HEL cells became contaminated with HBC-1 cells, and that the HBC-1 cells were subsequently transformed. Therefore, a subculture of CMV carrier HEL cells frozen before the introduction of the human bladder lines into the laboratory was thawed, cultured, and followed to see if a transformed line would again arise. Only a fraction of the frozen cells could be cultured. After 3 weeks, foci of transformed cells appeared (Fig. 1) and a stable cell line was developed. CMV-specific antigens could be demonstrated in this line (designated CMV-Mj-HEL-2) by immunofluorescence reagents. Furthermore, spleen cells sensitized to CMV were cytotoxic for the cells (Table 1). Thus far, no virus has been rescued from the cells. The cells induced progressively growing tumors in weanling athymic nude mice.

In summary, the present work indicates that human cells can be transformed by a recent isolate of CMV, and that such cells produce nondifferentiated tumors following transplantation to athymic nude mice. As such, the results constitute further evidence of the transforming capacity of CMV, and raise the possibility that the virus may have oncogenic potential in its natural host.

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Fossilized Eggs in a Pennsylvanian Blastoid

Abstract. *A single specimen of* Pentremites rusticus *(Echinodermata, Blastoidea) from the Early Pennsylvanian of Oklahoma has hundreds of apparent eggs in one of its abnormal anal hydrospire groups. This rare occurrence suggests that female blastoids in this sexually dimorphic species had modified their anal hydrospires for brooding eggs instead of for normal respiration.*

A single sectioned specimen (*1*) (out of a total of 658) of the fossil blastoid *Pentremites rusticus* Hambach (*2*) from the Early Pennsylvanian (Morrowan) of northeastern Oklahoma was found to contain several hundred small translucent spheres (Fig. 1, A to D) that are probably fossilized blastoid eggs. These eggs are confined to one group of the blastoid's anal (posterior) hydrospires, which are abnormally developed in *P. rusticus* [and in *P. angustus* Hambach (*2*), apparently a synonym (*3*)]. Hydrospires are the five pairs of thin-walled longitudinal calcite folds (Fig. 1B) hanging into the coelomic cavity from the ambulacral margins; most authors (*4*) have considered them respiratory structures. Our discovery is the first convincing example of echinoderm eggs known from the fossil record, and their presence in abnormal anal hydrospires suggests that these structures had been modified for reproduction.

The egglike spheres range from 0.12 to 0.20 mm in diameter and show a normal size distribution with mean and mode at 0.16 mm (Fig. 2). Their size is in the proper range for eggs in living echinoderms (*5*). They are uniformly smooth, yellow, translucent, spherical to slightly ellipsoidal objects showing no internal structure except for radiating contacts where calcite crystals have grown together (Fig. 1D). Electron microprobe analysis of these spheres indicates that their

