Table 3. Ratios of palmitic to pristanic acid (I) and pristanic acid to pristane (II).

Source of lipid	I	II (3)
Wilkinson Basin	15	130
Volden Fjord	30	47
Tarpaulin Cove	21	
Tuna (6)	32	13
Calanus spec.	50	0.5–0.2

methyl groups are displaced by one carbon atom along the chain have ECL values outside the range measured here (*10*).

Olefinic isoprenoid acids, for example, phytenic acid (*9*), are minor constituents of some lipids and could conceivably be present in the sediments. A different isolation procedure, excluding hydrogenation, was chosen to establish the presence in the extracts of the saturated acids. The esters were fractionated on an active silica gel, containing 2 percent water, at a high ratio of adsorbent to sample. Pentane and pentane of low benzene content served as eluents. The C_{16} isoprenoid ester appeared just ahead of the normal C_{14} and C_{15} esters; pristanic and phytanic esters appeared together with the C_{16} through C_{20} straight-chain esters and well ahead of the monounsaturated straight-chain esters. This establishes the presence in the recent sediments of the saturated C_{16}, C_{19}, and C_{20} isoprenoid acids. The great complexity of the olefinic fractions that were eluted later did not permit the identification of possible traces of olefinic isoprenoid esters.

For quantitative determinations, separate samples were processed; to minimize losses, urea clathration was omitted. Hydrogenation was necessary to reduce the complexity of the chromatograms. The values of Table 2 therefore include the concentration of any unsaturated isoprenoid esters that might be present in the sediments.

Terpenoid acids from C_{11} to C_{22} occur in ancient sediments (*15*). Acids other than those reported here were not detected in the recent sediments; if present, they amounted to less than 10 percent of the trimethyltridecanoic acid.

The ratio of palmitic to pristanic acid is similar to that encountered in typical marine lipids (Table 3); this suggests that the isoprenoid acids of recent sediments are directly derived from living organisms. Compared to pristanic acid, pristane is much more abundant in the lipids of *Calanus* than in the sediments. However, the high pristane content of *Calanus* lipids is unusual; it exceeds that of most other marine fats and oils by about two orders of magnitude. The ratio of pristanic acid to pristane in the sediments is much closer to that in the tuna lipids than to those in calanid copepods. This implies that the direct contribution to the sedimentary lipids by *Calanus* is minor, even in regions rich in these copepods.

In these sediments, the absence of the wide range of isoprenoid acids common to ancient sediments suggests that their formation is a slow postdepositional process. This is analogous to our earlier finding that the biochemically derived pristane is present in recent marine sediments but that the phytane of ancient sediments is formed slowly and presumably at depth. These results extend our knowledge of the biogeochemistry of phytol and of the dissemination of its degradation products through the biosphere and geosphere.

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Synovial Cell Synthesis of a Substance Immunologically Like Cartilage Proteinpolysaccharide

Abstract. *Fluids from joints contain a substance that reacts immunologically like one of two known antigenic components of articular cartilage proteinpolysaccharide. This newly recognized substance occurs in the lining cells of synovial membranes as shown by indirect immunofluorescence. The localization of this substance in tissue culture cells derived from synovial membranes and its identification in the culture medium supports the suggestion that it is synthesized by lining cells. Rheumatoid synovial cells contain less of this substance.*

The matrix of articular cartilage consists largely of collagen and proteinpolysaccharides. These substances are synthesized by the chondrocytes—cells which share a common mesenchymal origin with fibroblasts and surface (lining) cells of the synovial membrane (*1*). Synovial membrane lining cells appear to secrete (*2, 3*) hyaluronateprotein, the major proteinpolysaccharide of synovial fluid. Alterations in these cells derived from mesenchyme and in their secretory products may be significant in the pathogenesis of rheumatoid arthritis. We now describe the apparent synthesis by synovial cells of a substance immunologically identical to a component of articular cartilage matrix, and we note a diminished synthesis of this substance by rheumatoid cells.

The proteinpolysaccharides of cartilage have been extracted, purified (*4*), and shown to be antigenic (*5*). Moreover, they possess at least two antigenic components (*6*). One is present in the proteinpolysaccharides from bovine, porcine, and human cartilage and has been called the common component; the other appears to be unique to each species and has been termed the species-specific component. Antiserums were prepared by immunization of rabbits with purified cartilage proteinpolysaccharides from each species. Species specificity was demonstrated by reactivity between antigen and antiserum that

persisted after the antiserum was thoroughly absorbed with proteinpolysaccharide from heterologous cartilage.

