A HISTOCHEMICAL STUDY OF THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN VARIOUS NORMAL AND NEOPLASTIC TISSUES*

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Phosphatases having an *in vitro* P_H optimum of about 9 have been found in kidney, intestine, bone, and in smaller amounts in other tissues and in serum.[1] They have been stated to be involved in the absorption of glucose through the intestinal wall and reabsorption of glucose in the tubules of the kidney (Lundsgaard[2-4]), and in osteogenesis (Robison[5] and Kay[6]). Quantitative analytical measurements for the estimation of serum phosphatase have been developed by A. Bodansky and Jaffe,[7] and changes in serum phosphatase level were found in rickets, osteogenic sarcoma, obstructive jaundice, hyperthyroidism, and in other conditions.[1] Phosphatases were shown to differ by O. Bodansky,[8] who observed that the activity of bone and kidney phosphatases was retarded by bile acids, whereas intestinal phosphatase was unaffected.

Recently the introduction of a histochemical method by Takamatsu[9] and independently by Gomori,[10] for demonstrating the presence of phosphatase in tissues, has made it possible to study the location of phosphatase in cells and its distribution in different tissues.

The present study was undertaken to determine the presence of phosphatase in various normal and malignant tissues, and especially its relation to bone formation in a transmissible osteogenic sarcoma of fowls.[11] The data on the distribution of phosphatase were accumulated before we were aware of the studies of Takamatsu.[9] The histochemical method has also been used to study the inhibiting effect of various substances on the action of phosphatase in tissue sections.

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METHOD

The procedure outlined by Gomori [10] and by Takamatsu [9] is based on the deposition of $Ca_3(PO_4)_2$ at the site of enzyme action, when a section of tissue is incubated with an organic phosphate ester in the presence of calcium ions. In order to insure optimum activity of the enzyme, constant conditions and maximum histological definition, the following procedure was used:

Tissues were fixed in 95 per cent alcohol and paraffin sections were mounted on slides. The paraffin was removed with xylol and the xylol with absolute alcohol. The sections were then dipped in a dilute collodion solution, allowed to dry, hardened in 90 per cent alcohol,[10] and washed with distilled water. Alcohol under these conditions does not affect the stability of this enzyme. The sections were then transferred to substrate solution and incubated at 37° C. for 2 hours. Control serial sections were placed in a dilute (0.1 per cent) calcium nitrate solution. Stock solutions of 3.2 per cent sodium-β-glycerol-phosphate, 2 per cent calcium nitrate, 10 per cent sodium barbital, and 0.1 molar magnesium sulfate, were prepared. The solution used was made up by diluting 6 cc. of sodium-β-glycerol-phosphate, 9 cc. $Ca(NO_3)_2$, 6 cc. sodium barbital, and 6 cc. $MgSO_4$ to 60 cc., to give a final solution which was 0.01 molar with respect to glycerol phosphate and magnesium sulfate and had a P_H of 9. After incubation, each section was placed in the solution with its control and both stained for calcium by von Kossa's method, which replaces the calcium phosphate by metallic silver and gives a brown color at the site of phosphatase action. The sections were washed in absolute alcohol to remove the collodion, stained with hematoxylin and counterstained with light green. In the finished section the silver deposit (indicative of phosphatase) is brown, the cell nuclei blue and the cytoplasm green (Figs. 1-6).

Sodium barbital was used as a buffer since preliminary experiments showed very marked reduction and variability in the intensity of enzyme action in unbuffered solution. Magnesium, used in the concentration found to be optimal by O. Bodansky,[12] produced a slight increase in the intensity of the phosphatase reaction.

THE DISTRIBUTION OF PHOSPHATASE IN NORMAL TISSUES

The histochemical reaction showed the presence of large amounts of phosphatase in osteogenic tissues, renal epithelium and epithelium of the small intestine; that is, in tissues which form bone or are concerned with glucose absorption or elimination. In addition, small or moderate amounts of phosphatase were present in the endothelium of the blood vessels of most organs,

in certain nerve cells, in nerve fibers, in cells of the pituitary and in a few other types of cells.

Bone. The femur of a stillborn baby and bones of young chicks and of a mouse embryo were studied. Adult human bone could not be investigated because the usual procedure of decalcification results in the destruction of the phosphatase, but extracting the calcium salts from bones of a stillborn baby with diammonium citrate does not interfere with the demonstration of phosphatase.* The characteristic osteoblasts and the spindle-shaped periosteal fibroblast-like cells, wherever present, gave strong phosphatase reactions. Particularly instructive was the study of the embryonal bones. In the mesenchyme of the tail (Figs. 7 and 8) and leg rudiments large amounts of phosphatase were found in cells, which did not differ in appearance from the usual non-bone-forming mesenchymal cells. Proliferating cartilage cells in the chicken femur showed conspicuous phosphatase reactions. Cartilage in adult human trachea was free from phosphatase. Large amounts of phosphatase were found in the mandible and tooth anlage of a mouse embryo (Fig. 3).

Bone Marrow. Normal blood-forming elements, precursors of erythrocytes, granulocytes and megakaryocytes, did not give the phosphatase reaction. Some endothelial cells contained a small and variable amount of phosphatase. There were occasionally fine granules, indicating phosphatase action, scattered about the fat vacuoles.

