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 A, 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 axenic[2] 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 (Euflavine[3]), 1: 5000; α, α′-azobis (chloroformamidine) (Azo-

[1] With financial assistance from the National Research Council of Canada and Swift & Company.

[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.

chloramide[4]) 1: 5000; dihydrostreptomycin sulfate 40 mg. The azochloramide and dihydrostreptomycin were added as dry powders to the broth solution just before it was dispensed into the flasks. The flasks, each containing a single *Ascaris*, were then placed in large vacuum desiccators (18 flasks/desiccator) containing 300 ml of freshly prepared 20% alkaline pyrogallol solution, and these were evacuated quickly to a residual pressure of 60 mm Hg. Atmospheric pressure was restored with cylinder nitrogen gas, and the process was repeated twice. The pyrogallol served to remove oxygen present as impurity (0.2%) in the nitrogen gas. Desiccators and contents were incubated in a water bath in a constant temperature room for 4 hr, after which 1 ml of penicillin solution (30,000 units) was added to each flask and anaerobic treatment continued for an additional 4 hr. The treatment solution was then drained off and replaced with 50 ml of nutrient broth containing 0.45% NaCl and adjusted to pH 7.8. The worms in this broth were incubated either aerobically or anaerobically for 36 hr.

Most flasks at the end of 36 hr showed no visible bacterial growth. Transfers (0.15 ml) were made to broth tubes for aerobic and anaerobic culture. At the same time the worms were placed in fresh broth (pH 7.0) and incubated for a further period of 24 hr, after which fresh transfers were made. Observation of flasks and transfer tubes was continued for at least 96 hr, and usually for one week or longer. Proof of sterility was taken as the continuing absence of growth from flasks and transfer tubes. Since the microorganisms dealt with were derived solely from the intestinal flora of the host (pig), culture media other than nutrient broth were unnecessary.

Repeated experiments, using 9–27 worms per experiment, showed clearly that an average of 85% of the individually treated worms could be made axenic. In a very few instances yeasts persisted in the absence of bacteria, but growth of molds and fungi was never observed. For reasons that are not understood, 8 hr *aerobic*, rather than *anaerobic*, treatment gave consistently poorer results. Continuing efforts are being made to produce a more completely efficient method.

The decontamination procedure as described has no apparent harmful effects upon the parasite. Motility and viability are unimpaired, and the intestine appears normal when examined histologically. Eggs are produced, and develop motile embryos, but no statistical comparison of egg production and development in treated and untreated females has been made.

It is hoped that this method, or a modification thereof, will prove useful when applied to other nematodes. In a single experiment we were able to prepare axenic cestodes (*Raillietina cesticillus*) in apparently unharmed condition, after 10 min aerobic exposure to the treatment solution.

[4] A generous supply of Azochloramide N.D.A. was kindly provided by Wallace and Tiernan Products, Inc., Belleville, N. J.

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Book Reviews

Physico-Chemical Constants of Pure Organic Compounds. J. Timmermans. New York: Elsevier, 1950. 693 pp. $12.50.

According to the author, "this work records, as completely as possible, those physico-chemical constants of organic compounds which have been measured with sufficient care to warrant their acceptance as data established with a precision worthy of contemporary science." The criteria which qualify a substance by its purity, and a constant by its exactness for acceptance are stated in the initial pages of the volume. Necessarily, both go together, and the principles followed by the author in the selection make highly instructive reading for anyone interested in the problems that arise when compounds are purified and constants are measured in accordance with rigorous standards. These few pages are followed by more than 600 pages of tables which give a critical selection of the constants of more than 1,600 substances. The compounds are arranged according to a lucid chemical system, and their location in the tables is further facilitated by an empirical formula index and by a subject index. A bibliography completes the book.

It cannot be the objective of this review to analyze in detail the tables of constants. For each individual compound are given the preparative data relating to purity, and the constants listed include critical constants, vapor pressure, boiling point, freezing point, density, specific heat, latent heats, viscosity, surface tension, refractive indices, and other data like heat of combustion, critical density, etc., depending on the available values. Where more than one independent measurement qualifies for listing, the results of each are given.

Anyone who has faced the job of finding in the literature the most reliable physical constants for the most highly pure compounds will be grateful to the author for the stupendous task he has accomplished. The work is one of the fruits of a quarter-century's activity of the International Bureau of Physico-Chemical Standards, of which the author is director. It combines the results obtained in the laboratories of this institution with the data provided by a systematic survey of the literature by a man who is familiar with the intricacies of pure compounds and of exact measurements, This has been accomplished with the support of the Belgian Chemical Industry and the Belgian National Fund for Scientific Research. The user of the book will benefit from the