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

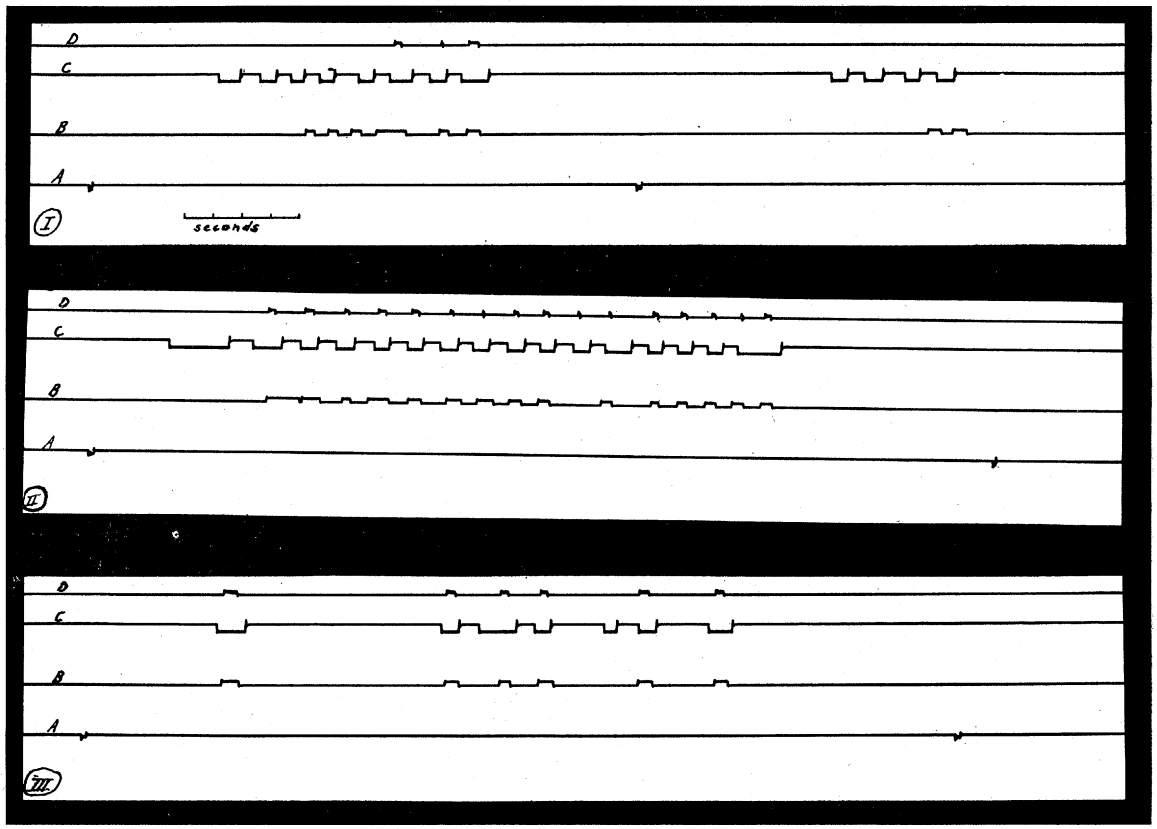

FIG. 1. Three segments of record paper. A mark on *A* indicates the start of a trial; the end of a trial of course comes where two coordinated reactions precede another mark. Line *B* moves up for every reaction of one subject; line *C* moves down for every reaction of the other subject. A mark on *D* indicates that the two reactions below, on *B* and *C*, were the same, i.e., the same lever was pulled by both subjects.

I shows an early trial of two subjects and the beginning of the following trial. *II* shows a later trial by the same two subjects on the same problem. Note the increase in frequency of marks on *D* and the improved coordination, or timing of pulls. *III* is a trial by two different subjects who have had a great deal of practice with the cooperative multiple choice apparatus.

box in the study of individual learning and the various problems which have been used are described in previous studies (*2, 3*). The cooperative multiple-choice apparatus consists of two of these boxes wired in such a way that the correct lever will not work unless pulled on both boxes simultaneously. The wiring necessary to accomplish this coordinated action is relatively simple and can be worked out in a few moments by anyone familiar with electric circuits.

According to the procedure used, the two patients whose cooperative learning is to be studied are first brought to a high practice level in the solution of multiple-choice problems. These consist of first a single lever problem, in which the same lever works every trial, an alternation from trial to trial of two of the levers, the rotation of three of the levers, the middle one of odd numbered levers, and so forth.

In the study of cooperation, two subjects attack the same problems, beginning with the simplest one. They are seated at one side of a table, about 3 ft apart. Preliminary instructions, which are very brief, consist of demonstrating to the two subjects that a "correct lever" will not release the tray when pulled on only one apparatus, but that if it is pulled simultaneously on both, both of the trays will be released.

The subjects' responses are recorded by a simple polygraph with four electrically activated markers (Fig. 1). One of the markers, *A*, is used by the experimenter to mark the start of a trial; another, *B*, shows a mark for every lever-pulling response of one of the subjects; a third, *C*, shows the same for the other subject. A fourth marker, *D*, is activated when the same levers on both boxes are pulled simultaneously. The only one of these markers which is operated at the will of the experimenter is the first.