Kidney. Adult human, and adult and embryonal mouse, tissues were examined. The cells of the convoluted tubules contained large amounts of phosphatase (Figs. 1 and 2). Their brush border was most intensely stained. The distal convoluted tubules, loops of Henle and collecting tubules contained no phosphatase, or traces only. The epithelium of Bowman's capsule gave an intense reaction if cuboidal, but was unstained if flat. The epithelium covering the glomerular tufts was free from phosphatase and only traces of phosphatase were found in the glomeruli.

Salivary Gland. In the lumen of a salivary gland about the acini, small amounts of phosphatase were demonstrated.

Intestine. Adult human, adult chicken and embryonal mouse

* Citrates have been used by J. Salk to dissolve calcium phosphate precipitates on which viruses have been adsorbed. (Personal communication.)

tissues were studied. The epithelium of the small intestine gave an intense phosphatase reaction (Fig. 6), most conspicuous in the upper layers of epithelial cells, while the deeper ones contained only a small amount of phosphatase. Autolyzed and desquamating epithelial cells also gave a marked reaction. A large amount of phosphatase was found in the endothelial cells of the submucosa. Occasional fibroblast-like cells of the submucosa likewise gave the reaction, while the muscle cells were free from phosphatase.

The epithelial cells of the large intestine did not give the reaction except a few cells in close proximity to the lumen. There were amorphous masses containing phosphatase in the lumen of the gut and also a few desquamated intestinal epithelial cells which contained only traces of phosphatase. Endothelial cells of the submucosa and of the lymph follicles almost invariably contained phosphatase and a thin film of phosphatase was found in the serosal layer.

Stomach. Epithelium of human gastric mucosa and that of the mouse embryo did not give the reaction.

Muscle. Adult human, and adult and embryonal mouse tissues were examined. Neither the heart nor the voluntary and smooth muscles examined contained phosphatase but the endothelium of capillaries gave a strong reaction.

Lung. Human adult, and mouse adult and embryonal tissues were examined. The usual alveolar epithelium did not contain phosphatase, but cuboidal alveolar epithelium contained some of this enzyme. In one specimen there was much edema fluid in the alveoli and this gave a faint phosphatase reaction. The endothelium, particularly of the large vessels, was well marked by this reaction. The epithelium of the bronchi and of the trachea contained small amounts of phosphatase. The cartilage of adult human trachea was phosphatase-free. Calcifying tracheal cartilage from an old individual contained no phosphatase about the calcium deposits. Much phosphatase was found in the basement membrane of the bronchial epithelium of an adult mouse. A small amount was found in the lumens of mucous glands and at the margin of this glandular epithelium in the trachea.

Liver. Adult human, chicken and mouse livers, and an embryonal mouse liver were examined. The endothelium of the sinu-

soids and of other vessels gave a conspicuous phosphatase reaction. The entire capillary bed, as a rule, was mapped out by this reaction. The bile ducts and liver cells contained no phosphatase, or only traces, although occasionally fine granules were seen scattered throughout the section but not identified with any cell type. In human liver of a young adult the liver cells contained a large number of very fine granules.

In order to distinguish between endothelial and Kupffer's cells a chicken was given intravenous injections of fine carmine particles. The animal was killed, the liver and spleen were fixed in alcohol and tested for phosphatase by the technic described. In the sections thus prepared, Kupffer's cells were filled with carmine particles, but failed to give the phosphatase reaction; whereas the endothelial cells of the liver contained large amounts of phosphatase but were free from carmine (Fig. 4).

Spleen. Adult human, mouse and chicken spleens and the spleen of a mouse embryo were studied. The endothelium, as in other organs, was well marked by the phosphatase reaction but none of the other elements of the spleen gave the reaction. There was some phosphatase in fibroblast-like cells about the margins of the trabeculae. The spleens of the mice contained erythrogenic and myelogenic foci with megakaryocytes, but none of these cells was phosphatase-positive. In the chickens that received intravenous injections of carmine the histiocytes of the spleen were seen to contain large amounts of carmine and were free from phosphatase, whereas the endothelium gave a phosphatase reaction but was free from carmine.

Lymph Node. Endothelial cells of blood vessels contained much phosphatase, while endothelial cells of most lymph sinuses were free from phosphatase. Most lymphocytes were free from phosphatase, though occasional groups of lymphocytes contained small amounts. Also occasional endothelial cells of lymph sinuses contained traces of phosphatase. The occurrence of phosphatase in lymphocytes and endothelial cells of the lymph sinuses was variable, and, if present, the amount of enzyme was small.

Adrenal. In sections from a mouse embryo the capsule and some cells of the zona glomerulosa gave the phosphatase reaction; the remaining cells of the cortex and those of the medulla did not. In the adrenal of a human adult, cells of the zona reticularis con-

tained the largest amounts of phosphatase, which was present in smaller amounts in both the fascicular and glomerular layers. The relative intensity of staining reaction, however, varied in different fields. In another section, from a woman who died *post partum,* large amounts of phosphatase were found in cells of the zona reticularis, moderate amounts in cells of the zona fasciculata and small amounts in cells of the zona glomerulosa. Cells of the adrenal medulla were phosphatase-free.

Pituitary of the Human Adult. In a pituitary from a man 71 years of age, neurogenic cells of the posterior lobe, the capsule and capillaries contained much phosphatase, as did clumps of epithelial cells in the posterior lobe. In the anterior lobe, only the capillaries and occasional clumps of epithelial cells gave the reaction. The type of epithelial cells which gave the reaction has yet to be identified, but their presence in large numbers in the posterior lobe of the pituitary of an old man makes it probable that they are basophils.

