trials, it was observed that when 60 mg of methylcholanthrene were incorporated into each 100 g of the basal diet, growth is inhibited. Although the rats do continue to grow, the rate of weight increase is markedly less than that observed in control animals ingesting the basal ration alone.

The incorporation of l-cystine (400 mg) or dl-methionine (500 mg) into each 100 g of the basal diet containing the methylcholanthrene results in a prompt stimulation of growth, with a resulting daily weight increment approximating that observed on the basal diet alone. On the other hand, supplements of glycine (500 mg), taurine (500 mg), or anhydrous sodium sulfate (500 mg), each added to 100 g of the basal diet containing methylcholanthrene, produced no increase in growth rate. Some typical data are presented in Table I. It appears, therefore, that cystine and methionine exhibit a specific ability to overcome the growth-inhibitory effect of methylcholanthrene. These results suggest that methylcholanthrene may produce a deficiency in the sulfur-containing amino acids, possibly by virtue of the involvement of these amino acids in the detoxication of the hydrocarbon. The metabolic fate of methylcholanthrene is being studied in order to obtain further evidence bearing on this suggestion, and similar methods are being extended to other carcinogenic substances.

10261

"Acid" Phosphatase and Functional Activity of the Prostate (Man) and Preputial Glands (Rat).

ALEXANDER B. GUTMAN AND ETHEL BENEDICT GUTMAN.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

While investigating the source of marked outpourings of "acid" phosphatase in the urine of adult men, Kutscher and Wolbergs[1] discovered that normal adult prostate tissue is extremely rich in a phosphatase, apparently specific,[2] with optimal activity at approximately pH 5.0. At about the same time, Moore and Hanzel[3] noted that prostate tissue extracts split off inorganic phosphorus from sodium nucleinate, a "nuclease" effect attributable to nucleotidases, which

1 Kutscher, W., and Wolbergs, H., *Z. f. physiol. Chem.*, 1935, **236**, 237.

2 Kutscher, W., and Wörner, A., *Z. f. physiol. Chem.*, 1936, **239**, 109.

3 Moore, R. A., and Hanzel, R. F., *Arch. Path.*, 1936, **22**, 41.

are now classified as phosphatases.[4, 5] Prostate phosphatase has since been found also[6, 7] in the primary tumor, at the site of distant metastases and in the blood serum of patients with metastasizing carcinoma of the prostate gland. The implications of these observations have been considered elsewhere.[6, 7]

The prostate gland of children, we find, contains little "acid" phosphatase (4.5 units at birth, 1.5 units at 4 years, 73.0 units at 13 years, per g fresh tissue). But adult prostate glands, though varying widely in phosphatase activity at pH 4.9 (from 2,284 to 522 units per g fresh tissue, in our series) consistently exceed by far the "acid" phosphatase activity of liver, kidney, duodenal mucosa and bone (Table I)—organs generally regarded as so rich in phosphatase as to imply some significant rôle of the enzyme in their respective functions. The presence in adult prostate tissue and in the semen of such high concentrations of this active enzyme (with optimal pH approximating that obtaining in the adult vagina) would suggest, similarly, that prostate phosphatase exercises some significant function in reproduction.

TABLE I.

Comparison of "Acid" and "Alkaline" Phosphatase Activity of Prostate Tissue with That of Other Tissues of the Adult Human.

(Phosphatase activity expressed in units/g fresh tissue.)

pH 4.9: *M*/200 monophenylphosphate substrate; *M*/10 citrate buffer; 37°C; 1 hr.

pH 9.0: *M*/200 monophenylphosphate substrate; *M*/20 Na veronal buffer; 37° C; 1 hr.

		Prostate pH		Kidney pH		Liver pH		Duodenum pH		Vertebra pH	
Case	Age	4.9	9.0	4.9	9.0	4.9	9.0	4.9	9.0	4.9	9.0
1	43	522	1.3	4.6	2.9	1.6	1.2	3.1	11.3	2.5	8.8
2	45	792	0.8	2.4	6.7	2.4	2.1	0.9	5.9	1.9	3.0
3	45	2,284	0.9	3.1	2.3	2.6	10.6	0.8	4.0		

An attempt to investigate this function further in experimental animals gave unexpected results. Repeated determinations at pH 4.9 revealed little phosphatase activity in the prostate gland of the sexually-mature dog (35.6 units per g fresh tissue), cat (2.8 units), rabbit (1.9 units), guinea pig (3.9 units), and rat (2.0 units).* Rat

[4] Folley, S. J., and Kay, H. D., *Ergebn. d. Enzymforsch.*, 1936, **5**, 159.

[5] Bredereck, H., *Ergebn. d. Enzymforsch.*, 1938, **7**, 105.

[6] Gutman, E. B., Sproul, E. E., and Gutman, A. B., *Am. J. Cancer*, 1936, **28**, 485.

