contrast. Only 4 percent of the total mutants were asporogenic after heating at 85°C for 15 minutes (no spores per 10^9 cells). In the experiments at 90°C, only 1.7 percent of all mutants were auxotrophic, whereas 12 percent of all mutants were auxotrophic in the 100°C experiments. This difference is in contrast to the similarity of sporulation mutant types at the two temperatures.

Many sporulation mutants have been obtained by this heating procedure. Almost all mutants can form a complete spore, but the sporulation frequency in most is lower than in the wild type. These mutants are oligosporogenic (OSP) (*2*), and their sporulation frequencies vary from ½ to 10^{-9}. The OSP mutants also have a range of cellular and colonial morphologies, as well as differences in pigmentation, at virtually every degree of sporulation observed. Another characteristic of the OSP mutants is that either spores or vegetative cells of any one mutant form cultures sporulating at identical frequencies. Thus a mutant which forms one heat-resistant spore per 10^7 vegetative cells produces spores which germinate and yield cultures with only one heat-resistant spore per 10^7 vegetative cells. Similar OSP mutants have been obtained when vegetative cells of *Bacillus subtilis* were treated with sublethal concentrations of acridine orange (*3*).

Zamenhof and co-workers, using a different heating procedure (shorter times and higher temperatures), have shown that temperatures above 100°C can produce large numbers of auxotrophic mutants and considerable killing of cells and spores (*4*). They have proposed that these mutations are due to depurination. Our results at temperatures greater than 100°C are consistent with their hypothesis, since we observed that spores were killed at a logarithmic rate and that many auxotrophic and sporulation mutants were among the survivors. At lower temperatures we observed virtually no killing and very few auxotrophs, whereas sporulation mutants were still present in significantly high numbers. This result suggests that there may be two mechanisms by which heat causes mutation. Depurination, resulting in random chromosomal mutation, could account for the data obtained at temperatures greater than 100°C, but probably does not explain the low killing and the predominance of sporulation mutants at lower temperatures. This phenomenon may be due to the elimination of (or damage to) a number of cytoplasmic factors (*5*). Possibly our data are relevant to the finding that spore DNA differs from vegetative DNA in some physicochemical properties (*6*).

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3 January 1967

Human Synovial Fluid: Detection of a New Component

Abstract. *A new component has been detected in synovial fluid by the agar double-diffusion technique. This component is closely related immunologically to the proteinpolysaccharides of cartilage, and is the first probable degradation product of the cartilage matrix to be consistently identified in human synovial fluid.*

Synovial fluid is the viscous fluid which bathes the adjacent articular cartilage. The role of synovial fluid in the metabolism of articular cartilage is not well understood. Articular cartilage is relatively avascular, and nutrients may gain entrance to cartilage from the synovial fluid (*1*); also metabolic products might be removed from cartilage by way of the synovial fluid. It is possible to produce precipitating antibodies to the proteinpolysaccharides of cartilage. In my study synovial fluids were examined by immunological methods to determine whether degradation products of the proteinpolysaccharides of cartilage were released into synovial fluid.

Synovial fluids were obtained from diseased knee joints of five patients with gout, 18 with classical rheumatoid arthritis (*2*), three with septic arthritis, two with psoriatic arthritis, 15 with acute rheumatic fever, two with lupus, and one with pigmented villous synovitis. Synovial fluids were also obtained immediately after death from five young subjects with apparently normal knees. Fluids were stored at 4°C.