Thyroid. Thyroid epithelium from a young adult did not contain phosphatase. The capillaries in the same section contained phosphatase.

Testis. In a section from a human adult, most spermatogenic cells contained a small amount and the basement membrane contained moderate amounts of phosphatase.

Prostate. In the prostate of a young adult 18 years of age, the epithelium was found to be free from phosphatase. Some muscle cells contained traces of phosphatase. In an adult 64 years of age, numerous epithelial cells of the prostatic acini contained slight or moderate amounts of phosphatase. The staining reaction of the cells lining the ducts was of the same intensity. There appeared to be less reaction in the hyperplastic than in the non-hyperplastic acini.

A phosphatase with an optimal P_H in the acid range, the so-called acid phosphatase, was present in large amounts in prostatic tissue. Attempts are being made to develop a staining technic for acid phosphatase characteristic of this organ.

Seminal Vesicles. In a section from a human adult the epithelium was found free from phosphatase; the endothelium contained much phosphatase and the muscle traces of it.

Uterus. The myometrium was free from phosphatase. The

epithelial cells of the endometrium contained moderate amounts of phosphatase.

Bladder. The epithelium of the renal pelvis and of the urinary bladder of a mouse embryo gave a strong phosphatase reaction.

Pancreas. The epithelial cells of the pancreas from a mouse embryo and from a human adult contained no phosphatase.

Breast. In sections from the breast of a young woman *post partum,* and from another in the puerperium, the epithelial cells gave a strong phosphatase reaction, as did those in a fibroadenoma that will be described. There was also a small amount of phosphatase about the basement membrane and in the spindle-shaped, loose connective tissue cells between the glands. The colostrum contained only traces of phosphatase.

Ovary. In a section of ovary taken *post partum,* a very large amount of phosphatase was found in the corpus luteum, while the epithelium of adjacent primordial follicles contained none. Spindle-shaped, theca lutein cells surrounding the epithelial cells of the corpus luteum contained considerable amounts of phosphatase. The intensity of the phosphatase staining decreased gradually peripherally and none was found in the spindle-shaped cells distant from the corpus luteum. Capillaries almost invariably contained phosphatase; germinal epithelium was free from it. In a follicular cyst, granulosa cells lying free in the lumen contained no phosphatase, whereas several layers of polygonal and spindle-shaped cells about the cyst contained very large amounts of phosphatase. In a corpus albicans no phosphatase was found.

Phosphatase in Tumor Cells

I. Chicken Tumors

Osteogenic Sarcoma. The sarcoma studied is readily transmissible in chickens and is probably produced by a filterable agent.[11] Numerous samples were examined, some showing osteoblasts with almost no intercellular substance, and others showing mature bone, cartilage, and various stages of bone and cartilage formation by the malignant osteoblasts (Figs. 15–18). These osteoblasts were large round cells, slightly larger than large lymphocytes, with a large vesicular nucleus and a scant amount of cytoplasm. If seen with no osseous or cartilaginous matrix, their

osteogenic character was not evident. The phosphatase tests showed, however, that they contained phosphatase in increasingly large quantities when cartilage or bone formation was taking place.

Fibrosarcoma. Cells of a transmissible fibrosarcoma (strain 11 [13]), produced by a filterable agent, contained no phosphatase. This enzyme was also absent in cells of sarcoma and malignant endothelioma produced by another filterable agent.[14] Transmissible sarcoma cells originating in a growth produced in the breast of a chicken by methylcholanthrene likewise failed to give the phosphatase reaction.

Fowl Leukosis. The leukemic cells in the liver, bone marrow and spleen of chickens injected with viruses of fowl leukosis (strains 1 and 2 [15]) contained no phosphatase.

II. Mouse Tumors

Cells of a transmissible mouse sarcoma derived from mice that had been injected with methylcholanthrene failed to give the phosphatase reaction.

Adenocarcinomata of mice arising spontaneously in stock C_3H were likewise phosphatase-negative.

A spindle cell sarcoma, the osteogenic character of which was at first not recognized in the usual hematoxylin and eosin preparation, was found to contain very large amounts of phosphatase (Figs. 19 and 20). A careful study of the control sections showed, however, deposits of calcium granules in several places among the spindle-shaped, fibroblast-like osteogenic cells.

III. Rat Tumors

A liver carcinoma produced by feeding a rat with butter yellow (dimethyl-amino-azobenzene) was examined. The cytoplasm of the tumor cells contained a fine dust of brown granules. The necrotic parts of the tumor contained amorphous masses with granules. Most liver cells were free from phosphatase. The basement membranes of some cells, however, contained small amounts of phosphatase. The endothelial cells between the newly formed ductlike structures contained large amounts of phosphatase, while those of the sinuses of the relatively normal liver tissue contained none or only traces.

IV. Human Tumors

Carcinoma of the Breast. Sections from 4 carcinomas of the human breast were examined. None contained phosphatase.

Fibro-adenoma. A section showing the characteristic appearance of a fibro-adenoma of the breast, contained brown granules in large amounts in the cells of both acini and ducts and particularly abundant about the basement membrane (Fig. 5).