It has been reported (*7*) that human synovial fluids contain products derived from breakdown of articular cartilage. A previous study to demonstrate the presence of cartilage proteinpolysaccharide in human synovial fluids with the use of a rabbit antiserum to the human articular cartilage antigen indicated that normal fluids and a variety of nonrheumatoid but pathological effusions contained a newly recognized substance (*8*). This substance was identified by methods that involved the use of absorbed antiserum as noted above; there were indications that it was immunologically identical to the species-specific component of human articular cartilage proteinpolysaccharide. A reduced or barely perceptible amount of this substance was found in fluids from involved joints of patients with chronic rheumatoid arthritis. These results were interpreted to indicate that a component of cartilage was released into synovial fluids—an apparent cartilage degradation product—and that, for some reason, its concentration was reduced in rheumatoid arthritis.

Phagocytic activity of synovial cells has been described (*9*); changes observed in rheumatoid synovial membrane lining cells (*10*) suggest that these cells may have greater than normal phagocytic function. Enhanced phagocytosis of joint fluid components seemed to be a reasonable explanation for the diminished amount of the new substance in synovial fluids from rheumatoid joints; therefore, we planned an immunofluorescent study to localize this component in synovial membrane lining cells.

Synovial membranes were obtained at surgery from normal (*11*) knee joints as well as a number of pathological ones, and the specimens were transported to the laboratory in cold, sterile tissue culture medium (*12*). A portion from each specimen was removed aseptically for tissue culture, and the remainder was saved for histological study by indirect immunofluorescence. The latter portions were frozen, sectioned, fixed, and reacted with antiserums as described (*3*). We incubated synovial sections for 30 minutes with a globulin fraction of rabbit antiserum to proteinpolysaccharide from human articular cartilage; the antiserum was prepared and absorbed according to the methods of Sandson *et al.* (*6*). This process was followed by incubation, again for 30 minutes, with a globulin fraction of goat antiserum to rabbit γ-globulin conjugated with fluorescein isothiocyanate (*13*).

All normal synovial membranes and a number of pathological membranes, including those from patients with pigmented villonodular synovitis, Reiter's syndrome, and suspected early rheumatoid arthritis showed diffuse and bright fluorescence in the cytoplasm of the lining cells (Fig. 1a). In striking contrast, the lining cells of synovial membranes from joints involved by chronic rheumatoid arthritis showed absence of fluorescent staining (Fig. 1d). Control and antibody absorption studies were carried out with each membrane tested. When the rabbit antiserum to human cartilage proteinpolysaccharide was absorbed with homologous antigen prior to incubation, only faint fluorescence of lining cells or none at all was observed (Fig. 1b). Negative results were also obtained when a globulin fraction of normal rabbit serum was substituted for the specific antiserum or when the fluorescein-labeled, goat antiserum to rabbit γ-globulin was applied directly to the tissue. Absorption of the specific antiserum with heterologous antigen (bovine proteinpolysaccharide) (Fig. 1c) or with human serum resulted in the same bright fluorescence of lining cells as with unabsorbed antiserum (Fig. 1a). Thus, synovial lining cells contain a substance that reacts immunologically like the species-specific component of human cartilage proteinpolysaccharide, and rheumatoid synovial lining cells seem to contain less.

