termine the conditions necessary for nitrate production, in an atempt to evaluate the importance of heterotrophic nitrification by fungi.

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A Method for the Quantitative Determination of Hyaluronic Acid in the Human Intervertebral Disk

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The presence of acid mucopolysaccharides in certain tissues and cells has been established by various well-known histochemical methods (*1–3*). The most widely used procedure is that which demonstrates metachromasia when specific basic dyes, e.g., toluidine blue, combine with the acid mucopolysaccharides present in the interfibrillar substance of the mesenchymal tissue (*4*). Furthermore, it has been shown that N-acetylglucosamine and glucuronic acid are formed in equimolecular proportions as a result of the depolymerizing action of hyaluronidase on these hyaluronate substrates. In an attempt to evaluate the potential role of hyaluronic acid in the pathogenesis of ruptured intervertebral disk, we have used a procedure based on the colorimetric method for the determination of N-acetylglucosamine, as described by Mueller (*5, 6*).

Fragments of fresh intervertebral disk obtained at operation are washed with copious amounts of physiologic saline solution until they are free from gross blood. These fragments are then frozen and sectioned at 25 μ using the usual tissue microtome. The masses of sectioned material, weighing 0.1–3.0 g, are then divided into 3 parts and carefully weighed in large test tubes; 1.0 ml of 0.1 *M* phosphate buffer, pH 6.8, is added to each specimen, and 2 *M* NaCl solution is added in sufficient quantity to obtain a relatively fluid mixture, the amount varying with the quantity of disk tissue. Six to eight thousand viscosity units of crystalline testicular hyaluronidase are added to this mixture, with constant shaking. The samples are agitated in a constant temperature water bath at 37–38° C. After 6 hr incubation, 2–4 thousand viscosity units of hyaluronidase are again added. The mixture is incubated 2 hr more, and filtered through No. 1 semiquantitative filter paper. Colorimetric determination of N-acetylglucosamine in the filtrate is carried out in the following manner on each sample.

One milliliter of filtrate and 0.5 ml *N*/2 Na_2CO_3 solution are placed in a large test tube and carefully mixed by shaking. The tube is placed in a boiling water bath for 5 min, then rapidly cooled in tap water. To this solution are added in order: 6.5 ml glacial acetic acid, 1.0 ml acidulated solution of recrystallized *p*-dimethylaminobenzaldehyde (*6*), and 1.0 ml glacial acetic acid. The solution is mixed thoroughly and permitted to stand for 45 min; colorimetric comparison is made, using a standard solution of N-acetylglucosamine. The amount of hyaluronic acid per gram of intervertebral disk may then be calculated from the quantity of N-acetylglucosamine released by similar enzymatic hydrolysis of a known quantity of purified hyaluronic acid.[1]

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[1] Purified hyaluronic acid supplied by the Schering Corp.

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A Cooperative Multiple-Choice Apparatus

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During an extensive investigation of learning in the chronic schizophrenic (*1–3*), the authors required an apparatus, or problem-solving material, for studying the process of cooperation. As the major project was an investigation of the possibilities of learning (over a three-month period) as therapy in chronic schizophrenia, and as the absence of interpersonal relationships is an obvious characteristic of these patients, the need for some form of cooperative learning seemed evident. In this work the conventional learning materials of experimental psychology were used. These permit objective recording of errors and correct responses, the timing of separate trials, and a study of the gradual improvement of performance with practice. The method described here is, the authors believe, the only one so far developed for studying human cooperation in the same way.

The apparatus consists of two identical multiple-choice boxes. In individual learning with one box the subject is faced with a bank of 10 levers each of which can be pulled toward him a distance of 3 in. (A picture of one of these boxes in use appeared in *Life*, Oct. 20, 1952, p. 80.) The subject pulls the levers in any order he wishes until he hits upon the "correct" one, which of course is determined by the experimenter on the other side of the apparatus. Reward for correct responses is given in the form of candy in a tray which moves forward from beind a transparent plastic screen. The operation of one multiple-choice