Carcinoma of the Gastro-intestinal Tract. Sections from a carcinoma of the stomach and from 7 carcinomas of the large bowel were examined. The latter included a mucinous carcinoma and 3 well differentiated adenocarcinomas. Phosphatase was absent in the tumor cells but present in the endothelium and in occasional fibroblast-like cells of the stroma (Figs. 13 and 14).

In a tumor metastatic from the sigmoid colon to the liver, no phosphatase was found in the tumor cells, while the connective tissue and endothelial cells of the stroma contained large amounts.

Liposarcoma. The section was taken from a tumor on the thigh of a man. The tumor cells were free from phosphatase. The endothelial cells contained a moderate amount and scattered cells resembling histiocytes between the malignant cells contained a large amount of phosphatase.

Malignant Melanoma. Sections from two examples of metastatic melanoma were studied. One was in an axillary lymph node, with the primary site undetermined. The other, in the small intestine, was a metastasis from the vulva. Neither contained phosphatase.

Adamantinoma. In this tumor from the lower jaw of a man, the endothelial cells and many of the connective tissue stroma cells contained small amounts of phosphatase, while the tumor cells contained none.

Hypernephroma. The tumor cells of a hypernephroma of the kidney, surgically removed, were free from phosphatase, while the capillaries were well marked by brown granules indicative of this enzyme.

Wilms' Tumor. Most tumor cells contained moderate amounts of fine granules indicative of phosphatase, while the stroma contained moderate or large amounts. The quantity of phosphatase in the stroma was sufficient to produce enough granules to obscure

the cells and it was not determined whether the phosphatase was free in the stroma or was in the fibroblast-like cells.

Perineural Fibroblastoma. The tumor cells of a malignant perineural fibroblastoma involving the sacral plexus showed no phosphatase.

Human Leukemia. Malignant lymphoid cells infiltrating the liver, spleen and other organs in a case of acute lymphoid leukemia contained no phosphatase.

Discussion

The following observations indicate that the histochemical method of Takamatsu [9] and Gomori [10] for phosphatase is specific for this enzyme. When this method is followed, except for the presence of sodium-β-glycerol-phosphate, only tissues containing calcium are stained; no non-calcium-containing tissues give the von Kossa reaction. The histochemical reaction is most pronounced in those organs (kidney, intestine and bone) which have been shown by chemical determinations on aqueous extracts to contain large amounts of phosphatase.

By the use of this histochemical method, the presence of phosphatase in tissues and organs not previously known to contain the enzyme has been established, and its relation to cells clarified. Thus, in the kidney the phosphatase is contained in cells of the proximal convoluted tubules. The endothelium, the cells surrounding embryonal hair follicles (Figs. 9 and 10), the myelin sheath of nerves (Figs. 11 and 12), the spermatogenic cells of the testis, and cells of the adrenal cortex have been shown to contain phosphatase. It will now become of considerable interest to determine what rôle phosphatase plays in these tissues.

The occurrence of phosphatase in vascular endothelium, for example, is of considerable interest in relation to the origin of the serum phosphatase. Armstrong and Banting [16] observed that removal of the viscera did not affect the serum phosphatase level and concluded that most of the serum phosphatase comes from bone. The presence of phosphatase in vascular endothelium may indicate either that this tissue contributes to the serum phosphatase, or that endothelial cells selectively remove phosphatase from the blood.

The presence of glycine had an inhibiting effect on the activity

of phosphatase. Thus, 0.25 molar glycine added to the substrate solution completely inhibited the action of phosphatase in bone, intestine and endothelium and almost completely inhibited phosphatase in the kidney in tissue sections. This is in agreement with the findings of O. Bodansky,[12] who demonstrated that glycine in concentrations higher than 0.00625 molar inhibited the action of kidney, intestine and bone phosphatase. This observation furnishes additional evidence for the specificity of the histochemical method.

No histochemical studies have hitherto been made on bone phosphatase, owing to the difficulty of decalcifying bone under conditions which did not destroy the enzyme. By the use of a 10 per cent solution of diammonium citrate, it was possible to decalcify bone without affecting the phosphatase. Kidney phosphatase was shown to be unaffected if the fixed tissues were kept for a week in diammonium citrate solution. Bone decalcified in this manner, however, gave poorer preparations than are usually obtained by other methods of decalcification. Additional data on bone phosphatase were obtained from developing mouse embryos, which can be cut with or without decalcification.

The method enables a differentiation between endothelial cells, on the one hand, and Kupffer's cells, histiocytes or monocytes, on the other. In one experiment Kupffer's cells and histiocytes of the spleen of a chick were marked by intravenous injection of carmine particles. Sections of liver and spleen showed no phosphatase in the carmine-laden cells, but the endothelial cells gave a good reaction (Fig. 4).

Lundsgaard [2-4] observed that phlorhizin, in addition to producing glycosuria, inhibits the phosphorylation of glucose, as well as the absorption of glucose, from an intestinal loop. These observations led him to postulate that glucose absorption in the intestine and its reabsorption in the kidney tubule are associated with intermediate phosphorylation, and that the phosphatase subsequently dephosphorylates the glucose phosphate. Lundsgaard subsequently became more cautious about this hypothesis with respect to reabsorption of glucose in the kidney because the dose of phlorhizin which completely inhibited glucose reabsorption per gram of kidney tissue was less (about one-fifth) than that necessary to prevent esterification in tissue. The histochemical

findings indicate that in the kidney the phosphatase is localized only in the proximal convoluted tubule,[9, 10] and if phlorhizin is also selectively localized in the proximal convoluted tubules its concentration might be high enough to inhibit the phosphorylation. Lundsgaard has himself observed a selective concentration of phlorhizin in the cortex of the kidney, and Ellinger and Lambrechts [17] demonstrated that phlorhizin dyes which produce glycosuria accumulate in the proximal convoluted tubules. The occurrence of large amounts of phosphatase in the lining epithelium of the small intestine also supports Lundsgaard's original hypothesis. None of the data presented by other investigators [18] directly contradicts Lundsgaard's original hypothesis.