Proteinpolysaccharides were isolated from human articular cartilage by rapid homogenization in water (*3*). Rabbits were immunized with either the light (PP-L) or heavy (PP-H) proteinpolysaccharide of cartilage as described (*4*). The antiserums were absorbed with human serums (*4*) or plasmas. The antiserums were absorbed to remove antibodies produced to trace amounts of serum proteins which occasionally contaminate PP-L and PP-H (*4*). Agar double diffusion was performed with 1 percent agar (*5*) in barbital buffer (0.075*M*, *p*H 8.6). The PP-L and PP-H were digested with testicular hyaluronidase (*4*) before the above studies were made. Zone electrophoresis of several inflammatory synovial fluids were performed at 4°C on blocks (10 by 30 cm) of polyvinyl chloride in phosphate buffer (ionic strength 0.075, *p*H 7.4) at a voltage gradient of 15 volt/cm for 16 hours. After electrophoresis, the content of each segment (1 cm) of the block was eluted, analyzed for hexuronic acid, and after concentration (ten times) tested by agar double diffusion with the above antiserums. Synovial fluid from several patients was filtered through a Millipore cellulose filter (0.1 μ) at 4°C without stirring (*6*). Most of the hyaluronate was retained on the filter. The filtrate was placed on a column (50 by 4 cm, void volume 180 ml) of Sephadex G-200 (*7*) and eluted with a phosphate buffer (0.06*M* phosphate, 0.34*M* NaCl, *p*H 6.4) with an upward flow (20 ml/hour) provided by a peristaltic pump (*8*). The eluate from the column was monitored by continuously recording optical density at 254 mμ. Each fraction (10 ml) from column was concentrated (ten times) and was analyzed by agar double diffusion with antiserum to PP-L or PP-H.

After absorption with human serums (or plasmas) the antiserums to PP-L or PP-H produced one precipitin line with all 51 human synovial fluids (Fig. 1). In general, the antiserum to PP-H produced more intense precipitin lines with synovial fluid than the antiserum to PP-L did. This reaction with synovial fluid took place without prior digestion of the synovial fluid with testicular hyaluronidase. The precipitin line formed by this component of synovial fluid fused completely (Fig. 1) with

Table 1. Differences between the proteinpolysaccharides of synovial fluid and cartilage. H.D., Hyaluronidase digestion.

Material	Determinant		Precipitation in agar	Electrophoretic mobility	Filtration through 0.1-μ filter
	Species specific	Common			
New component	+	−	No H.D.	β-Globulin	+
PP-L	+	+	Requires H.D.	Prealbumin (*11*)	−
PP-H	+	+	Requires H.D.	Stays at origin (*11*)	−
NHP	−	+	Requires H.D.	Prealbumin	−

the line formed by the species specific determinant (*4*) of hyaluronidase-digested PP-L or PP-H. This component has not been previously reported, and I have called it the new component of synovial fluid. The new component has not been measured quantitatively. The precipitin line, however, was more intense in synovial fluids from acutely inflamed joints (gouty arthritis, septic arthritis, lupus, and acute rheumatic fever) than in synovial fluids from either normal or chronically inflamed joints (rheumatoid arthritis, psoriatic arthritis, and pigmented villous synovitis) (Fig. 1). The precipitin line was barely perceptible in some rheumatoid synovial fluids.

After zone electrophoresis on polyvinyl chloride, the hyaluronate-protein was completely separated from the new component. It migrated faster than albumin, whereas the new component migrated in the β-globulin zone. The new component also migrated in the β-globulin zone after electrophoresis (0.075*M* barbital buffer, *p*H 8.6) in 1 percent agar. It passed through the 0.1-μ Millipore filter, whereas PP-L, PP-H, and normal hyaluronate-protein (NHP) were retained by this filter. The new component did not pass through the Sephadex G-200 column with the large-molecular-weight components (19*S* and greater) of synovial fluid. It was eluted after 90 ml of the material constituting the void volume had passed through the column, in the region where the 7*S* γ-globulins of synovial fluid were eluted.

My studies indicate the presence in synovial fluid of a new component which is closely related immunologically to the component containing the species-specific antigenic determinant in hyaluronidase-digested PP-L or PP-H. It does not contain the common antigenic determinant present in PP-L and PP-H, and has not yet been isolated in a sufficiently pure state to determine its chemical composition. The differences between the new component and PP-L, PP-H, and NHP are summarized in Table 1.

