

The corneal test was used in a similar experiment with streptomycin and potassium iodide in mice in which only one eye was infected. The results were assessed both on the incubation period and on the subsequent progress of the corneal lesions. There were 6 untreated control mice, 7 mice on potassium iodide (400 mg/k of the diet), 14 mice on potassium iodide combined with streptomycin (4 mg daily subcutaneously) and 12 mice on streptomycin (4 mg daily). Treatment was started on the day of infection and maintained for 28 days (Fig. 3).

In order to compare the concentrations of potassium iodide produced in the present experiments with those used by Woody and Avery, experiments were done with radioactive iodine. Guinea pigs (as used by Woody and Avery) were given sodium iodide (containing I^{131}) by stomach tube in doses of 80 mg/k. Rabbits and mice

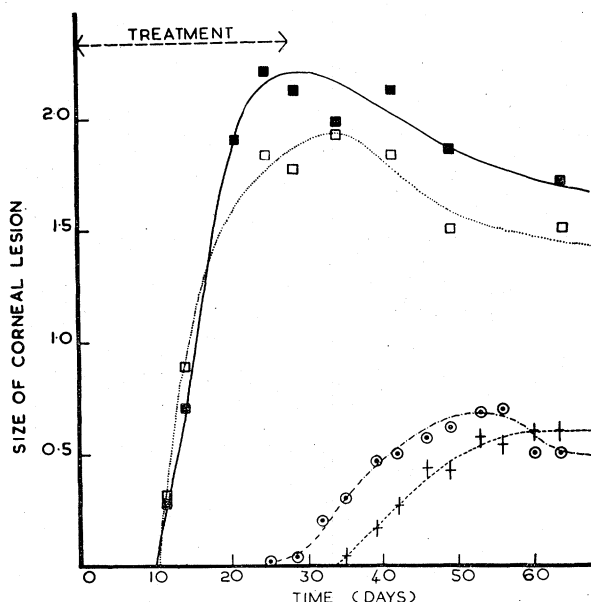


FIG. 3. Effect of combined streptomycin and potassium iodide on the development of tuberculous corneal lesions in mice. (Legends as in Fig. 1.)

were treated with I^{131} as described above. The results show that the concentrations of iodine in the guinea pig and mouse were of the same order, but that the iodine in the aqueous of the rabbit's eye was considerably higher.

Our results with combined streptomycin and potassium iodide confirm those of Woody and Avery. The enhancement of the action of streptomycin, though not marked, is most definite in established caseous tuberculosis (Experiment 2 on rabbits) and becomes more evident only after prolonged treatment. The effect in very early lesions (first experiment on rabbits and mice) is much less definite, and this probably explains the negative results recently described by Levaditi and his co-workers (7) in acute tuberculosis in the mouse.

An experiment on mice using a combination of potassium iodide and *p*-aminosalicylic acid (2% in the diet) showed no enhancement of the effect of *p*-aminosalicylic acid, thus confirming the work of Bavin (8).

Streptomycin p-aminosalicylate compound. In this experiment 33 mice were infected intracorneally and divided into four groups. Streptomycin was given in a dose of 4 mg/day and the streptomycin *p*-aminosalicylate compound in daily doses containing 4 mg of streptomycin. *p*-Aminosalicylic acid was given as 2% in the diet. The treatment was begun within a few hours of infection and maintained for 28 days. The results, as judged by prolongation of the incubation period and by the number of eyes remaining free from tuberculosis at the end of the experiment, show clearly that all three forms of treatment had produced a beneficial effect, but of the three treatments the streptomycin *p*-aminosalicylate was the least effective. Hence, even if streptomycin salicylates reduced the development of resistant strains of tubercle bacilli in man, combined streptomycin and PAS treatment definitely can (9), and at the same time it has greater chemotherapeutic activities.

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Use of Selective Fluorescent Stains to Detect Insect Egg Plugs on Grain Kernels¹

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A major need in the cereal-processing industries is a method for the detection and elimination of grain which contains insects at various stages of development within the kernels. Methods yielding presumptive evidence of internal infestation have been proposed. These involve staining the gelatinous insect egg plug of the granary weevil, *Sitophilus granarius* L., and the rice weevil, *S. oryzae* L., with Lugol's solution (1), acid fuchsin (1), or gentian violet (2) in order to render them visible. Such procedures have not proved entirely satisfactory, since these reagents usually stain the starchy endosperm and frequently other tissues of the grain as well, and thus render difficult the adequate differentiation of infested kernels from those that are only mechanically injured. A selective stain specific for the egg plug is highly desirable.