Our results indicate that phosphatase occurs in cells of several different malignant tumors, the most important of which is osteogenic sarcoma. Malignant osteoblasts contain phosphatase even though there is no formation of cartilage or bone. This may be of some value in differentiating osteogenic from other tumors. For example, a sarcoma of the right arm, which was regarded as probable myosarcoma largely on the basis of its location, was found to contain large amounts of phosphatase. The malignant cells were fibroblast-like and because normal muscle cells are devoid of phosphatase it is unlikely that these malignant cells were derived from myoblasts.

Of four different strains of fowl tumors examined, only the cells of the osteogenic sarcoma contained phosphatase. Primitive blood cells from leukoses, like normal immature blood cells, were devoid of phosphatase.

Phosphatase was also found in a fibro-adenoma of the breast (Fig. 5), but not in four carcinomata of the breast. It is noteworthy that lactating breast also contains large amounts of phosphatase. These observations suggest that malignant transformation of mammary gland epithelium is accompanied by loss of this enzymatic function.

There are certain irregularities. Connective tissue, for example, is, as a rule, phosphatase-free. Occasionally, however, it contains small amounts of phosphatase. The fibrous connective tissue stroma of tumors often contained large amounts of phosphatase, even though the tumor cells contained none. These observations

suggest that young or proliferating cells contain more phosphatase than adult or resting cells. The phosphatase content of cells of the adrenal cortex varied greatly in different individuals and it is possible that this was due to changes in the functional state of the gland.

Muscle cells, as a rule, are phosphatase-free. Occasionally the sarcolemma takes the phosphatase stain, so that in cross section muscle fibers are surrounded by a bright brown honeycomb marking the sarcolemma sheaths.

Our observations with normal tissues are in essential agreement with those of Takamatsu[9] and of Gomori,[10] but there are minor discrepancies which will have to be cleared up by future work. For example, Takamatsu found that fibroblasts were free from phosphatase whereas in some of our sections these cells gave a definite phosphatase reaction. Takamatsu described strong phosphatase reactions in the epithelium of the large intestine, which were not seen in our preparations.

It is significant, as Takamatsu[9] noted, that endothelium of lymph vessels is phosphatase-free, while that of vascular endothelium contains phosphatase. This may be interpreted by assuming that the phosphatase of endothelium of blood vessels is derived from the plasma phosphatase which is absorbed by or deposited on the cells, or that the reaction is subtle enough to distinguish between endothelium of blood and of lymph vessels.

The observation that cells of a liver tumor induced in a rat by the feeding of butter yellow contained phosphatase was unexpected because liver and bile duct epithelium do not contain the enzyme.

It is noteworthy that embryonal precursors of phosphatase-containing adult cells also contain large amounts of phosphatase at the stage of embryonal development when there is no evidence of functional activity. Robison[5] has shown that mesenchyme of the mandibular anlage, incubated *in vitro* in tissue cultures, assumes histiogenic activity.

The histochemical reaction indicates that phosphatase is localized in the cytoplasm of cells. There is nothing known about its relation to other substances present in the cell. Recent observations[19] indicate that the enzyme is bound to a substance

sedimentable at high speed (approximately 27,000 r. p. m. for 1 hour), but upon autolysis the enzyme is liberated in an active, nonsedimentable form.

SUMMARY

The technic described by Takamatsu and by Gomori for the histochemical demonstration of phosphatase is specific for this enzyme.

Alkaline-phosphatase activity is characteristic of certain cells. Among normal cells, the epithelium of the small intestine and of proximal convoluted tubules, osteoblasts, and endothelium are particularly rich in phosphatase.

Of the tumors studied, phosphatase is present in conspicuous amounts in the malignant osteoblasts of a transmissible chicken sarcoma, and in an osteogenic tumor of the mouse. In the osteogenic chicken sarcoma, phosphatase is particularly abundant about the sites of bony and cartilaginous deposits. In three non-bone-forming strains of transmissible chicken sarcoma, phosphatase is absent.

Human fibro-adenoma of the breast contains much phosphatase, as does lactating breast, while this enzyme is absent in carcinoma of the breast.

In the liver and spleen, endothelial cells alone contain phosphatase. Kupffer's cells and histiocytes do not contain phosphatase.

The presence of phosphatase in normal and malignant osteoblasts supports the view that this enzyme is important in bone formation and may aid in the histological identification of osteogenic cells.

The presence of large amounts of phosphatase in the intestinal epithelium is in accord with the views of Lundsgaard on the relation of glucose absorption to phosphorylation. Similarly, its presence in the proximal convoluted tubules of the kidney reopens the question of a similar mechanism explaining the reabsorption of the glucose secreted by the glomeruli.

Glycine, a known inhibitor of phosphatase action, inhibits also the phosphatase reaction in tissue sections.

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DESCRIPTION OF PLATES

All sections were counterstained with hematoxylin and light green. The granules demonstrating the presence of phosphatase appear brown or black in the color photomicrographs.

