to overcome the antidiuretic effect of exercise. Since water diuresis, itself a stress of a sort, might conceivably affect renal plasma flow in the human subject, it was necessary to obtain specific information on the point.

Full details of the method have been reported elsewhere (*1*). Renal plasma flow was determined under basal conditions in 9 normal young men by the *p*-aminohippurate clearance technique. There was a total of 59 experiments and 140 basal clearance periods. Water was given by mouth in varying amounts before and during the clearance determinations. Urine samples were collected by voluntary micturition.

Fig. 1 shows that there is no significant trend in renal plasma flow at rates of urine flow varying from 5 to 20 cc/min. Renal plasma flow at lower rates of urine flow were not studied, but the mean figure obtained in the present work (613 ± 107 cc) is not significantly different from that obtained by other workers (*2, 4*) employing low rates of urine flow. This suggests that neither moderate water diuresis nor substitution of voluntary micturition for catheterization affects renal plasma flow in healthy young men.

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Acid Phosphomonesterase Activity of Human Neoplastic Tissue[1]

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Since the development of a method of histological demonstration of enzymes hydrolyzing mono-phosphate esters in acid hydrogen ion concentrations (*3*), it has been shown that the nuclei of cells of almost all human tissues react strongly (*10*). Neurones and prostatic epithelium alone exhibit heavy cytoplasmic staining (*1, 2, 10*). In most of this work, however, the deleterious effects of protein denaturation during fixation and heating for paraffin embedding have been ignored, resulting in inconstant staining and variable localization of the precipitate forming during incubation. In developing our method for simultaneous quantitative estimation of activity of this enzyme along with its cytological localization, we noted, as did others (*1, 3*), these factors which are to be avoided in precise work. In addition we have attempted to preserve cellular integrity to a greater extent than that usually attained in biochemical methods used in the past to measure tissue phosphatases (*11*).

Utilizing the procedures outlined below we have been able to obtain reproducible measurements of activity of this universal nuclear component of human tissues on specimens no larger than those obtained in the usual surgical biopsy. We have found that it appears definitely related to rate of tissue growth and secretion. Moreover, human cancers exhibit a uniform increase in nuclear acid phosphatase when compared with homologous tissues of origin.

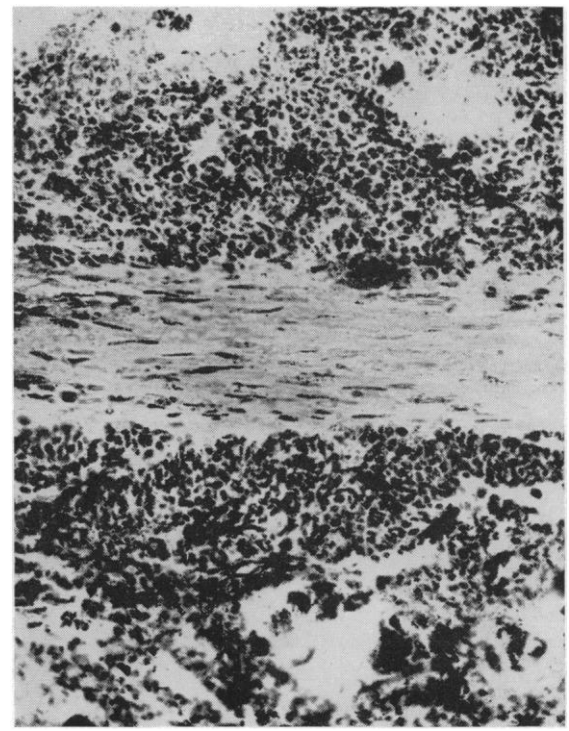

FIG. 1. Advancing margin of carcinoma of stomach, stained for acid phosphatase after 30-min incubation. Central area of normal muscularis with infiltrating tumor on each side. The black areas indicate lead sulfide precipitate within nuclei, at the site of maximum phosphatase activity. The cytoplasm has been lightly counterstained with fast green. (400×).

