mixtures of the two in any proportion can readily be prepared. Mixtures of the two liquids increasingly rich in the solute are made by adding more and more of the solution to the nonvolatile solvent. The mixture is tested from time to time by smearing a drop of it onto a glass microscope slide with a stirring rod. The volatile solvent rapidly evaporates, leaving a thin film of the substance dissolved in the nonvolatile solvent. As the ratio of the solute to the nonvolatile solvent is increased, a point is reached at which the residue appears milky because of the formation of large numbers of small crystals. This indicates that there is a sufficiently high degree of supersaturation in the residual film to produce spontaneous nucleation. If, then, a slight amount of the nonvolatile solvent is added to the mixture, it will be found that the residual solution film on the slide is highly supersaturated but that few, if any, crystals form.

The solution mixture obtained in this manner can be easily regulated to give a film of any desired degree of supersaturation by controlling the ratio of the solute to the nonvolatile solvent. The solution mixture is itself unsaturated and can be kept in a bottle indefinitely. A great many films can be prepared from a small quantity of the solution.

The supersaturated films of solution prepared by this technique are a sensitive tool for detecting small crystalline particles with respect to which the solution is supersaturated. When such a crystalline particle comes into contact with the solution film, it rapidly grows to a size sufficiently large to be seen and counted with a microscope. In this laboratory, these supersaturated films have been used to detect and count aerosol particles of sodium chloride and silver iodide having particle diameters of the order of 100 A.

In the case of the sodium chloride solution, water was used as the volatile solvent and glycerine as the nonvolatile solvent. In the case of silver iodide, a solution of sodium iodide in acetone was used as the volatile solvent, and triethylene glycol was used as the nonvolatile solvent.

The simplest way to use the films to detect these aerosols is to expose the film directly to the aerosol and to allow the particles to land on it by diffusion. In general, this method works quite well; it is, however, subject to certain drawbacks. If the film is to be exposed for more than a few minutes, the first few particles which land will grow and thereby reduce the supersaturation of the solution to such an extent that particles arriving later will not grow. A better system for examining particles collected over a period of time is to precipitate them first on a clean surface and then to bring this surface into contact with a freshly prepared supersaturated film. Each particle then starts growing at the same time, and all grow to an equal size.

In general, the supersaturation of films prepared from a solution will vary somewhat with temperature and humidity. For reproducible results, it is desirable that the films be formed at some standard temperature and humidity. By using the technique in which a sample of particles is first taken on a clean slide and then brought into contact with the supersaturated film, it is possible to use the films under standardized temperature and humidity.

Another method of forming supersaturated solutions for detecting particles on a surface is to spray the solution mixture from a spray nozzle onto the surface. By using a very volatile solvent (such as acetone) the spray can be arranged so that the volatile solvent evaporates as the spray drops pass through the air and the drops are supersaturated as they land on the surface.

One would expect, by analogy with the action of silver iodide (*1*) as an ice-forming nucleus, that supersaturated films of one substance might be nucleated by other substances having a similar crystalline structure. This fact will doubtless cause some ambiguity when the films are used to identify particles, but in some cases it may permit the use of a solution of one substance to identify particles of another insoluble substance having a similar structure.

The preliminary work which has been done with supersaturated films suggests that the technique may have general use as a tool for particle counting and identification and as a method for investigating the kinetics of homogeneous and heterogeneous nucleation.

Reference

1. VONNEGUT, B. *J. Applied Phys.*, **18**, 593 (1947).

Manuscript received April 7, 1952.

Algebraic Relationships Between Digestion Coefficients Determined by the Conventional Method and by Indicator Methods

H. L. Lucas

Institute of Statistics, University of North Carolina, Raleigh

In studies on the usefulness of indicator methods for the determination of coefficients of apparent digestibility, it is often desired to compare the results obtained by the indicator method with those obtained by the conventional method in the same series of trials. The indicator methods require an assumption as to the recovery of the indicator. Hence, it is desirable, if using the indicator method alone, to ascertain the consequences of making an incorrect assumption about recovery. There obviously exist algebraic relationships between the two methods of determining digestibility. If applied, these may be saving of computational time when comparing the two approaches, and they also allow an assessment of the consequences of an erroneous assumption regarding recovery of the indicator. These relationships seem generally not to be known.

In the conventional approach, the percentage apparent digestibility of a given nutrient is computed by the formula

$$d = 100 - \frac{100\, pw_0}{cw}, \qquad (1)$$

in which d = percentage apparent digestibility of the nutrient determined by the conventional method; p = percentage content of the nutrient in the feces; w_o = weight of feces excreted; c = percentage content of the nutrient in the feed; and w = weight of feed consumed.