PLATE 59

FIG. 1. Mouse kidney. × 600.

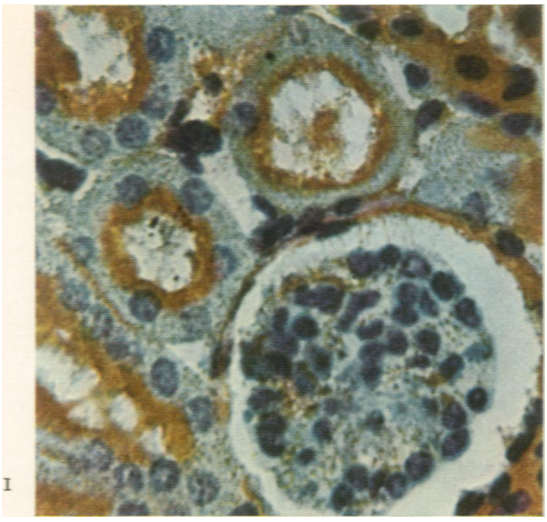
FIG. 2. Mouse kidney, control. × 600.

FIG. 3. Mandible and tooth anlage of a mouse embryo measuring 20 mm. in length. × 98.

FIG. 4. Chicken liver (carmine particles injected ante mortem). × 300.

FIG. 5. Fibro-adenoma of the breast (human). × 98.

FIG. 6. Small intestine of a mouse embryo. × 67.



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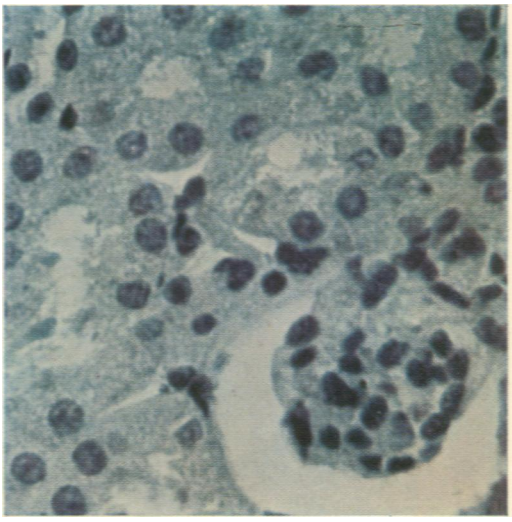

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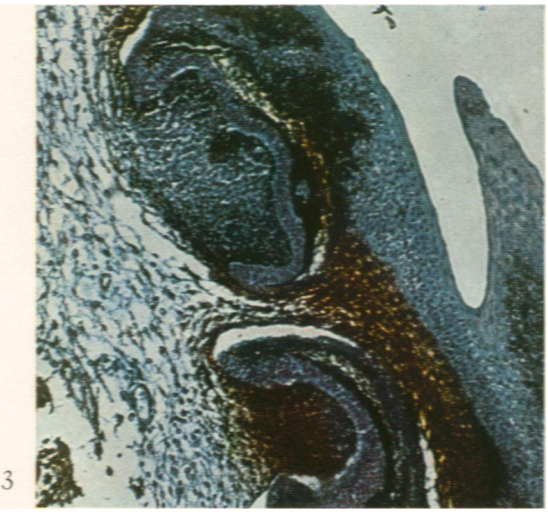

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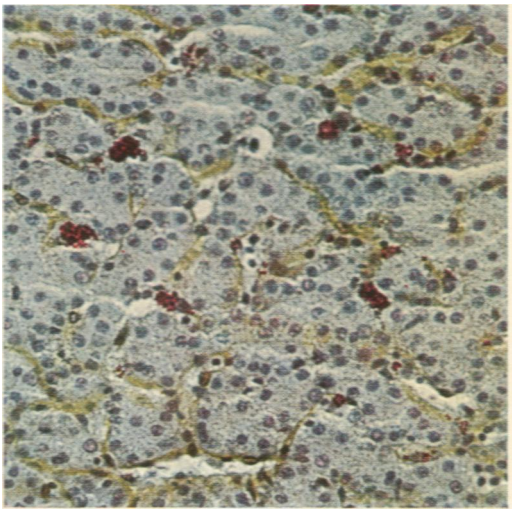

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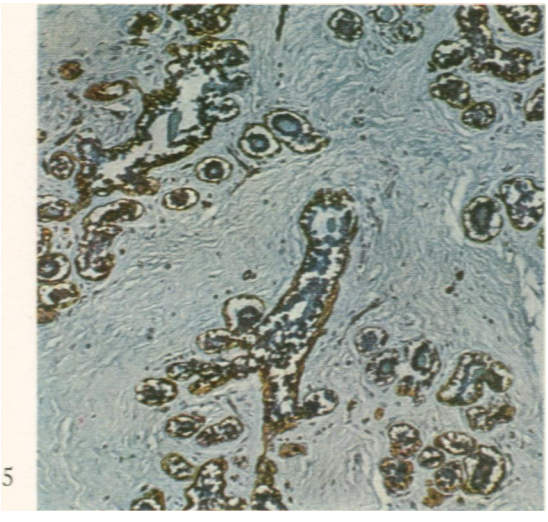