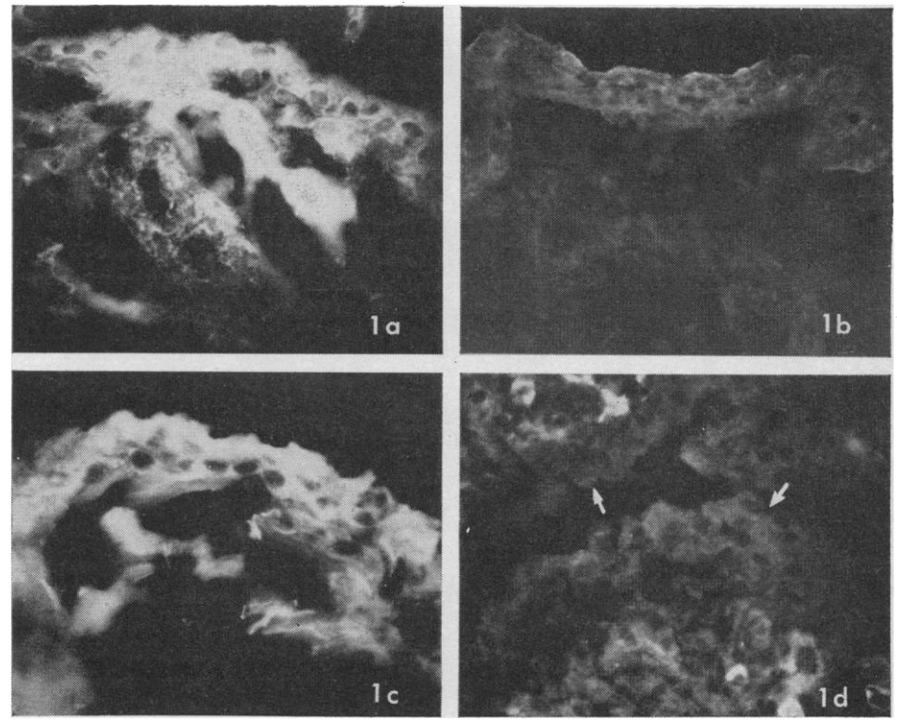

Fig. 1. Sections of normal (a–c) and rheumatoid (d) synovial membranes. (a) The section was first incubated with rabbit antiserum to human cartilage proteinpolysaccharide and then with fluorescein-labeled, goat antiserum to rabbit globulin. Cytoplasm of the surface (lining) cells is brightly fluorescent (yellow-green on the original color photograph) and offers sharp contrast to the negatively reacting nuclei. Small blood vessels beneath the lining cell layer show some endothelial cell fluorescence. The broad fiber running diagonally beneath the lining cell layer is collagen, which exhibits strong blue autofluorescence. (b) The section was first incubated with the rabbit antiserum that had been previously absorbed with purified human cartilage proteinpolysaccharide; then it was incubated with the fluorescein-labeled, goat antiserum to rabbit globulin. Essentially no fluorescence is shown. (c) The section was treated similarly, except that the rabbit antiserum had been previously absorbed with purified bovine cartilage proteinpolysaccharide. Fluorescence of the lining cells is as bright as in (a). (d) A section of synovial membrane from a patient with chronic rheumatoid arthritis was incubated with the same antiserums as in (a). Fluorescence of the lining cells (arrows) is extremely faint. (In the areas deep to the lining cells, occasional plasma cells show bright cytoplasmic fluorescence. This phenomenon, observed after the application of any rabbit serum to this tissue, suggests the presence of rheumatoid factor in these cells.) All magnifications are approximately × 300.

Hence, the previous observations (*8*) of reduced content of this species-specific component in rheumatoid synovial fluids could not have been the result of enhanced uptake by the lining cells, as we had speculated. The new observations suggested that this new substance might normally be produced by synovial membrane lining cells and secreted in diminished amount by rheumatoid cells. To determine this, we studied synovial membrane cells in tissue culture.

Portions of the lining surfaces were dissected from the surgically removed synovial membranes, explanted onto sterile petri dishes, and nourished with modified Eagle's medium (*12*). Incubation was carried out at 37°C in an atmosphere of 10 percent carbon dioxide and room air. Each cell strain was subcultured two to four times, representing at least ten generations in culture plus an unknown number of generations required to establish the primary outgrowth. No culture was more than 2 months old. The cells were grown as monolayers onto cover slips and were washed free of medium just prior to the experiments. They were then fixed for 5 minutes in neutral buffered formalin; antiserum to human cartilage proteinpolysaccharide and then fluorescein-labeled antiserum to rabbit globulin were applied in the same manner as with the synovial membrane sections.

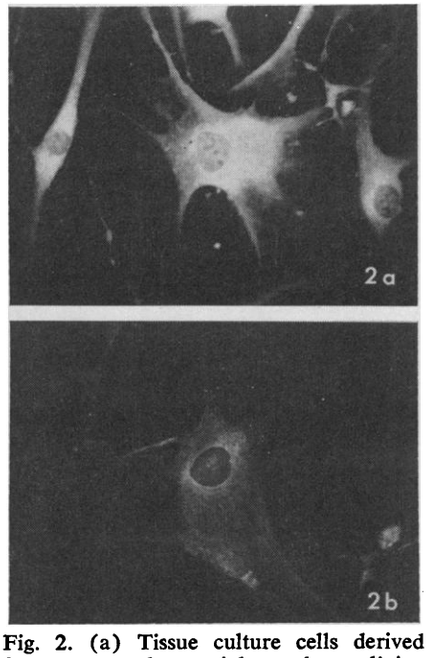

Fig. 2. (a) Tissue culture cells derived from a normal synovial membrane lining surface. The cell cytoplasm is brightly fluorescent after incubation with rabbit antiserum to human cartilage proteinpolysaccharide and fluorescein-labeled, goat antiserum to rabbit globulin. (b) Tissue culture cell derived from a rheumatoid synovial membrane. Fluorescence is faint after incubation with the same antiserums as in (a). Magnifications are approximately × 300.