[7] Gutman, A. B., and Gutman, E. B., *J. Clin. Invest.*, 1938, **17**, 473.

* These results are in agreement with Wolbergs' recent findings[8] that unlike man and the apes, "in the steer, dog and several other animals, only very small amounts of phosphatase could be detected" (in the prostate gland).

[8] Quoted by Kutscher, W., and Pany, J., *Z. f. physiol. Chem.*, 1938, **255**, 169.

prostates, unlike the prostate glands of these other animals, were found to contain considerable amounts of "alkaline" phosphatase: 54.8 to 15.5 units per g fresh tissue, at pH 9.0, in animals with liver phosphatase not exceeding 3.6 units at that pH. But no significant phosphatase activity was found in adult rat prostates buffered at more acid levels, as shown in the following example: 3.2 units at pH 7.3, 3.9 units at pH 5.5, 3.1 units at pH 4.9, 0.1 unit at pH 2.2.

Rats were treated with appropriate steroids known to cause more or less specific proliferation of certain cellular components of the rat prostate gland.[9]

A. (1) Mature rats were injected with 2 mg of testosterone propionate twice at 4-day intervals, then killed after 5 to 9 days. The prostates showed 2.4 mean units of phosphatase activity per g fresh tissue at pH 4.9; 30.3 units at pH 9.0. (2) The prostate glands of immature rats treated in the same way yielded 2.8 mean units per g fresh tissue at pH 4.9; 27.8 units at pH 9.0.

B. Mature rats were injected with 200 rat units of estradiol benzoate in oil twice at 3-day intervals, killed 4 days later. The prostates showed mean values of 2.4 units per g fresh tissue at pH 4.9; 49.4 units at pH 9.0.

C. Immature rats were injected with 100 rat units of Follutein (Squibb) 3 times at 2-day intervals, killed 3 days later. The prostates showed mean values of 2.3 units per g fresh tissue at pH 4.9; 49.0 units at pH 9.0.

Although the enlargement of accessory sex glands caused by these agents resulted in some increase in the total content of "acid" prostate phosphatase, it will be noted that the "acid" phosphatase content per g fresh prostate tissue was not significantly elevated.

Other sources of "acid" phosphatase in the adult male genital tract of the rat were then sought. Little phosphatase activity at pH 4.9 was found in the testis (1.1 to 4.2 units per g fresh tissue), seminal vesicles (0.4 to 2.8 units) and Cowper's glands (0.9 to 1.6 units), even after injection of the steroids mentioned; and only testis showed significant values at pH 9.0 (12.2 to 18.6 units). On the other hand, rat preputial glands proved unexpectedly rich in "acid" phosphatase. At pH 4.9, 27.4 to 104.0 units per g fresh tissue were found, including a value of 43.2 units in a sexually-immature rat of 4 weeks. The steroid-treated animals showed no definite deviation from the controls, per g preputial gland, but the increased size of the glands caused an increase in total preputial gland phosphatase. The pH-activity curve of this "acid" phosphatase approximates that of

[9] Bühler, F., *Z. f. d. ges. exp. Med.*, **1938, 104, 249.**

human prostate phosphatase. Both are inhibited by sodium fluoride and by propyl alcohol, neither is accelerated by Mg ion: an extract of rat preputial gland with 102.5 units of "acid" phosphatase activity showed 65.0 units with *M* propyl alcohol; 7.0 units with *M*/200 NaF, and 99.0 units with *M*/50 $MgCl_2$. Rat preputial glands, like rat prostate glands, also contain significant amounts of "alkaline" phosphatase (11.6 to 50.1 units per g fresh tissue at pH 9.0).

These results suggest another approach to problems of the accessory sex glands. It is interesting to note in this connection that Disselhorst long ago,[10] on purely morphologic grounds, postulated a specific function of the rat preputial glands.

Methods. The ground glands were usually extracted with 10 times their weight of distilled water for 24-48 hours in the refrigerator. Further dilution of the extract as high at 1:500 was necessary for optimal hydrolysis in the cases of human prostate and rat preputial glands. Phosphatase activity at pH 4.9 was determined by the adaptation of the King and Armstrong method[11] described elsewhere.[7] The unit employed in this study is defined as that degree of phosphatase activity which at pH 4.9 and 37°C will liberate from the specified buffer-monophenylphosphate substrate solution 1 mg of phenol in 1 hour.

10262 P

Effect of Calcium in Quantitative Determination of Prothrombin.*

JOHN K. STEWART AND FREDERICK J. POHLE. (Introduced by W. S. Middleton.)

From the Departments of Clinical Pathology and Medicine, University of Wisconsin Medical School.

Recent investigations,[1, 2] indicate that satisfactory methods for the quantitative determination of prothrombin have been developed.

10 Disselhorst, R., in Oppel's *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere*, 1904, **4**, 280.

11 King, E. J., and Armstrong, A. R., *Canad. M. A. J.*, 1934, **31**, 376.

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1 Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

2 Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.