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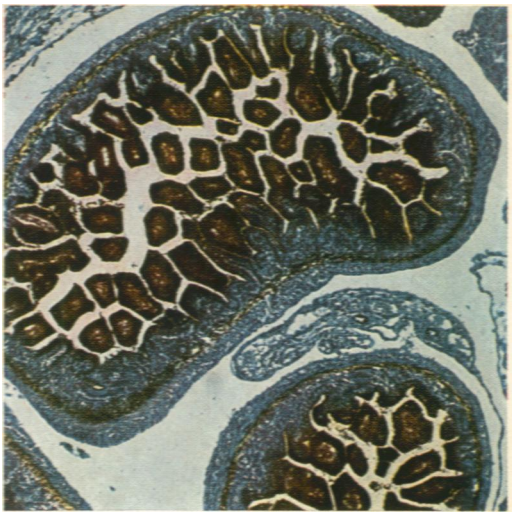

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PLATE 60

FIG. 7. Osteogenic mesenchyme of the anlage of the tail of a mouse embryo measuring 20 mm. in length. × 150.

FIG. 8. Control section for that shown in Figure 7. × 150.

FIG. 9. Hair follicle of the scalp of a mouse embryo. × 180.

FIG. 10. Control section for that shown in Figure 9. × 180.

FIG. 11. Myelinated nerve fiber from chicken. × 350.

FIG. 12. Control section for that shown in Figure 11. × 350.

FIG. 13. Carcinoma of rectum (human). × 500.

FIG. 14. Control section for that shown in Figure 13. × 500.

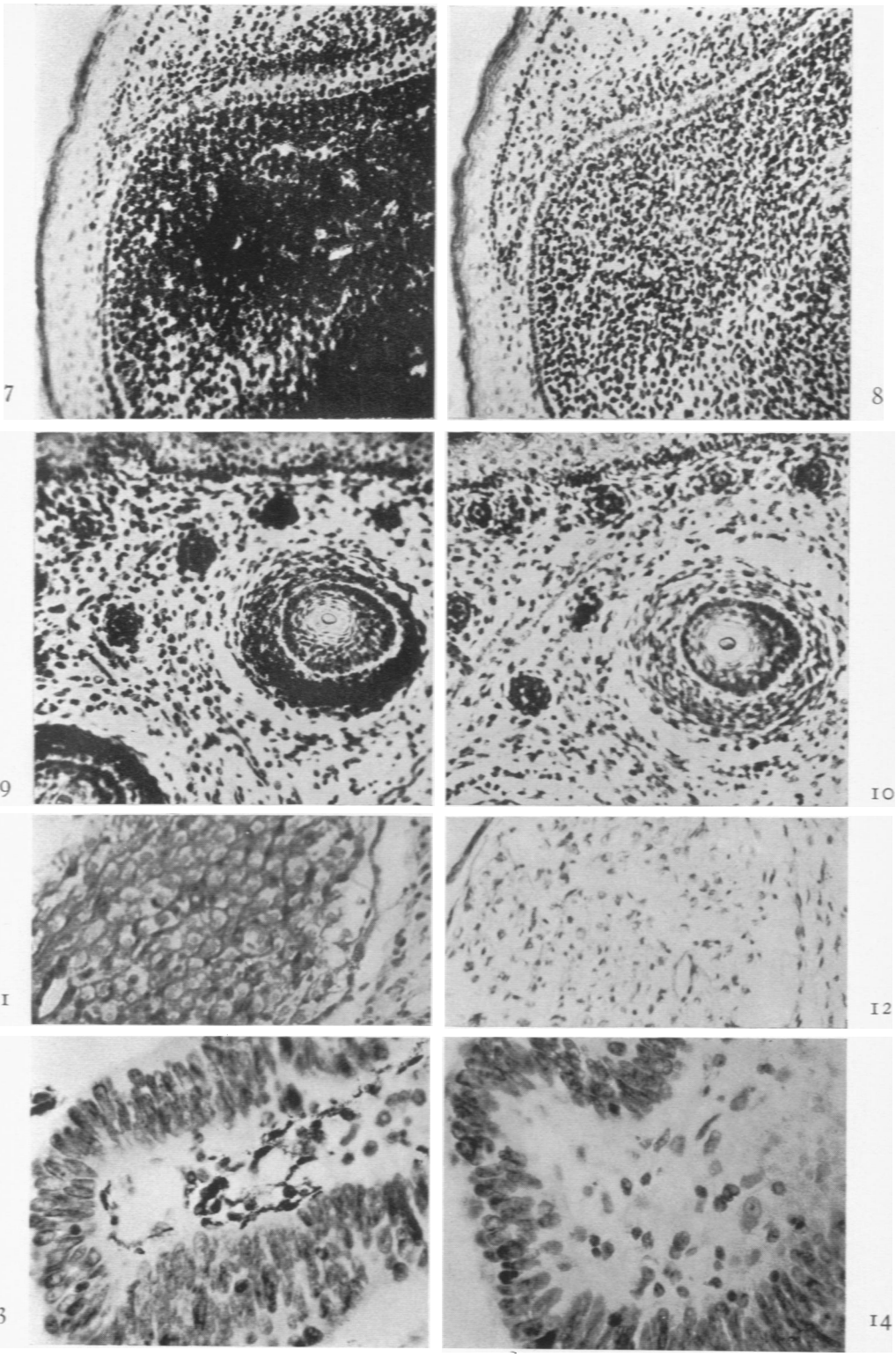
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PLATE 61

FIG. 15. Osteogenic chicken sarcoma. × 380.