The cooperative multiple-choice apparatus has the features of learning materials used in conventional experimental work: (1) there is an unlimited number of problems of the same quality but of roughly graded difficulty; (2) the process of learning is divisible into separate trials, thus permitting a study of parts of the process; (3) errors and correct responses are precisely defined and are objectively distinguishable.

The unique feature of the apparatus is its isolation

of cooperation as a process which has a measurable improvement and objective criteria of success and perfection. The preliminary practice level eliminates the individual learning phase. Each subject is equally necessary to the solution of the problem, and each is equally rewarded. Competition between subjects is eliminated, although the usual factors of the cooperative relationship, such as dominance, submissiveness, and initiative, are also allowed to operate. The level of cooperation can be measured in at least three ways: (1) by the number or proportion of responses in unison per trial; (2) by the number or proportion of the same levers pulled simultaneously; and (3) by the time gap between patients' pulls. The first two of these clearly tend to increase with practice.

With this method, it was found that extremely regressed schizophrenics, at least those who have previously been brought individually to a high practice level at multiple-choice learning, can learn to cooperate with one another. Qualitative features of their interaction behavior are also evident and tend to point up a fixed pattern for each individual's cooperative behavior. These features are observed and tallied on a specially prepared trait sheet during the experimental sitting. They include watching the levers pulled by partner, telling him which levers to pull, holding back a lever until partner pulls the same, and actually telling the other patient the principle of the solution. It is possible to wire together more than two of the multiple-choice boxes, thus permitting the study of cooperation in a group of several individuals.

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The Potential Value of Sulfaguanidine in Urology[1]

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Following a review on absorption and excretion of sulfaguanidine, a new therapeutic rationale for the use of sulfaguanidine is suggested.

Since 1940, texts have generally stated that sulfaguanidine is slowly and/or poorly absorbed from the gastrointestinal tract. Dosages of approximately 20 g in a day have been used for thousands of persons with bacillary dysentery, and as much as 60 g in a day have been given (*1*). The facts that blood levels remain relatively low (*2–5*) and that toxic manifestations occur infrequently (*2–10*) have probably contributed toward false impressions about the actual situation in regard to absorption of sulfaguanidine.

[1] This paper contains material submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at New York University.

[2] Appreciation is expressed for aid from Horace W. Stunkard, and advice from Charles Willey, Harold Fay, Harry Bergman, and Benjamin Slivko.

TABLE 1. Excretion of sulfaguanidine by five persons.

Experimental individual	Oral dose (g)	Av. conc. free drug in urine during stated time interval following administration	Yield of dose in urine during stated time
Ingalls	4	107 mg % at 3–6 hr	15.0% in 8 hr
Pearl	2	126 mg % at 1–3½ hr	11.5% in 3½ hr
Parker	2	168 mg % at 1¾–6½ hr	13.5% in 6½ hr
Greenberg	3	176 mg % at 1¾–3 hr	6.0% in 3 hr
Slivko	3	210 mg % at 4½–13 hr	42.0% in 13 hr

There is considerable literature dealing with the absorption and excretion of sulfaguanidine. Beling and Abel (*11*) found concentrations of sulfaguanidine in the urine varied from 25 to 200 mg %, while concentrations of the drug in the blood remained within the narrow limits of 1.5 to 1.8 mg %. Anderson and Cruickshank (*2*) found concentrations in urine as high as 240 mg %; and in blood, 3 mg %. Jamieson, Brodie, and Stiven (*8*) found as much as 154 mg % in urine. Fairley and Boyd (*12*) mentioned that sulfaguanidine is absorbed to a large extent when very small doses are given, but to a small extent when larger doses are given. Hawking (*13*) considered the possibility that sulfaguanidine appears to be poorly absorbed because it is in fact first absorbed from the intestine and then excreted from the blood back into the intestine, but he demonstrated that this hypothesis was not valid. Hubbard, Butsch, and Aaron (*14*) thought that the apparent failure of absorption of sulfaguanidine might be due to removal of the drug from the blood stream by the liver and its return to the intestine in the bile. They proved this is not the case. Rose and Spinks (*15*) postulated that poor absorption of sulfaguanidine might be accounted for on the basis of its molecular structure. They failed to find direct evidence to substantiate this idea.

Investigations, into excretion in 45 normal healthy young men, indicate that sulfaguanidine is often well absorbed and rapidly absorbed. The combined effect of absorption from the gastrointestinal tract and excretion into the urinary tract is such that higher titers of

TABLE 2. Rapidity with which drug appears in urine.

Experimental individual	Oral dose (g)	Conc. of free drug in urine at time after administration
Slivko	3	101.0 mg % at 1¾ hr
Greenberg	3	71.5 mg % at 1¾ hr
Parker	2	59.0 mg % at 1¾ hr
Pearl	2	50.5 mg % at 1 hr
Ingalls	4	6.5 mg % at ¾ hr
Ingalls	2½	2.5 mg % at ½ hr