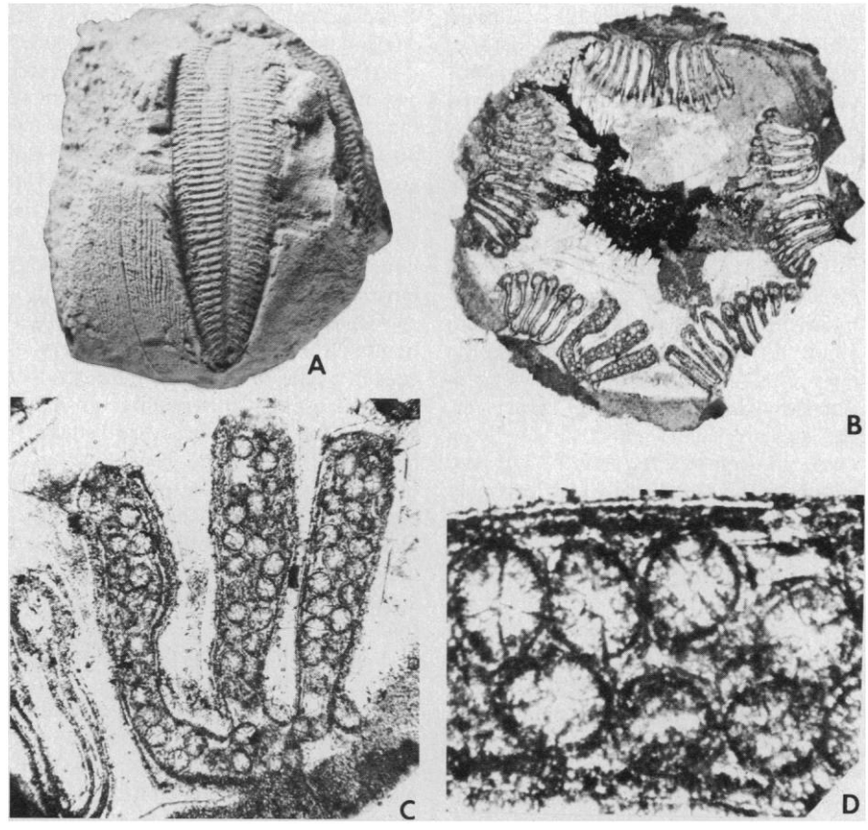

Fig. 1. *Pentremites rusticus* Hambach, plesiotype specimen OU 8372. (A) Reference photograph of complete blastoid (side view) before sectioning; note wide ambulacrum and slightly damaged calyx plates (× 3.7); (B) transverse thin section near middle of blastoid showing calyx plates and ambulacra on exterior surface (plates at lower right lost during grinding), thin calcite hydrospires hanging into coelomic cavity, and abnormal anal hydrospires (bottom) with left anal group containing numerous spherical eggs (×5); (C) enlargement of left anal hydrospire group showing eggs completely filling the three folds (× 23); (D) greatly enlarged eggs in one anal hydrospire fold showing degraded egg membrane and internal contacts where calcite crystals have grown together (× 92).

chemistry ($CaCO_3$, 96.1 ± 3.5 percent; $MgCO_3$, 0.8 ± 0.2 percent; $FeCO_3$, 0.9 ± 0.3 percent, and $MnCO_3$, 0.3 ± 0.1 percent) is nearly identical to that of the surrounding calcite cement, the thin hydrospire walls, and the overlying calyx plates and ambulacra (*3*).

The spheres do not appear to be oolites, pellets, algal bodies, spores, dolomite rhombs, sand grains, any kind of known parasite, or other foreign material that might have entered the blastoid after, or perhaps causing, its death. The spheres were probably present in the living blastoid, for when foreign particles entered a blastoid calyx after death, as has occurred frequently in other specimens, the particles entered the coelomic cavity (through the mouth) as well as the hydrospires. In this unique specimen, the spheres are present only in the abnormal left anal hydrospires; they are completely absent from the coelomic cavity and from the normal hydrospire groups. The abnormal right anal hydrospires (Fig. 1B) apparently did not contain any spheres at the time of fossilization.

Hambach (*2*) has made the only previous reference to possible blastoid eggs, which he reported in a Mississippian species of *Pentremites*. However, his spherical bodies were located in the coelomic cavity between normal hydrospire folds, and are so poorly substantiated (generalized drawing; original specimen now missing) that there is considerable doubt as to the validity of his interpretation.

The egglike spheres in our specimen of *P. rusticus* are larger than the incurrent hydrospire pores (about 0.12 mm wide) along the overlying ambulacrum, so the eggs could not have been lost to the exterior through these openings. However, the eggs are small enough to have been extruded from the blastoid's supposed gonopore (up to 0.5 mm wide), probably located within the anispiracle at the top of the anal hydrospire groups, as in other genera (*6*). The eggs are also small enough to have been ejected through the large excurrent anispiracle (about 1.0 mm wide) on the summit when the period of brooding was terminated.

The rarity of fossilized echinoderm eggs results from the fragility of these tiny unmineralized objects and the fact that most fossil echinoderms probably reproduced by external fertilization. Also, *P. rusticus* probably brooded eggs for only a few days or weeks out of the entire year, further reducing the chance of finding a fossilized female blastoid with eggs. Brooding is used by some members of all living echinoderm classes, but has been reported only in a few fossil echinoids (*7*)

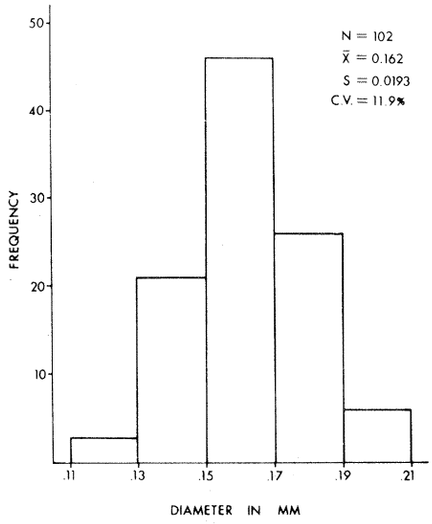

Fig. 2. Histogram of egg diameter for all measurable eggs in thin sections of specimen OU 8372 showing a nearly normal size distribution. *N*, number of eggs measured; $\bar{X}$, mean value; *S*, standard deviation; and *C.V.*, coefficient of variation.

based on sexually dimorphic features.

