THE FORM AND FUNCTION OF SYNOVIAL CELLS IN TISSUE CULTURES

II. The Production of Mucin

By ERNST VAUBEL, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

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The object of this communication is to present evidence that mucin is actively produced by synovial cells. The demonstration of such a capacity by synovial cells in tissue cultures would be of importance because heretofore there has been no unanimity of opinion concerning the site and mode of formation of the synovial fluid, as is shown by the following brief review of the literature bearing on this subject.

In conformity with the idea that the synovial membrane was an epithelial structure the older authorities designated the joint fluid as a product of secretion in the narrower sense, a process observed only with genuine epithelial elements. The opinion was expressed by both Winslow (1) and Havers (53) that the synovial fatty tissue was a mucin-producing gland; but later Bichat (3) advanced opposing views, and he, together with other authors, saw in the joint fluid merely a transudate which percolated through the walls of the numerous blood vessels of the synovia (Bichat, Todd (54), Drechsel (55) and others); von Kölliker (2), on the other hand, thought that it was a fluid originating in the vessels but altered by some action on the part of the "synovial epithelium." Frerichs (56), who in 1846 first discovered mucin in the synovial fluid, and who considered the synovial membrane as anatomically composed of serosal cells, opposed the possibility of a secretory origin in the narrower meaning of the word, because these cells like those of all other serous membranes are capable of producing only an exudate. Thus the so called epithelial lining of the joint capsule was supposed to desquamate as a result of motion and then gradually to dissolve in the alkaline serum. This process was thought to be favored through the constant friction in the joints, a unique condition not existing in other serous membranes.

Frerichs' opinion that the mucinous nature of the synovial fluid was the result of cellular disintegration, and conditioned by the purely mechanical factor of motion, influenced many subsequent investigators, particularly those, such as Hammar (11), who regarded the lining of the joints as a simple fibrous connective tissue. Heuter (5), on the other hand, spoke of a specific action of the cells upon the

nourishing fluid which transuded from the blood vessels, and found in the microscopic picture of the synovia no support for the idea that the cells are dissolved *en masse*. The secretion theory was, nevertheless, by no means dislodged. Thus, Soubbotine (12) stated that the synovial fluid was formed by goblet cells which he frequently encountered among the epithelial elements of the synovia; and Mayeda (13) described certain zones of the joint lining as secretory areas, characterized by glandular cells which he thought he could clearly differentiate both anatomically and functionally from those found in other places. Both Aschoff (14) and Kaufmann (15) speak of the synovial fluid as a secretion. Müller (57), basing his opinion on Lubosch's conceptions, expresses the contemporary viewpoint that the joint fluid must not be considered as entirely a secretion from the synovial membrane, but rather that it is chiefly a solution of cellular elements of the joint lining—a mucoid degeneration—that arises from a constant stream of fluid poured out from the articular surface. While some disciples of Frerichs still exist who consider the synovial fluid only as a substance arising from the mechanical destruction of the synovial cells, recently other voices have again been raised advancing arguments in favor of the secretion theory. These are based in part upon the results of histologic investigations (Mayeda (13), Kling (46)) and in part upon clinical and experimental researches. Noteworthy also is a theory advanced by Banchi (58), who regards the synovial fluid as originating from the articular cartilage rather than from the synovial membrane.

A study of the literature dealing with the embryologic phases of the subject, reviewed more extensively elsewhere (see footnote 1 in previous article), throws little light upon the question. Most of the authors subscribe to one or the other of the two viewpoints considered above. Only in Retterer's (59) writings does one encounter this interesting observation confirmed by all embryologic data, namely that the first trace of the synovial fluid takes its origin from a liquefaction of the hyaloplasm, and he concludes from this observation that a similar, peculiar liquefying condition must exist in postembryonic life.

In summary, then, it may be stated that there are two theories concerning the mode of formation of synovial fluid: the one tries to explain it as a secretion, the other considers it a product of cellular degeneration, in other words, a detritus dissolved in tissue juices. A satisfactory clearing up of these differences is not available; and because somewhat confused and unphysiologic contributions have led to theories not in accordance with contemporary knowledge, the whole subject requires revision.

