carbon-coated grids. These preparations contained many bizarre cylindrical particles (Fig. 1) with a uniform diameter of 90 to 100 nm. Their length varied from 130 to more than 2600 nm. Particles had rounded ends, but many had large blebs of membranous material attached to one end. Many bent into looped or "fish hook" configurations around their terminal bleb. Rarely, branched particles were observed (Fig. 2). Cross-striations and an inner cylindrical structure were observed in particles penetrated by the stain; the cross-striation interval was approximately 5 nm, and the core diameter was 45 nm. Electron microscopy (thin sections) of infected cell cultures revealed extracellular particles which, when sectioned transversely, consisted of concentric electron-opaque rings consonant with the surface and core appearing in negative-contrast preparations. Sectioned particles appeared to be covered with an additional irregular surface layer.

The same kind of particles was observed in ultrathin sections of liver from guinea pigs infected with Porton material that had been passaged twice through guinea pigs or with Flak isolate passaged four times through guinea pigs. In areas of periportal necrosis, particles accumulated in large numbers within the lumina of debris-filled sinusoids (Fig. 3). Infrequently, budding of particles was observed from the cytoplasmic membrane of hepatocytes (Fig. 4). This budding, which is observed in thin sections, may mean that the blebs attached to free particles in negative-contrast preparations were a likely consequence of avulsion of attached membrane at the time of particle release. These particles were not observed in uninoculated cell cultures or normal guinea pig tissues examined in parallel with infected specimens.

Because we often found the particles in passage materials from Porton specimens as well as in passage materials from human specimens received directly from Frankfurt and observed the spatial and temporal relation between these particles and cytopathological changes both in guinea pig liver and in cell cultures, we conclude that these particles are probably the etiological agent of the fatal human disease. This conclusion is supported by our serological results. Moreover, the characteristics of ether and heat lability, resistance to the metabolic inhibitor 5-bromodeoxyuridine, and the cross-striated cylindrical structure suggestive of helical symmetry, indicate that the agent is viral. Similarities to viruses of the Stomatoviridae or rhabdovirus (*6*, *7*) family are evident. Cross-striation interval, core structure, suggestion of surface projections, and mode of maturation are similar to vesicular stomatitis virus, the prototype virus of the family. However, the particle diameter measured here (90 to 100 nm as compared to 65 to 75 nm for vesicular stomatitis virus) and, primarily, the remarkable length and extreme variation in length among particles serves to emphasize the uniqueness of this agent.

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20 February 1968

Dihydromatricaria Acid: Acetylenic Acid Secreted by Soldier Beetle

Abstract. *The aposematic cantharid beetle* Chauliognathus lecontei *produces a defensive secretion, from glands in its thorax and abdomen, containing 8-cis-dihydromatricaria acid. Similar acetylenic compounds are known only from certain fungi and flowering plants.*

Chauliognathus lecontei is a large red and black beetle (Fig. 1A) of the family Cantharidae (soldier beetles). A native of the southwestern United States and northwestern Mexico (*1*), it commonly occurs in dense and conspicuous aggregations on various flowering herbs and shrubs, sometimes in association with mimics (*2*). Like other members of its genus, it possesses a series of exocrine defensive glands in pairs on the prothorax and on the first to eighth abdominal segments where their openings are visible as small pores near the lateral margins of the segments. Each gland, a sac-like invagination of the integument, consists of a cuticular lining, a secretory epithelium, and a surrounding layer of compressor muscles. When the beetles are handled, pinched with forceps, or otherwise disturbed, they discharge their glands, and droplets of a white secretion emerge from the openings (Fig. 1, B and C) (*3*). We now report isolation and identification of an acetylenic acid that is a major component of the secretion.

Beetles (1500) were "milked" by gentle squeezing with forceps and absorption of the secretion with pieces of filter paper. Extraction of the papers with methylene chloride yielded a yellow viscous oil showing strong infrared absorption at 3.43, 3.48 (shoulder), 5.72, 5.79 (shoulder), and 8.80 μ, in addition to a region of weaker absorption between 2.85 and 4 μ, indicative of a carboxylic acid. Thin-layer chromatography also suggested the presence of an acidic function; the material was therefore separated into acidic and neutral fractions with aqueous sodium bicarbonate.

The neutral fraction consisted of a semisolid polymeric material and a nonpolar component that, isolated by preparative thin-layer chromatography, showed strong infrared maxima at 5.75 and 8.70 μ. On standing, the latter compound was shortly converted to a polymeric species and was examined no further (*4*).

In contrast, the bicarbonate phase, after thorough washing with methylene chloride and acidification, afforded colorless, odorless platelets (6.4 mg); the melting point was 55° to 57°C after crystallization from pentane. Inspection of the infrared spectrum [bands at 2.86, 3.13 to 3.85 (weak), 3.44, 5.73 (shoul-