The formula for obtaining apparent digestibility by the indicator procedure is

$$d^* = 100 - \frac{r^* p c_i}{p_i c}, \qquad (2)$$

in which p and c are defined as for (1) and d^* = percentage apparent digestibility of the nutrient determined by the indicator method; r^* = the assumed recovery of the indicator in percentage; c_i = percentage content of the indicator in the feed; and p_i = percentage content of the indicator in the feces. If the true recovery of the indicator were taken into account, (2) would be

$$d = 100 - \frac{r p c_i}{p_i c}, \qquad (3)$$

in which r = true recovery of the indicator in percentage, and the other symbols are defined as before.

It is evident that (1) and (3) give identical results because

$$r = 100\,\frac{p_i w_o}{c_i w}.$$

The relationship between d^* and d can be obtained by solving for $\frac{p c_i}{p_i c}$ in either (2) or (3), substituting in the other, and rearranging. This yields

$$d^* = 100 - \frac{r^*(100 - d)}{r} \qquad (4a)$$

or

$$d = 100 - \frac{r(100 - d^*)}{r^*}. \qquad (4b)$$

It follows from (4a) and (4b) that the difference between the results of the conventional and indicator methods—i.e., $d - d^*$—is given by

$$d - d^* = \frac{(r^* - r)(100 - d)}{r} \qquad (5a)$$

or

$$d - d^* = \frac{(r^* - r)(100 - d^*)}{r^*} \qquad (5b)$$

From (4a) and (4b) it is seen that both basic formulas need not be used when comparing the conventional and indicator methods in the same series of trials. Either d or d^* may be computed, and the other may be obtained by (4a) or (4b), using the observed and assumed recoveries of the indicator. The possible errors due to an erroneous assumption about recovery when using the indicator approach alone can be ascertained from (5b) by inserting reasonable limits for the true recovery, r.

The usual assumption is that the recovery of the indicator is 100%; i.e., $r^* = 100$. The formulas above apply, however, for any assumed values of r^* and any observed or postulated values of r.

Manuscript received March 31, 1952.

Influence of pH on Phosphatase Activity of Rat Seminal Vesicles

J. C. Porter and R. M. Melampy
Iowa Agricultural Experiment Station, Ames[1]

The physiological significance of the phospho-monoesterases has been reviewed by Moog (*1*) and Sumner and Myrbäck (*2*). Also, Mann and Lutwak-Mann (*3*) have recently emphasized the importance of these enzymes in the functioning of the male accessory organs. Stafford, Rubinstein, and Meyer (*4*) made quantitative estimations at pH 5.4 and 9.7 of acid phosphatase (AcP-ase) and alkaline phosphatase (AlP-ase) in rat seminal vesicles, using a modification of the Huggins-Talalay procedure.

This investigation was undertaken to determine the pH optima for AcP-ase and AlP-ase of rat seminal vesicles, since a survey of the literature indicates an absence of such data for these glands. Tissue was obtained from 21 male rats (mean and SE: 255 ± 3 g). After separating the coagulating glands from the seminal vesicles, the latter were removed. The vesicles were split and then blotted to remove the secretion. After weighing each lobe, one was used for dry-weight determination and the other ground in 0.9% NaCl. The resulting mixture was diluted to 20 ml and allowed to extract for 5 min. The mixture was then centrifuged, and the supernatant decanted.

A modification of the phosphatase procedure described by Greenberg, Lucia, and Weitzman (*5*) was used in this investigation. For determinations between pH 2 and 8, 0.5 ml of tissue extract was mixed with 5 ml of the Michaelis (*6*) acetate-veronal buffer containing 15 mg disodium phenylphosphate, and for reactions between pH 9 and 12.7, 0.2 ml was mixed with 5 ml of the Sorensen glycine buffer (*7*) containing the same amount of substrate. Final pH was adjusted with a Beckman pH-meter and corrected for the Na ion effect. The reaction mixtures were incubated in a water bath for 1 hr at 37° C. After incubation, the tubes containing the reaction mixtures were chilled in an ice bath, and 2.5 ml of 20% trichloracetic acid was added to each. Controls were run for each determination by incubating the appropriate buffer-substrate for 1 hr at 37° C and then adding either 0.5 or 0.2 ml of test solution before protein precipitation. After precipitation, all tubes were allowed to stand for 1 hr before centrifuging. Five ml of the supernatant solution was transferred to a Klett colorimeter tube and neutralized with *M* NaOH, followed by 1 ml of 2 *M* NaOH. The tubes were placed in a water bath for 10 min at 37° C before adding 2 ml diluted (1:3) Folin-Ciocalteu phenol reagent. The contents of the tubes were then diluted to 10 ml and allowed to stand in a water bath at 37° C for 20 min

[1] This project is supported in part by funds furnished by Dairy Genetics, Des Moines; Eastern Iowa Artificial Breeding Association, Cedar Rapids; and Northwest Iowa Federated Breeders Cooperative, Sheldon, Iowa. Project 936. Journal paper No. J-2090.