The presence of eggs and the use of brooding may explain the abnormally developed anal hydrospires in *P. rusticus* (*3*). Abnormal hydrospires in this species are of two types: type 1 (38 percent of the studied specimens) has the anal hydrospire folds reduced in number and greatly expanded in shape (Fig. 1, B to C), whereas type 2 (62 percent) has the anal hydrospire folds only reduced in number. No specimens of *P. rusticus* have completely normal anal hydrospires, as do all other spiraculate (closed hydrospire) blastoids. The discovery of eggs in a type 1 *P. rusticus* indicates that this hydrospire "abnormality" is an adaptation for internal brooding instead of for normal respiration. These two types of abnormal anal hydrospires apparently represent a sexually dimorphic character, with type 1 being females and type 2 males (*3*). This is the first known instance of sexual dimorphism in blastoids.

A further implication of fossilized eggs is that the blastoid genus *Pentremites*, which had normal hydrospire morphology and apparently reproduced by external fertilization in all known Mississippian species, had changed its hydrospire morphology and reproductive strategy to internal fertilization and brooding in this Early Pennsylvanian species. Ripe eggs, extruded through the gonopore in the anispiracle of females, would collect in the enlarged anal hydrospires where they would be protected and bathed in slowly circulating seawater. Sperm released by nearby male blastoids would enter the egg-laden anal hydrospires with the circulating seawater and fertilize the brooded eggs. The eggs could then be brooded until they became either free-swimming larvae or, through direct development, immature adults ready to settle and attach to the substrate. They could then be expelled through the anispiracle by increasing the current velocity through the anal hydrospires.

The brooding of eggs (and perhaps larvae) would reduce the severity of predation on these early stages and would prevent their drifting into nearby unfavorable environments. Modern predators and suspension or filter feeders that thrive on echinoderm eggs and larvae include dinoflagellates, coelenterates, copepods, amphipods, barnacles, bivalves, other echinoderms, and some fish (*8*). The Morrowan fauna in northeastern Oklahoma contains several of these groups (*9*), but so do Late Mississippian faunas which contain normal species of *Pentremites*. Perhaps more significant is the report of numerous, small, isolated, moundlike bioherms in the Morrowan of northeastern Oklahoma (*10*). *Pentremites rusticus* is abundant in and around these structures (*3*), in contrast to Mississippian species of *Pentremites* which do not commonly occur near bioherms. Perhaps internal brooding in *P. rusticus* was useful in preventing the offspring from drifting away from these local "ideal" environments.

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Thrombosis in Association with Atherosclerosis Induced by Dietary Perturbations in Dogs

Abstract. *The distribution, severity, and complications of diet-induced atherosclerosis in dogs can be altered by changing the source of fat in the diet. Thrombosis and thromboembolic disease associated with atherosclerosis occurred with diets containing beef tallow and lard or coconut oil but were absent in dogs fed cottonseed oil as a source of fat. Experimental animals with and without thrombosis are of value as models in elucidating the role of platelets and thrombostatic mechanisms in atherosclerosis.*

Atherosclerosis is the major cause of mortality and morbidity in the United States. It is characterized by local fibromuscular proliferation and lipid deposition in the tissue layer that lines the arteries. These overgrowths of tissue are called atherosclerotic plaques. The disease becomes symptomatic when the size of the plaques causes significant obstruction to blood flow or when thrombi form on the plaques. Despite the identification of various dietary, metabolic, and environmental factors as contributors to the genesis of this disease, little is known on a molecular or cellular level about its causative mechanisms. The roles of the platelet and hemostatic mechanisms in the etiology of the disease have been debated for decades. Most investigators agree that thromboembolic events are frequent and often fatal sequels of atherosclerotic lesions, but there is major controversy concerning the contribution of platelets to the primary or secondary events of the disease process (*1*). Diets high in saturated fat have been implicated in causing thrombosis, and it has been suggested that increased sensitivity of platelets to aggregation results from altered platelet membrane cholesterol or fatty acid content, or both (*2*). However, investigations of the roles of the platelet and coagulation in atherosclerosis have been hampered by lack of a suitable animal model. We now describe an animal model that develops significant thrombosis in association with atherosclerosis and suggest that such a model may be useful in the elucidation of the role of hemostatic processes in atherosclerosis.

