TABLE 1. Parameter estimates and standard errors of depigmentation dosimetry equation.

Months after irradiation	Number of mice	λ_0	λ_1	Standard error of λ_1	Standard error of estimate about line
7	138	313.22	85.97	2.71	51.42
17	116	253.75	92.30	4.05	62.80

ing this period. The same increment in expected dosage per unit of increase in depigmentation existed at both times, but at 17 mo the threshold was 254 r (reading from the line), whereas at 7 mo there was no discernible graying below 313 r.

The correlation between dose and depigmentation varied markedly with different regions of the coat, and occasionally gave rise to a mottled appearance of the fur. Sharply demarcated areas of brown (grade 0) and white (grade 4) hair were at times in juxtaposition to one another. This is best explained by postulating the existence of differences in the radiosensitivity of hair follicles of various areas at the time of irradiation. Chase has shown that the susceptibility of individual hairs to graying varies markedly, and depends upon such factors as the stage of the growth cycle and the type of the hair follicle (*4*). No attempts were made to characterize the development of the hair follicles of LAf_1 mice or to determine by microscopic count the anatomical distribution of the various types of follicles, but the observed pattern of depigmentation suggests the contours of the moulting patterns described by Dry (*5*). As the mice of this experiment were irradiated at the age when moulting was presumably in progress, it is logical to attribute regional variations in degree of depigmentation, as observed, to differences in radiosensitivity of hair follicles due to moulting.

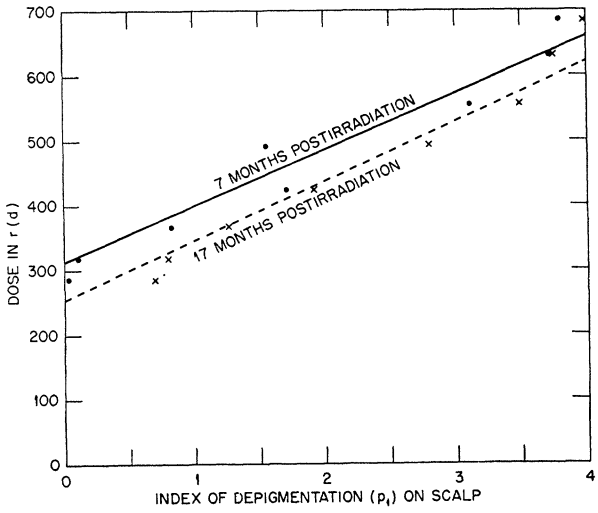

FIG. 1. Estimated dose as a function of depigmentation.

It may be concluded that the degree of depigmentation of fur of mice of the LAf_1 strain is closely enough correlated with the dose of radiation to constitute a convenient biologic dosimeter. This correlation, however, varies markedly with different regions of the coat, and for the mice studied it is more constant for the fur of the top of the head than for that of other areas examined. The greater constancy for the top of the head is attributed to the tendency of all hair follicles in this region to be resting during the age at which the mice were exposed (*2, 5*). The progression of depigmentation of various areas probably resulted from gradual replacement of old colored club hairs by postirradiation depigmented hairs, as several hair generations are usually represented in each follicle at any given time (*2, 5*).

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Nitrate Formation by a Soil Fungus[1]

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Nitrification in soil is a biological process of paramount importance to the nutrition of green plants, for the nitrate nitrogen that is the end product of this process serves as the principal form in which nitrogen is used by photosynthetic plants. Ever since the classical researches of Winogradsky around 1890, the process of nitrification has been attributed to the activity of a few genera of highly specific, strictly autotrophic soil bacteria which oxidize ammonium nitrogen to nitrate in a two-step reaction. In recent years evidence has suggested that certain heterotrophic microorganisms isolated from soil may participate in the first stage of nitrification, the production of nitrite nitrogen. Quastel *et al.* (*1*) reported the isolation of three species of soil bacteria capable of oxidizing pyruvic acid oxime and the oximes of certain other alpha-keto acids to nitrite. Jensen (*2*) found isolates of two additional genera of soil bacteria, and numerous isolates of the actinomycete species *Nocardia corallina* capable of producing nitrite from pyruvic oxime. The oxidation of ammonium nitrogen to nitrite has been observed also for a soil actinomycete of the genus *Streptomyces* (*3*), various unidentified heterotrophic

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TABLE 1. Production of nitrite and nitrate from organic nitrogen by a strain of *Aspergillus flavus* isolated from soil.