A consideration of the foregoing opinions concerning the source of the synovial mucin leads to the following possibilities for experimental investigation. If the synovial fluid and especially its high content of mucin is a detritus arising from mucoid degeneration and cellular

destruction then one would expect to find no mucin in a tissue culture composed of healthy synovial cells, but rather in a culture of degenerating synovial cells and possibly in one of fibroblasts grown from periarticular tissue. If, on the other hand, the mucin is a specific product of the synovial membrane one would expect to find a certain amount of it in a normally growing culture of synovial cells, and none in a degenerating growth or in a culture of fibroblasts. Finally, if the mucin arises as a result of degeneration of the cartilaginous portion of the joint then none would occur in a culture composed entirely of synovial cells.

EXPERIMENTAL

The same cultures, both of synovial membranes and of control tissues, used in the morphologic studies (60) were employed for the present study, for in this manner some of the forms and functions of the cells could be correlated.

The investigation of the soluble material originating in the tissue cultures was carried out in a simple manner. If the flasks contained no free fluid, either because no fluid phase had been supplied or because the plasma had absorbed the fluid previously added, the plasma coagulum was separated from the bottom of the flask thus allowing it to contract and squeeze out a certain amount of fluid; at times this process was hastened by centrifuging. The fluid so obtained was decanted or pipetted into small tubes and tested for mucin as described below. All control cultures were handled in the same manner; and in case the growth had progressed to the stage of complete degeneration similar procedures were employed for obtaining fluid. During a period of active growth when attempts were made to correlate possible mucin formation with a certain stage of growth a modification in the manner of collecting the fluid was introduced: the flasks were placed upright for half an hour when the expressed liquid which had collected at the dependent portion was removed with capillary pipettes; then that from each flask was tested separately or the product from a number of flasks was pooled and tested.

In order to eliminate the possibility of introducing mucin into the original culture media no embryonic extract was used. Furthermore, all fluids used in making and washing the cultures were tested for the presence of mucin with negative results. As already mentioned, all synovial membranes were washed half an hour in Tyrode's solution in order to remove adherent joint fluid before being explanted. Several times the explants before being placed in the plasma were washed with Tyrode's solution made slightly alkaline with sodium hydrate; and as an additional control, bits of synovial membrane were placed in similar alkalinized Tyrode's solution and incubated at 37°C. for 24 hours; the supernatant liquid tested with 2 per cent acetic acid never gave a positive reaction for mucin.

The test for mucin in a fluid under investigation was carried out as follows: to the clear solution, contained in small tubes, 2 per cent acetic acid was added

drop by drop. In the presence of either mucin or nucleoprotein a precipitate is formed; but these precipitates have distinguishing characteristics: that formed by both appears to be composed of coarse threads, which, if the acetic acid is added carefully, gathers into a sack or tube-like membrane, as Kling recently demonstrated. If a glass rod is placed in the fluid the precipitate gathers around it in a pasty, elastic mass. If the precipitate is due to nucleoprotein it is soluble in an excess of acetic acid, while the presence of mucin is indicated by a failure of the precipitate to go into solution in an excess of the acid. The precipitated mucin is, however, readily soluble in alkaline media and can be repeatedly precipitated and redissolved by altering the reaction of the surrounding fluid.

Von Holst (61) states that the mucin of the synovia is free from phosphorus and yields a reducing substance after long heating with hydrochloric acid. Kling (46), in agreement with Salkowski (69), could demonstrate no noteworthy reduction with copper sulfate even after prolonged hydrolysis of the synovial mucin with dilute hydrochloric acid. Extensive chemical investigation of the mucinous material derived from tissue cultures was impossible because of the small amount of the substance available from such sources.

RESULTS

Before reporting in detail on the results with fluid obtained from synovial cultures it seems well to record the reaction obtained with that from controls. With cultures of healthy fibroblasts of different origin there occurred in no single case a precipitate of the coarse thready type; the solution usually remained clear following the addition of 2 per cent acetic acid or there occurred only a slight turbidity; such finely dispersed precipitates formed very slowly, often only after an interval of 24 hours. Fluid from the degenerated and dying cultures at times yielded a thread-like precipitate, small in amount, which redissolved upon the addition of an excess of acetic acid; usually, however, only a slight clouding was observed.