The cultured synovial cells derived from a normal membrane (Fig. 2a) showed uniformly bright and diffuse cytoplasmic fluorescence. In contrast, the cells derived from a rheumatoid synovial membrane (Fig. 2b) showed considerably less cytoplasmic fluorescence. The illustrations (Fig. 2, a and b) are representative of the findings in all cultures of normal and rheumatoid synovial cells studied. Controls for specificity of the immunofluorescent reactions gave the same results as those described for the membrane sections. The fact that this newly recognized substance can be localized in synovial cells in culture is strong evidence that it is synthesized by these cells.

At the time these cells were removed for the immunofluorescent experiments, the culture media in which they grew were harvested, concentrated by ultrafiltration, and examined for the presence of this new substance by agar-gel diffusion (*8*) with the use of the same rabbit antiserum to human cartilage proteinpolysaccharide (Fig. 3). The single precipitin line formed between the synovial fluid from a gouty joint in well 1 and the antiserum in the center well is similar to those observed previously (*8*) in normal fluids and fluids from pathological but nonrheumatoid joints; it indicates the presence of a substance immunologically like the species-specific component of human cartilage proteinpolysaccharide. The precipitin lines in 2 and 3—indicating that this substance is also present in the medium of synovial culture cells—and absence of precipitin reaction with nonincubated medium (well 4) offer good evidence that synovial cells synthesize and secrete this substance. The reduced intensity of the precipitin reaction between the antiserum and the medium from a rheumatoid cell culture (well 3) and the diminished cytoplasmic fluorescence of the rheumatoid cells in the synovial membrane (Fig. 1d) and in tissue culture (Fig. 2b) suggest that these cells secrete less of this substance.

These results indicate a need to modify the earlier proposal (*8*) that this newly recognized substance in synovial fluid is a probable breakdown product of cartilage and suggest that it may also arise from cells of the synovial membrane. It appears to be neither chondroitin sulfate, which has recently been identified in some rheumatoid synovial fluids (*14*), nor the proteinpolysaccharide—apparently from cartilage—that has been isolated from bovine synovial fluids (*15*); chondroitin sulfate, hyaluronate, and the "light" fraction of cartilage proteinpolysaccharide migrate ahead of albumin on zone electrophoresis (*8, 16*), whereas the substance that we have identified, which is immunologically similar to the species-specific component of cartilage proteinpolysaccharide, migrates in the α- and β-globulin zones. Because the biological role of this substance is not clear, and its chemical characterization is not yet complete, any speculation about the significance of its diminished synthesis by rheumatoid cells would be premature.

The results also indicate that a trait of synovial membrane lining cells, which is demonstrable in tissue sections, can be perpetuated by synovial cells through several generations in culture. Other traits that appear to be maintained in tissue culture of rheumatoid cells are the increased size and number of lysosomes containing acid hydrolases (*17*) and the ability of these cells (but not normal ones) to degrade cartilage matrix (*18*).

Castor and Dorstewitz (*19*) showed that rheumatoid synovial tissue cul-

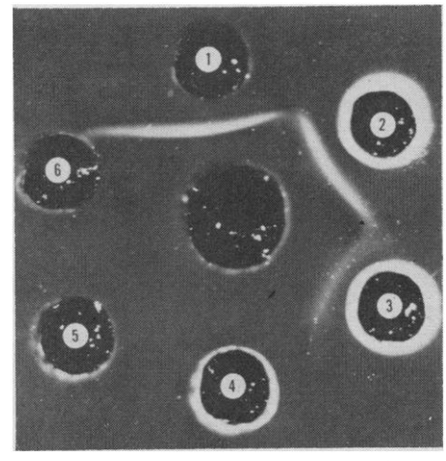

Fig. 3. Agar-gel diffusion in which the center well contains rabbit antiserum to human cartilage proteinpolysaccharide, previously absorbed with human serum; well 1 contains a sample of synovial fluid obtained from a gouty joint; wells 2 and 3 contain 200-fold concentrated samples of nutrient medium removed from cultures of normal (well 2) and rheumatoid (well 3) synovial membrane cells; in well 4, there is a 200-fold concentrated sample of nonincubated culture medium. Wells 5 and 6 are empty.