The methods used have been as follows: small blocks of tissue 1–2 cm in length and a few mm wide and thick are removed from freshly obtained surgical specimens, usually adjacent to blocks obtained for pathological diagnosis. Normal gastrointestinal epithelium for control studies in gastrointestinal cancers is dissected free from the muscularis. In the case of fibroids, blocks from the central but nondegenerated part of the fibroid are removed for comparison with adjacent blocks of homogeneous and grossly uninvolved myometrium. In malignant tumors it is always a problem to obtain a block which will be rich in viable cancer tissue, approaching the epithelium of origin in density of cells. We have usually sampled the

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edge of the advancing tumor, at times including a thin margin of uninvolved normal tissue (Fig. 1). We have learned to avoid the sclerotic base of many cancers which are relatively poor in malignant cells, and have had best results with sampling the soft fungating surface of polypoid lesions when such are available.

phosphatase activity after the procedure devised by Gomori (*3*) and with hematoxylin and eosin. We have used 0.01 molar sodium glycerophosphate (52% alpha)[3] for the substrate in all our tests, buffered at a pH of 5.0 + 0.1 with acetate buffer. We have found that 0.060 molar sodium acetate buffer gives best results for histological

TABLE 1

QUANTITATIVE ACID PHOSPHATASE ACTIVITY OF HUMAN NEOPLASMS COMPARED WITH TISSUES OF ORIGIN*

Source of tissue	Normal			Benign tumors Qap	Carcinoma Qap	Ratio
	Qap whole tissue	Qap smooth muscle	Qap epithelium or mucosa			
Stomach, ileum, colon, and rectum (mean of 10 cases)		.020 ± .009	.093 ± .034			4.7 ± 2.1
Uterine myometrium compared with leiomyomata (mean of 7 cases)		.018 ± .008		.012 ± .008		0.69 ± 0.21
Breast (not including skin)	.002				.008	4.0
	.029				.398	14
	.004				.073	18
Bronchus	.024				.214	8.9
	.066				.077	1.2†
	.022				.040	1.8
	.017				.051	3.0
Skin	.010				.063	6.6
	.029				.102	3.5
	.016				.028	1.8
Bladder	.009				.046	5.1
Esophagus	.012				.106	8.8
Stomach	.013				.030	2.3
						mean = 6.1 ± 4.6
Stomach, colon, and rectum			.066		.104	1.6
			.083		.193	2.3
			.084		.210	2.5
			.030		.027	.90‡
			.175		.287	1.6
			.018		.034	1.9
			.078		.173	2.2
			.067		.167	2.5
			.038		.067	1.8
			.081		.140	1.7
			.071		.119	1.7
			.054		.072	1.3
			.152		.192	1.3
						mean = 1.8 ± 0.5

* Each pair of values represents a different consecutive case; ± indicates standard deviation of observations from mean.
† Nitrogen mustard injected into pulmonary artery of isolated lung just prior to resection.
‡ Estimated proportion of neoplastic cells in tumor block only 52%.

The unfixed tissue specimens may be quickly frozen in serology tubes immersed in a mixture of dry ice and acetone, and then stored in a dry ice chest without marked decline in activity. At the time of test they are thawed and sections are cut at 20–30 μ on a freezing microtome. Four to twelve such sections are then incubated at 37° C in 6–8 ml of buffered substrate for periods of 15 to 60 min, at the end of which time phosphate analyses of filtered supernatant solution are carried out by Fiske and Subbarow's method. Duplicate sections are stained for

studies, but have preferred 0.162 molar buffer for quantitative microchemical analysis. We have reason to believe this preserves cellular integrity to a greater extent. We have used 0.0022 molar lead acetate in the incubating solution to precipitate the hydrolyzed phosphate for cytochemical localization; in this case the sections are not fixed on slides until after complete conversion of the precipitate to lead sulfide has been obtained. The only

[3] Obtained from Eastman Kodak Company.

counterstain used for the latter was a 2% solution of fast green in 70% alcohol, after fixation. All quantitative microchemical enzyme studies have made use of control preparations inhibited with 0.01 molar sodium fluoride, whose free phosphate content is subtracted from that of the test preparations. The net milligrams of phosphate hydrolyzed per hour has been divided by the milligrams of total tissue nitrogen in the sections separated from supernatant by centrifugation, as determined by the micro-Kjeldahl method of Wong. All our results are expressed in terms of this quotient (Qap), which usually reflects a nitrogen content of 0.1–0.4 mg.

