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

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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.

for color development before reading in a Klett-Summerson colorimeter, using a 540 filter. The instrument was set at 0 with distilled water. A phosphatase unit is the mg of tyrosine equivalent to the phenol liberated from the substrate per g dry weight of tissue per hr. A standard curve was prepared with L-tyrosine according to Greenberg, Lucia, and Weitzman (*5*).

The activities of AcP-ase and AlP-ase of seminal vesicle tissue at various pH values is shown in Fig. 1.

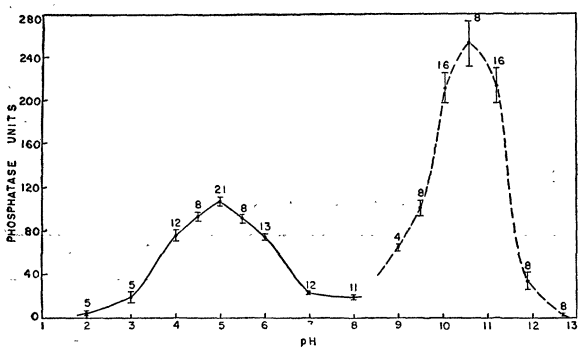

FIG. 1. Phosphatase activity of rat seminal vesicles as influenced by pH. Phosphatase units expressed as means and SE. Number of animals used for each pH value is indicated above mean.

Maximum AcP-ase activity was obtained at pH 5.0, whereas maximum for AlP-ase was at 10.6. It is significant that within the pH range usually associated with intracellular functions the activities of these enzymes are considerably less than their maxima.

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Manuscript received April 7, 1952.

Differentiation of Nucleic Acids and Acid Mucopolysaccharides in Histologic Sections by Selective Extraction with Acids[1]

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The markedly basophilic substances in sections of fixed tissues prepared by the paraffin technique fall into two principal groups, nucleic acids and acid mucopolysaccharides. The affinity of the former for basic dyes is attributable to their phosphoric acid moiety, whereas the staining of the latter is due to their component sulfuric or glucuronic acid (*1*). The histochemical methods devised for the microscopic demonstration of these substances have been based on one of two principles: identification by properties other than basophilia—e.g., the Feulgen nucleal reaction for desoxyribonucleic acid; or the use of basic dyes in conjunction with the differential extraction of various of the stainable substances. The present investigation is concerned only with the latter method.

The first tinctorial method developed for the histochemical demonstration of RNA was based on its removal from tissue sections by the enzymatic action of relatively crude preparations of ribonuclease (*2*). Sites of RNA were determined by comparing the distribution of stainable material in parallel digested and undigested sections. Although the technique was refined by subsequent investigators (*3*), it has remained of limited usefulness because of the difficulties involved in the preparation of ribonuclease and because of the high cost of the commercial enzyme. No satisfactory method has been devised for the removal of acid mucopolysaccharides as a group, although the preliminary results obtained with pectinases (*4*) warrant further investigation. The distribution of hyaluronic acid has been demonstrated successfully in a number of mammalian tissues by the use of hyaluronidase (*5*).

The disadvantages of the enzymatic method for the extraction of RNA from tissue sections appeared to have been eliminated by the recent introduction of perchloric acid ($HClO_4$) (*6–8*), based on the procedure developed by Ogur and Rosen (*9, 10*) for the separation of RNA from DNA components in the residue of plant and animal tissue homogenates after the removal of alcohol- and acid-soluble compounds and phospholipids. The substitution of $HClO_4$ for ribonuclease in the differentiation of RNA from DNA in paraffin sections seems to be justified. However, the effect of $HClO_4$ on the subsequent stainability of acid mucopolysaccharides and, therefore, the value of the reagent in distinguishing cytoplasmic RNA from these substances have not been considered. Neither has the efficiency of $HClO_4$ been compared with that of hydrochloric acid and trichloracetic acid

TABLE 1

EFFECT OF FIXATION ON EXTRACTION OF RNA FROM LIVER

(*N* $HClO_4$ at 5° C for 18 hr)

Fixative	Untreated	H_2O	Cytoplasmic RNA	Chromatin
Zenker's	+++	+++	+++	+++
Carnoy's	+++	+++	+	+++
Ca-formal	+++	+++	+	+++
80% Ethanol	+++	+++	±	+++
PAF	+++	+++	±	+++

[1] This work has been aided by a grant from the Montgomery County Society for Cancer Control, Dayton, Ohio.

[2] The invaluable technical assistance of Louise L. Brown is gratefully acknowledged.