The data in Table 1 indicate that the new component is not identical with PP-L, PP-H, or NHP. The new component has a different electrophoretic mobility than PP-L, PP-H, and NHP. It contains only one of the two antigenic determinants present in PP-L and PP-H. The new component is smaller than PP-L or PP-H which are very large macromolecules with molecular weights greater than 10^6. The best interpretation of the data is that the new component consists of a portion of PP-L or PP-H or both. It is not likely that the new component represents a subunit of proteinpolysaccharide passing through the synovial fluid to be polymerized into native PP-L, or PP-H in the articular cartilage. It is much more likely that the new component represents a degradation product of cartilage proteinpolysaccharides which is normally released into synovial fluid.

The increased concentration of the new component that occurs in the synovial fluid of patients with lupus, gout, acute rheumatic fever, and septic arthritis suggests that more new component is released from articular cartilage during acute inflammation of the joint. Inflammatory exudates certainly contain enzymes capable of degrading cartilage proteinpolysaccharides (*9*). Barker, Hawkins, and Hewins (*10*) found chondroitin sulfate in some inflammatory synovial fluids. The decreased concentration of the new component in synovial effusions from some patients with rheumatoid arthritis is more difficult to understand. All synovial fluids which showed a decreased concentration of the new component were chronic effusions. Three possible explanations exist for the decreased concentration of the new component in these fluids: (i) less new component is being released into the synovial fluid; (ii) the new component is being more rapidly removed from the synovial fluid; and (iii) the immunological reactivity of the new component is altered.

This is the first demonstration that a probable degradation product of cartilage proteinpolysaccharides is normally released into human synovial fluid. The presence of such a component in human synovial fluid should be useful in studying the metabolism of cartilage proteinpolysaccharides in that human synovial fluid can be more readily obtained than human articular carti-

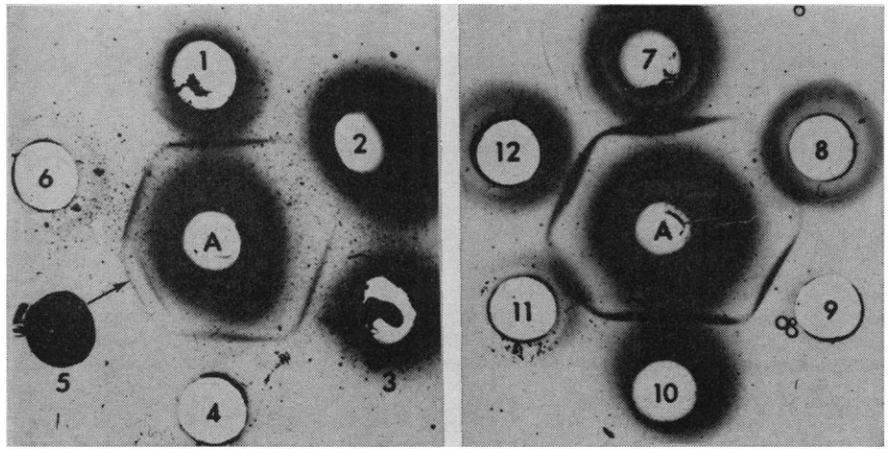

Fig. 1. Agar double-diffusion studies of human synovial fluids (SF). Wells A, antiserum to PP-H absorbed with normal human serum. Well 1, SF from patient with gout; well 2, normal human serum; well 3, SF from normal subject; well 4, SF from normal subject; well 5, human PP-H digested with testicular hyaluronidase; well 6, SF from patient with systemic lupus; well 7, SF from patient with septic arthritis; well 8, SF from patient with rheumatoid arthritis; well 9, SF from patient with systemic lupus; well 10, SF from patient with acute rheumatic fever; well 11, SF from patient with psoriatic arthritis; well 12, SF from patient with gout. The arrow indicates the precipitin line formed by the species-specific component of PP-H.

lage. Considerable knowledge might be gained about the effect of inflammation on the metabolism of human cartilage proteinpolysaccharides by comparing the amount, chemical composition, and turnover of this new component in normal and inflammatory synovial fluids.