Preliminary studies indicate a broad optimum pH range between 4.0 and 6.0, in which enzyme activity is roughly constant; the reaction increases with time of incubation and concentration of substrate, and does not seem markedly changed by small variations in concentration of the buffer. Heat denaturation at 100° C totally abolishes the reaction. We have worked with rather low substrate concentrations and short incubation periods (15–30 min) in the cytochemical preparations to preserve the nuclear localization of lead sulfide. The initial precipitate may become diffuse within or without the cell if the cells are damaged with heat or fixatives, or if incubation is prolonged.

The nuclei of all cells except prostatic epithelium and neurones react first in stained preparations, acquiring a dense brown precipitate of lead sulfide by the time a few scattered granules of precipitate appear in the cytoplasm. Staining appears selectively to demonstrate epithelium of all types before a reaction becomes marked in supporting muscular and connective tissue elements. The columnar duct epithelium of the breast, endometrium, epidermis, mucosa of the respiratory tract, and gastrointestinal tract all display a characteristic rapid response with gross staining possible after 15–30 minutes' incubation. We have noted even greater response in specimens of thyroid, parathyroid chief cell adenoma, pheochromocytoma, and ovarian corpora lutea. Quantitative analyses of the content of enzyme in the gastrointestinal tract confirm the staining technic in revealing a fivefold increase of enzyme in mucosa as compared with homologous smooth muscle (Table 1). Prostatic tissue, however, usually has a quotient of unity or above.

Gomori observed unusually heavy staining reaction in a number of cancers (*3*), and our studies to date, which include carcinomata of the breast, lung, skin, bladder, and all portions of the gastrointestinal tract, have shown a characteristic increase in acid phosphomonoesterase activity in the tumors when compared with their tissue of origin (Table 1). Analyses of different portions of the same gastrointestinal cancer have indicated that the ratio of phosphatase activity of the tumor specimen to that of the homologous muscle-free mucosa in the control is directly proportional to the percentage of neoplastic cells in each of the former specimens.

There is a considerable variation in the proportion of neoplastic cells in the tumor samples in Table 1. In those tests utilizing glandular epithelium free of muscle, the best control yet devised, the histological preparations must indicate nearly 100% viable neoplastic cells. If this condition is satisfied, then a mean twofold increase in enzyme activity has been demonstrated in all tests thus far. Certain histologically benign colonic polyps, which could be regarded as definitely premalignant by reason of close association with polyposis coli and adenocarcinoma, showed a fourfold increase in acid phosphatase. In contrast, we have thus far been unable to find any phosphatase increase in uterine leiomyomata, due possibly to the presence of large amounts of relatively nonreactive fibrous tissue in these benign tumors. Although Greenstein has noted similar increased acid phosphatase activity in rat and mouse hepatomas compared with regenerating and normal liver, no significant increase was reported for other transplantable malignant mouse tumors (*4*). Huggins and others have noted a marked reduction in the acid phosphatase of neoplastic human prostatic tissue compared with that of the normal adult gland (*5*). Since normal prostatic epithelium contains very large amounts of cytoplasmic acid phosphatase which it also secretes, it seems likely that the loss of activity noted was largely the result of anaplastic changes affecting specialized secretory mechanisms in the cytoplasm. To the best of our knowledge, increase in tissue acid phosphatase activity has not been previously reported in human neoplasms, and we believe our observations are of significance in providing a further indication of fundamental enzymatic disturbance in human cancerous tissue. It may be that the increased acid phosphomonoesterase activity of human cancer cells is related to the rapid uptake and retention of labeled phosphorus by malignant tissue (*6*), and it has been shown that the nuclei of malignant cells are more active than the cytoplasm in this respect unlike normal cells (*7*). The predominant nuclear location of the enzyme in cancers of organs other than the prostate probably prevents its escape to the blood stream with the same facility as is seen in carcinoma of the latter gland. Only 2 to 19% of advanced cancers of other organs produce even slight elevations in the normal serum level of this enzyme (*9*).

Simple technics such as we have used may aid a more intensive investigation of the quantitative microchemical changes involved in the malignant transformation of epithelial tissues in primates.

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