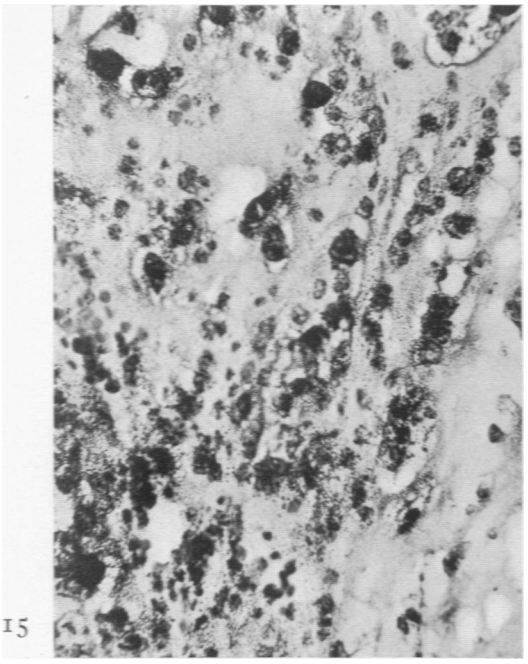
FIG. 16. Control section for that shown in Figure 15. × 300.

FIG. 17. Osteogenic chicken sarcoma. × 200.

FIG. 18. Control section for that shown in Figure 17. × 200.

FIG. 19. Osteogenic fibrosarcoma in a mouse. × 150.

FIG. 20. Control section for that shown in Figure 19. × 150.



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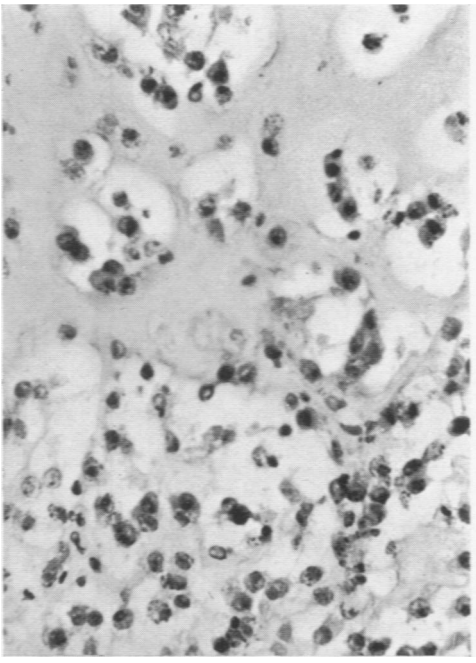

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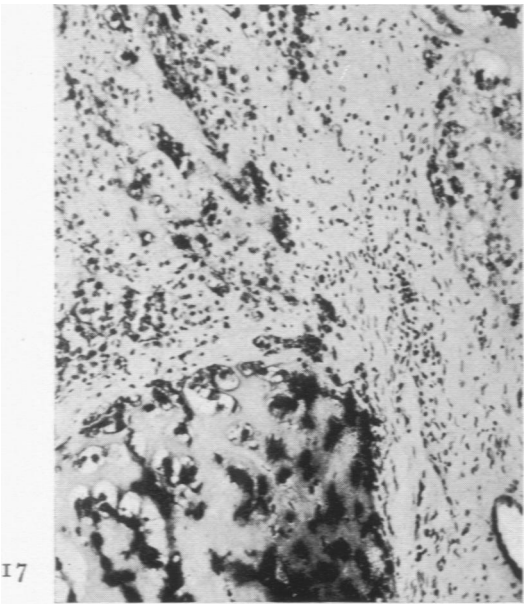

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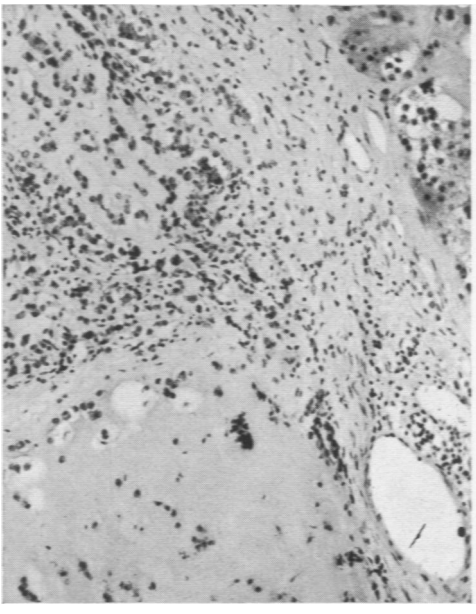

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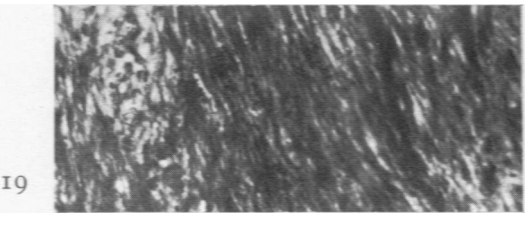

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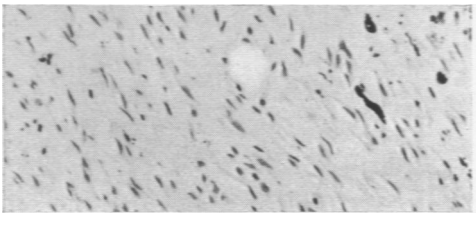

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