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13 December 1966

Direction-Selective Units in Rabbit Retina: Distribution of Preferred Directions

Abstract. *The preferred directions of 102 direction-selective ganglion cells in the rabbit retina have been determined. Cells of the "on-off" type form four nonoverlapping groups; cells of the "on" type fall into three groups. The on-off groups appear to correspond to the directions of apparent object displacement produced by contractions of the four rectus muscles. Each group of cells could, without further processing, provide the error signal for a visual servo-system minimizing retinal image motion.*

The vertebrate retina used to be regarded as a transducer of light into nervous impulses, but it has recently become clear that complex "editing" and selection of features from the retinal image are performed before any impulses are transmitted centrally. Individual nerve cells signal the presence of particular spatiotemporal features of the image, thus performing a primitive pattern-recognition operation. One important feature of the retinal image pattern is its movement, and certain retinal ganglion cells in frogs, rabbits, pigeons, and ground squirrels have been found to signal the direction of image movement (*1*). These cells respond maximally when the image moves in one particular direction, called the "preferred" direction, and not at all when it moves in the opposite, "null," direction.

This asymmetrical response is very striking, and when impulses from a single cell are recorded, the unit can be studied by simply moving a small hand-held target in different directions through the appropriate part of the visual field. The orientation of the preferred-null axis is a characteristic that can be determined in this way, and the distribution of these axes in the population of ganglion cells is of interest, especially since a single "direction-selective" cell could not signal direction of motion without ambiguity.

In the rabbit, these direction-selective cells have been further divided into two groups (*2*). Cells of the more common "on-off" type respond to small stationary light spots flashed in the receptive field with a discharge at both the beginning and the end of the flash. Cells of the second group, the "on" type, respond only at the beginning; they respond to slower movements and there is evidence that their mechanism is different. We report here the distribution of preferred directions of 79 "on-off" and 23 "on" type direction-selective ganglion cells from a total sample of 577 units isolated in the retinas of 21 rabbits. We excluded 48 direction-selective units from this study because they were insufficiently studied or were in the extreme periphery of the visual field.

Microelectrode recordings from ganglion cells, or their axons, were made by recently described methods (*3*). The preferred axis is not perturbed by a moderate amount of aberration (*4*), so it was permissible to use the rabbit's natural pupil to avoid vignetting of peripheral receptive fields by an artificial pupil. Receptive fields were plotted on a uniformly illuminated tangent screen (15 cd/m^2) placed 57 cm from the cornea and roughly normal to the pupillary axis. Since data from a number of animals were to be combined, it was necessary to relate the preferred directions to some observable retinal landmark whose position could be expected to remain fairly constant from animal to animal. For this purpose, we chose the large retinal blood vessels which travel a reasonably straight course over the band of myelinated nerve fibers. Two points on these blood vessels, 20° to 30° on either side of the optic disc, were projected onto the tangent screen with a reversible ophthalmoscope; these points established the reference line to which all data for that animal could be compared. A similar technique was used to determine the orientation of the blood vessels in six unanesthetized, but restrained, animals. The line of blood vessels is not horizontal, but has a mean rotation of 10.7° ± 2.25° ($N = 6$) from the horizontal, with the anterior end lower.

Figure 1 shows the preferred directions of on-off direction-selective units from the superior visual field within 40° of the pupillary axis. The receptive fields have been superimposed and the preferred directions drawn relative to the horizontal and vertical of object space for the normally oriented animal (*5*). The distribution has four lobes, which may be designated anterior (35 units), superior (9), posterior (17), and inferior (18), but the group means are slightly deviated from the horizontal and vertical, especially the posterior group. These conclusions have been verified statistically, and this prompted us to search for an explanation in terms of the use that is made of the direction information provided by these cells.

When a substantial portion of the visual field is moved in one direction, the rabbit shows optokinetic eye movements, whose slow phase follows the stimulus very precisely (*6*). These movements reduce the sweep of the image over the retina, and one thus has evidence of an optical servo-stabilization system. Clearly, the corrective eye movements require error-signals that indicate which direction the image is slipping over the retina, and the on-off direction-selective ganglion cells could serve this function.

In man and many other animals, the superior and inferior rectus muscles