With fluid derived from serosal cell cultures from the pericardium, pleura, peritoneum and tunica vaginalis, similar results were obtained. In spite of varying cultural conditions no mucin-like substances were demonstrable, with the following exception: in the liquid from two flasks containing growths from explants of peritoneal serosa a precipitate was observed having the morphologic properties of mucin and not soluble in an excess of the acid. The amount of precipitate was too small for further chemical investigation. With numerous other cultures of peritoneal origin it was impossible to repeat this observation; nevertheless, it is of distinct interest, especially when

considered in connection with the observation of von Holst, who demonstrated mucin-like substances in ascitic fluid.

In sharp distinction to the negative results noted above were the clearly positive findings with fluid obtained from cultures of synovial cells. By decanting and combining the liquid from several flasks a distinctly increased viscosity was observable; such fluid was like a thin glue and could be drawn into long threads; it was, indeed, very similar to a thin joint fluid. Upon the addition of 2 per cent acetic acid there was formed a coarse thready precipitate that quickly dropped to the bottom of the tube in the form of adherent clumps. This precipitate was insoluble in an excess of acid as well as in a neutral medium; it dissolved readily in slightly alkaline solutions, and again formed a precipitate upon the addition of an excess of acid to the alkaline solution; it was not coagulated by heat. At times a substance capable of reducing copper sulfate was obtained after prolonged heating of the precipitated material in dilute hydrochloric acid; but this result was inconstant, for the reaction was sometimes negative under experimental conditions apparently identical with those giving positive results.

The output of mucin was, naturally, small and not sufficient for extensive chemical studies; yet there seems little doubt of its identity with the mucin formed in joints. It did not give the reaction of nucleoprotein which dissolves in an excess of acid. Furthermore, in the fluids from degenerating synovial cultures there were found only traces of mucin or none, while the amount of thready precipitate of nucleoprotein soluble in an excess of acid was found to increase in such cultures.

The quantity of mucin precipitate varied, depending upon the proportion of producing cells and the number and extent of cultures in the flasks. It was especially marked with explants and with typically growing transplants, particularly in the older healthy cultures showing no cellular degeneration. The mucin could be demonstrated distinctly in fluid obtained from a sixth passage transplant, that is about 60 days after the original explantation. Its amount decreased with the increase of a fibroblast-like growth, and it disappeared with complete transformation of the synovial cells into typical fibroblasts, and no mucin appeared when such growths were

allowed to degenerate. The transformation into fibroblasts with failure of mucin production seemed to occur more readily with transplants of synovia from young animals than with those from older rabbits. With the latter, as a rule, typical growths with mucin formation persisted through several passages.

When a flask was washed out with 1 cc. of Tyrode's solution on the day following explantation, no mucin, or at least only traces, could be detected in the washings; and the same was true of the supernatant liquid obtained by centrifuging the plasma coagulum in which explants 1 day old had grown. With progressing growth of typical synovial cells there was an increase in mucin production; after about 10 days, when the growths were divided and transplanted, production of mucin appeared to be at its maximum. If the culture was stimulated by the addition of a large amount of splenic extract then there was early a large yield of mucin; but under this stimulating environment the dedifferentiation into fibroblast-like growth took place more rapidly with a corresponding diminution in mucin production. By conditioning the nutritive state with small amounts of splenic extract the appearance of typically growing synovial cells was better maintained and the specific function of these cells seemed to be held at its optimum.

DISCUSSION

From a review of the results obtained in tissue cultures the conclusion seems warranted that cell types develop in the growths from synovial membranes which are clearly differentiated from other mesenchymal cells, especially from those of the subcutaneous or interstitial varieties. The peculiarity of a synovial growth rests in part on the polymorphism of the cells, the contour of which ranges from round and spindle to polygonal and epithelioid, and especially on their tendency to form membranes composed of syncytial elements; this agrees with histologic investigations (Hammar (11) and others). Furthermore, these cells are peculiar in producing both mucin and a proteolytic ferment and in forming in their cytoplasm large highly refractive granules, that stain deeply with neutral red. Because of these functions they are differentiated sharply from fibroblasts and

brought into fairly close relationship with other cells of mesenchymal origin having special morphologic and physiologic features, namely osteo- and chondroblasts.