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In this laboratory it has been found that immersion of infested grain, such as wheat and corn, in aqueous solutions containing 20 ppm of the alkaloid berberine sulfate for a period of 1 min results in selective staining of the egg plug. The seed coat and other parts of the grain take up the stain in insignificant amounts; thus washing or further treatment of the seeds is unnecessary. On exposure of the treated kernels in the dark to a source of ultraviolet radiation having a predominant wavelength of 3,660 Å, the stained egg plugs will fluoresce intensely in the yellow range of the spectrum and may be identified easily without auxiliary visual aids. A light-tight viewing box, with hand-holes to which are attached sleeves ending in elastic cuffs, provides for manipulation and examination of samples under the ultraviolet light in ordinary room illumination.

Other alkaloids will also produce fluorescence of the egg plug. Chelidonium extract is very selective and causes the egg plug to fluoresce orange-yellow. Primuline causes a light-blue fluorescence of the plug but also stains the seed endosperm to a considerable extent. Thioflavin is selective for the egg plug and fluoresces a light-yellow color. The fluorescent stains described are being employed in the development of quantitative methods for the determination of insect-infested kernels in grain.

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Embryonic Death Rate and Sex Ratio in Chicks

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In chicks the male is the homogametic type and should exhibit a lower embryonic death rate than the female. Hays (1) pointed out that with a mild disease outbreak in the parents, female embryos are more likely to succumb at an early stage of development.

During the hatching season of 1950 there was no evidence of disease in the parent stock, and chick mortality was less than 3% up to 8 wks of age. This provided an opportunity to study the relation between the percentage of fertile eggs that hatched from each of 108 Rhode Island Red females and the sex ratio of the chicks at 8 wks of age. Table 1 shows these females grouped with respect to the hatchability of their eggs and the sex ratio of their chicks.

TABLE 1

Egg hatchability of dams (%)	No. dams	Sex ratio (percentage of males) of chicks at 8 wks
60-69	8	55.31
70-79	30	52.47
80-89	34	51.69
90-100	36	49.12

These summarized data appear to suggest a linear decline in the percentage of males as hatchability increases. The slope of the line representing this decline was found to be -1.935 ± 0.272 . The small magnitude of its standard error suggests a significant decline in sex ratio. These data strongly suggest that the greater portion of embryonic deaths occur in females and that the sex ratio approaches equality when few embryos die.

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Complete Elimination of Microorganisms from an Intestinal Parasite (*Ascaris lumbricoides*)¹

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In vitro physiological investigation of the parasitic nematodes (roundworms) is hindered seriously by the presence of contaminating microorganisms, particularly in the case of nematodes inhabiting the alimentary canal of higher animals. When quantitative information is desired concerning nutrition, excretion, secretion, and respiration of such species, and the *in vitro* experimental period extends for more than a few hours, as it usually must, the elimination of microorganisms is essential (1, 2).

The present communication describes for the first time a method by means of which axenic² preparations of a common intestinal nematode may be made. This method is particularly useful in that microorganisms are eliminated completely from the parasite's intestinal tract, as well as from all other external surfaces. As a result, the nutrition and the excretion products of this species are now being studied under controlled conditions. The method of preparation is given below.

Ascaris lumbricoides adults were collected at the slaughterhouse in insulated bottles containing warm 0.9% NaCl solution, and were taken immediately to the laboratory, where decontamination methods were initiated within 2-3 hr of removal of the parasites from the pig intestine. All procedures were carried out at 37° C, with strict observance of aseptic techniques.

The worms were washed collectively with saline, and medium-sized males and females (1.5-3.0 g) were transferred to individual 125-ml Erlenmeyer flasks containing 30 ml of 0.8% nutrient broth (Bacto) at pH 6.0. This broth contained the following substances: NaCl, 0.45%; sodium sulfathiazole, 1:250; neutral acriflavine (Euflavine³), 1:5000; α, α'-azobis (chloroformamidine) (Azo-

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² The term axenic was proposed by Baker and Ferguson (3) to describe an organism free from all demonstrable life apart from that produced by its own protoplasm.

³ British Drug Houses, Ltd., Toronto.