The dog is a suitable model for studies of atherosclerosis. There is much to recommend the species, including ease of handling, availability, and a size large enough to provide sufficient blood and arterial tissue for analyses. In addition, dogs do not develop spontaneous atherosclerosis; thus, any disease observed is the direct result of the imposed experimental conditions. However, dogs are resistant to the development of hypercholesterolemia and atherosclerosis and require experimental maneuvers beyond simply feeding them a high cholesterol diet. Two different protocols have been demonstrated to be successful in the production of canine atherosclerosis. In one protocol the hypothyroid dog is fed a high fat, high cholesterol diet in association with a bile acid (*3, 4*). We have used various dietary fats in this protocol to determine the effects of changes in triglyceride content on the type and distribution of atherosclerosis and the occurrence of thrombosis. In the second protocol (*5*), euthyroid dogs are fed a semisynthetic diet containing cholesterol and hydrogenated coconut oil as the only sources of fat. Dogs on this diet have not been reported to develop thrombosis.

Atherosclerosis was shown to develop (*4*) in surgically thyroidectomized dogs fed a commercial diet supplemented with cottonseed oil, cholesterol, cholic acid, and propylthiouracil. The most severe disease occurred in dogs with concentrations of cholesterol in the plasma in excess of 750 mg per 100 ml and a characteristic hyperlipoproteinemia. The atherogenic hypercholesterolemia was characterized by the occurrence of beta very low density lipoproteins (β-VLDL), an increase of low density lipoproteins (LDL), and the presence of a unique lipoprotein, the HDL_c (*6*). A similar type of hyperlipoproteinemia has been described in association with accelerated, cholesterol-induced atherosclerosis in swine (*7*). The atherosclerosis in the dog fed the cottonseed oil diet involved the thoracic and abdominal aorta and the coronary arteries with only moderate peripheral artery disease. The lesions were of the intimal proliferative type and were associated with lipid deposition. Evidence of thrombosis was not observed (*4*).

In sharp contrast to the above, when beef tallow or a mixture of beef tallow and lard was substituted for the cottonseed oil in the diet, there were significant changes in both the type and distribution of the disease. Moreover, thrombosis became a prominent component of the atherosclerotic disease process. To investigate the role of saturated fats in the production of atherosclerosis, 48 purebred foxhounds (NIH colony) were divided into two groups, which were maintained for 12 months on diet. One group of 24 euthyroid dogs was fed (*8*) a standard dog chow supplemented with fat (an equal mixture of lard and tallow). The other group of 24 dogs was surgically thyroidectomized and placed on the same diet except that cholesterol, taurocholic acid, and propylthiouracil were added (*8*). The euthyroid dogs did not develop significant hypercholesterolemia or atherosclerosis. The hypothyroid dogs developed atherosclerosis which was associated with a hyperlipoproteinemia that was similar to that of the dogs fed cottonseed oil. However, the distribution of this disease, unlike that in dogs fed the cottonseed oil, was restricted primarily to the terminal abdominal aorta, while the thoracic aorta was spared. An additional, not previously seen, difference in the pattern of disease was an extensive, often occlusive, involvement of the peripheral arteries, including the iliofemorals, mesenterics, internal carotids, and coronaries. Histologic study of the lesions of the terminal aorta revealed severe intimal proliferative disease with lipid deposition, occasional necrosis, calcification, and adventitial inflammatory response. The peripheral arteries showed an even more fulminant disease process associated with extensive necrosis, lipid deposition, and rapid progression to medial involvement. Of particular interest was the occurrence of thrombosis in association with many of these atherosclerotic lesions.

At autopsy, grossly visible thrombosis was found in association with arterial lesions in six of the 24 dogs. This occurred in the iliofemorals, terminal aorta, and internal carotids. A large, virtually occlusive thrombus in the iliofemoral artery is shown in Fig. 1A. Histologic examination of the arteries of many of the ani-