A comparison with the reports of other workers shows that these three types of cells appear to be related in several respects. Dolschansky's (62) descriptions and illustrations of growing osteoblasts and chondroblasts reveal a striking similarity to those given above for synovial cells. Fischer and Parker (18) confirmed Dolschansky's findings, and, in addition, caused both cell types under special cultural conditions to form an organized tissue with a hyalinized ground substance, that closely resembled bone or cartilage. The morphologic similarity of cultures of these three tissues is closely connected with their ontogenetic relationship: they arise from a common embryonic anlage.

The capacity of the synovial cells to dissolve the coagulated plasma under certain favorable circumstances may be regarded as a physiologic function of this tissue that may be likewise observed *in vivo*. For example, one finds references in the literature indicating that the synovial fluid is capable of dissolving bone when a bone pegging operation has been performed near a joint; and also when bits of bone have been broken off into the articular cavities. Coagulated blood in the synovial cavities becomes liquefied after a few days (Bier (63), Kaiser (64), Jaffé (65)). The poor healing of intraarticular fractures may also be cited in the same connection; and in order to explain this phenomenon Podkaminsky (66) investigated the lytic capacity of the fluid obtained from the joints of oxen and demonstrated *in vitro* a proteolytic ferment, a lipase and an amylase; he thought, therefore, that the occurrence of these substances accounted for the osteo- and chondrolytic power of the synovial fluid. The enzymes, especially those of proteolytic nature are doubtless active in supplying a medium suitable for the holding in solution of substances favorable to the peculiar function of the synovial membrane.

The demonstration of mucin in the cultures of synovial cells is important in indicating that the maintenance of the slimy property of synovial fluid is a peculiar function of these cells and not the result of their death and subsequent dissolution. The term "secretion" is

avoided because mucin formation in joints cannot be compared strictly with the enzyme-forming function of epithelial glands, for which this term, in its narrower sense, should be reserved.

As an analogue to the formation of mucin by the synovial membrane one must look for the production of similar substances by certain other mesenchymal elements. This subject is discussed much more fully in another place (see footnote 1 in previous article), where the viewpoint is advanced that one may regard the synovial fluid as a ground substance of the synovial tissue analogous to the intercellular substance of the cartilage which has become solid by the imbibition of chondroitinsulfuric acid, or to that of the bones which have become impregnated with lime salts. This viewpoint is in perfect accord with the modern conception of mesenchymal tissue, as formulated by Hueck (67), Studnička (68) and others. The feature distinguishing this ground substance from all others is its persistent liquid mucinous state, a physical condition that renders it most valuable for the purposes of lubrication.

Finally a word concerning nomenclature may be introduced. We designate the individual elements of bone as osteoblasts, and those of cartilage as chondroblasts according as these cells elaborate a peculiar intercellular substance, bone or cartilage respectively. If, therefore, one regards the synovial fluid as a specific ground substance elaborated by synovial membrane it is only logical to employ the designation "synovioblast" to characterize a specific type of cell with this peculiar function.

SUMMARY

1. Synovial cultures are differentiated in tissue cultures from other tissues of mesenchymal origin by their type of growth and cell function.

2. In these respects they are more closely allied to chondroblasts and osteoblasts than to fibroblasts.

3. Synovial cells in tissue cultures develop marked globular cytoplasmic granulations that stain easily with neutral red and sometimes with toluidine blue; they show marked polymorphism with all transitions from round to spindle, polygonal and star shapes and eventually form an epithelial-like membrane, composed of cells with numerous syncytial bridges.

4. In cultures of typically growing synovial cells a mucin-like substance is elaborated. Typical growth and maximal mucin production is best maintained in media containing a minimum of growth-stimulating substances. Transformation of synovial cell growths into fibroblastic growth is accompanied by a loss of mucin production. Dying cells apparently do not produce mucin.

5. Amitotic cell division and the formation of macrophage-like cells were observed.

6. Marked tendency to liquefaction of the plasma about the growths was observed and attributed to the elaboration of a proteolytic ferment.

7. The specific designation "synovioblasts" is proposed for these cells.

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