Days of incubation	pH		Dry wt of mycelium, mg		NO_2^-–Nγ/ml		NO_3^-–Nγ/ml	
	Shaken	Static	Shaken	Static	Shaken	Static	Shaken	Static
0*	7.2	7.2	—	—	0.01	0.01	2.0	2.0
4	8.1	6.9	192.1	124.3	0.10	0.55	8.0	11.1
7	8.6	7.7	178.8	154.8	0.27	1.14	27.0	28.0
11	8.7	8.3	148.0	157.0	0.21	3.20	17.6	26.8
16	9.2	8.4	131.9	149.8	0.35	1.13	20.2	24.5
25	8.2	8.2	133.9	160.6	0.45	1.32	26.3	26.5
32		8.1		155.7		1.50		34.0
39		8.5		154.0		1.51		35.0

* Control flask plus inoculum.

soil bacteria (*4*), and methane-oxidizing bacteria (*5*). In discussing the possible importance of nitrite production by heterotrophic organisms to the nitrogen economy of the soil, Jensen (*2*) emphasized that no organism other than the autotrophic *Nitrobacter* is yet known to accomplish the second stage of the soil nitrification reaction, the oxidation of nitrite to nitrate. The present communication records a soil fungus, *Aspergillus flavus*,[2] capable of producing nitrate nitrogen.

The *A. flavus* isolate was discovered during an investigation of the relationship of nitrate production in Minnesota soils to nitrate-induced methemoglobinemia in infants. The organism was obtained from dilution plates of a farmyard soil on glucose (1%), yeast extract (0.2%), and agar (1.5%) medium. Nitrification reactions resulted from the growth of the fungus in a liquid medium containing organic nitrogen. The culture medium consisted of dipotassium phosphate 0.1%, magnesium sulfate 0.15%, ferrous sulfate trace, manganous sulfate trace, glucose 0.2%, peptone 0.4%, and yeast extract 0.1%. Organic constituents of the medium were autoclaved apart from the mineral salts solution. Nitrite was determined colorimetrically by the Gries-Ilosvay method. Nitrate in the presence of nitrite was determined by first oxidizing the nitrite with a few drops of hydrogen peroxide and then measuring total nitrate by the phenoldisulfonic acid method. Culture filtrates were measured directly for pH and ammonia, and were decolorized with charcoal for nitrite and nitrate assays. Uninoculated control flasks were analyzed at the end of each incubation period and corrections were made for the slight positive values (0.01 γ/ml NO_2^-N, 2.5 γ/ml NO_3^-N) observed.

TABLE 2. Ammonium production during static culture in the course of the nitrification of organic nitrogen by a strain of *Aspergillus flavus* isolated from soil.

Days of incubation	pH	NO_2^-–Nγ/ml	NO_3^-–Nγ/ml	NH_4^+–Nγ/ml
0	7.2	0.14	3.0	8
7	7.9	0.29	7.4	116
12	8.3	0.83	21.3	366
18	8.4	0.45	9.2	282
24	8.1	0.74	19.3	258
30	8.4	0.67	16.1	132

[2] The author is indebted to C. M. Christensen, Department of Plant Pathology and Agricultural Botany, University of Minnesota, for the identification of the isolate.

The results summarized in Table 1 extend the list of heterotrophic microorganisms that produce nitrite nitrogen to include a fungus. Of much greater interest is the fact that nitrate nitrogen resulted from the growth of the *A. flavus* isolate. These data present the first indication of the existence of heterotrophic microorganisms capable of carrying the nitrification reaction to completion with the formation of nitrate. Nitrate concentrations were found to build up rapidly during the period of most active cell synthesis in both static and shaken cultures. Maximal nitrate concentrations however were produced after more prolonged incubation. Nitrite nitrogen remained at about the same level during the course of incubation, apparently due to oxidation of the nitrite to nitrate. Table 2 illustrates that only a small portion of the organic nitrogen was oxidized to nitrate. Ammonium nitrogen constituted the principal inorganic form of nitrogen present in the medium and accounted for the rise in pH. Subsequent to observations with the original isolate, additional isolates of *A. flavus* were obtained on rose bengal-streptomycin agar (*6*) from several different soils. Each of these isolates proved capable of producing both nitrite and nitrate nitrogen under the cultural conditions described.

The foregoing data clearly show the occurrence in soils of fungi capable of oxidizing a portion of an organic nitrogen substrate to nitrate. While such observation does not necessarily mean that heterotrophic microorganisms participate in nitrification as the process is carried out in soil, the existence of nitrate forming fungi greatly enhances the plausibility of this hypothesis. Studies are now underway to determine the abundance of nitrifying fungi in soils, and to de-

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