ture cells are less responsive to hydrocortisone suppression of hyaluronate synthesis than normal ones are; they also pointed out that this trait is propagated by the cells for more than 20 generations in monolayer culture. The possibility that the persistent differences in the rheumatoid cells are due to a genetic change or the serial passage of an infectious agent deserves consideration. Further studies that compare the properties of normal and rheumatoid synovial cells may reveal additional differences that could explain the pathogenesis of rheumatoid arthritis.

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Giant Axon of Myxicola: Some Membrane Properties as Observed under Voltage Clamp

Abstract. *A new space-voltage clamped giant (500 to 900 microns) axon preparation is described* (Myxicola infundibulum). *Normal value for resting and action potentials are 71 and 89 millivolts, respectively. This preparation under voltage clamp exhibits relations between current and voltage like those described for the squid axon. The early inward current component is reduced in a solution with low sodium concentration. This preparation, then, acts in all its essential features like the squid giant axon.* Myxicola, *however, can be made available the year around and should prove to be an extremely useful preparation for the study of excitable membranes.*

Much of the present understanding of excitable membranes is based on data and analyses obtained from the squid giant axon (*1*, *2*), which has many characteristics making it a favorable preparation, particularly for observation using the space-voltage clamp technique (*3*, *4*). However, squid are available only seasonally, and they do not survive well in the laboratory. It would be desirable, then, to have an axon preparation also suitable for observation under space-voltage clamp, which is available year around. We now describe voltage clamp studies on the giant axon of the marine polychaete *Myxicola infundibulum*, which is available the year around.

Animals (obtained from Maritime Biological Laboratories, St. Stephen, New Brunswick) were maintained in the laboratory for periods of several weeks to several months in a cold, aerated commercial seawater mixture (Instant Ocean). There was no difficulty in obtaining a good supply of fairly large animals (5 to 6 cm long, when contracted). The prepared axons from such animals have been up to 900 μ in diameter, but they were usually from 500 to 700 μ. To prepare the fibers we first anesthetized animals by immersing them in 5 percent ethanol in Instant Ocean for about 30 minutes. A median dorsal incision, just through the body wall, was made for the entire length of the animal, care being taken not to rupture the gut. The animal could then be pinned ventral side down in a paraffined dish; we exposed the nerve cord by gently moving the gut aside. Most of the nerve cord is the giant axon which dominates the dorsal aspect of the cord (*5*). In this preparation a problem is presented in the intersegmental constrictions in the cord and giant axon (*5*). These constrictions may reduce the diameter of the fiber to as little as one-fourth that of the segmental swellings and so make insertion of the long internal electrode impossible. However, we could relieve these constrictions by carefully stripping off the closely adhering dorsal blood vessel, taking care at each intersegmental constriction to include in the stripping the lateral branches of the blood vessel and the connective tissue bands adhering to the lateral branches. The fiber was then a more uniform cylinder. The fiber and a narrow strip of the body wall to which it was attached, the ends ligated with thread, were removed from the worm. Then, if the body wall was carefully torn away from the fiber, the fiber boundaries could be readily seen when lighted from beneath. Three centimeters of axon were usually prepared in this way.

The dissection probably cuts fine axon branches, as it does in the preparation of the squid giant axon. As judged from the values obtained for resting membrane potentials, action potentials, and the current records obtained under voltage clamp, these cut branches do not seem to have produced any particular problems. Stretch (*6*) was not controlled for, but it was never more than 1.5 times the length of the nerve *in situ*. Better results were obtained if the preparation was kept in cold (10° to 12°C) Instant Ocean during the dissection. The entire dissection usually required from 2 to 3 hours.

The internal coaxial electrode has been described elsewhere (*7*). The chamber and clamp were essentially the same as those used for the squid axon (*7*, *8*). Normal saline used in the recording chamber (ASW) had, in final concentration, the following composition: 430 m*M* Na^+, 10 m*M* K^+, 10 m*M* Ca^{++}, 50 m*M* Mg^{++}, 560 m*M* Cl^-;