body weight and produce a feather syndrome similar to the one here reported. This suggests that ACE may act indirectly on the embryo by depressing the thyroid to the extent of interfering with local feather and general embryonic metabolism.

Summary. 1. The blood picture of 14, 15 and 16 day White Leghorn chick embryos injected into the allantois with aqueous adrenal cortex extract, shows no significant change in the number of lymphocytes but a marked absolute polymorph leukocytosis, 24 hours after injection. The erythrocytes are not affected by the extract.

2. Large individual variation in the lymphocyte count and greater difficulty involved in their identification may have obscured the effect of the hormonal preparation on the lymphocyte count.

3. ACE treatment induced a tendency (not statistically significant) toward weight loss in the 14 day embryos, a significant decrease in the hatchability rate, and an apparent defective feather development. However the blood picture and weight in comparison with the controls was normal after hatching.

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Glycerophosphatases of the Normal and Tumorous Frog Kidney. (17436)

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Lucké[1] described a neoplastic growth in the kidneys of the leopard frog, *Rana pipiens* Schreber (Fig. 1), which was manifested sometimes as a circumscribed adenoma and, at other times, as an adenocarcinoma with considerable infiltrative and destructive activities. The incidence, as reported by Lucké[1] and corroborated by the present studies, may be as much as 2% among both sexes.

It has been demonstrated that the alkaline phosphatase of the kidneys of many animals shows considerable activity.[2,3] Since it is further known that the phosphatase content of an organ is frequently altered under various pathological conditions[4,5] and especially in neoplasia[6] it was felt that studies of the alkaline and acid phosphatase systems might add to our knowledge of the physiology of the normal and pathological frog kidney.

Experimental procedure. Kidneys, both normal and tumorous were treated with a modified Gomori method recommended by Kabat and Furth.[7] Pieces of fresh kidney were fixed in cold acetone at 0°C for 24 hours and embedded in paraffin-Bayberry wax. Sections cut at 5 μ were mounted, deparaffinized and transferred to the buffered sodium glycerophosphate mixture and incubated for 8 hours at 37°C. Control sections were maintained. Some sections were stained in the usual way with Harris' hematoxylin and eosin; others were stained with safranin.

Tissues which were to be compared, namely, normal, neoplastic and calcium controlled sections were incubated and stained simultaneously.

Acid phosphatase was demonstrated by the Wolf, Kabat, and Newman[8] modification of Gomori's original method.[9]

Stewart[10] has pointed out that in cross sec-

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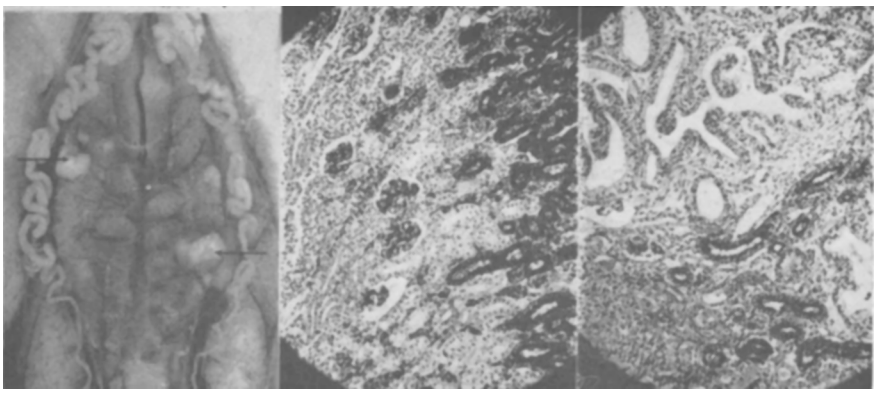

FIG. 1 FIG. 2 FIG. 3

FIG. 1. Bilateral tumor (T34) of a male *Rana pipiens* fixed in Kaiserling fluid. × 10.

FIG. 2. Alkaline phosphatase activity in the normal frog kidney (N6). The proximal convoluted tubules (at the right) show a +++ reaction, the glomeruli a +++ reaction, and the distal tubules (at the left) are negative. Safranin stained. × 110.

FIG. 3. Alkaline phosphatase activity in the tumorous frog kidney (T25). The gland-like tubules of the tumorous (upper) portion show a negative phosphatase reaction, whereas the tubules of the adjacent non-tumorous portion (lower quarter) indicate a reduced enzyme activity from that seen in the normal (Fig. 2). Safranin stained. × 110.

tion the frog's kidney is approximately hemicircular and may conveniently be divided into 3 crescent-shaped segments—the dorsal, intermediate and ventral zones. The proximal convolutions are quite uniformly restricted to the dorsal zone in which is located practically the entire system of collecting tubules. The distal convolutions were mainly found to lie in the ventral zone. In the central zone are grouped the glomeruli along with the transitions of the proximal to distal convolutions. Our results were tabulated using this description of the locations of the various kidney segments.

The intensity of the phosphatase reaction was graded by a system of pulses based on the size, distribution and density of granules in several sections: ++++ maximum reaction, *i.e.*, one in which the phosphatase deposition was so dense that the nuclear outline was just discernible, +++ large amount, ++ moderate, + slight, – negative.

Results. 1. *Sites of alkaline phosphatase activity in normal and tumorous kidney.* Alkaline phosphatase was consistently found to be heavily concentrated at the luminal borders of the proximal tubules and in the collecting ducts. A fair amount of the enzyme was also found in the glomeruli. However, the glomerular capsule and the distal convolutions were always found to be negative (Fig. 2).

There was no difference in the alkaline phosphatase picture between the male and female kidneys, neither did there appear any preferential staining for any portion of the same kidney, *i.e.*, serial sections of normal kidneys excluded the possibility of local variations in phosphatase activity.

The alkaline phosphatase content of tumor-bearing kidneys is quite unlike that of their normal controls. In the first place the neoplastic portions of all affected kidneys showed a negative alkaline phosphatase reaction. Secondly, areas adjacent to the neoplastic growth, although exhibiting the same general distribution of alkaline phosphatase as the normal controls, showed a marked reduction in enzymatic activity (Table I and Fig. 3).

It is noted in Table I, that the nontumorous portions of some tumor-bearing kidneys (36 and 39) showed a slightly greater phosphatase reaction than did the others. Upon examination we found that these tumors were simply solid masses of cells without lumina. This would indicate that the frog renal carcinoma does not exert its influence on the surrounding kidney tissue phosphatase in its earlier stages, *i.e.*, before the tumor has dif-

TABLE I.
Kidney Alkaline Phosphatase.

Frog expt.	Tumor	Non-tumorous portions of kidney: Proximal tubules	Collecting tubules	Glomerulus	Glomerular capsule	Distal tubules
			Normal			
1-14		++++	++++	+++	—	—
			Tumorous			
1-35 37-38, 40	—	+ to ++	+ to ++	+ to ++	—	—
36 and 39	—	+++	+++	++	—	—
Controls (technical)	—	—	—	—	—	—

The intensity of the phosphatase reaction was graded as follows: ++++ maximum, +++ large amount, ++ moderate, + slight, — negative.

ferentiated into the tubular form.

In all cases kidney sections at a distance from the neoplastic area showed a normal phosphatase content.

2. *Sites of acid phosphatase activity in normal and tumorous kidney.* Even when activated by 0.01 M manganous sulfate,[11] all portions of both the normal and neoplastic kidneys gave negative acid phosphatase reactions, except for an occasional positive glomerular reaction due to the phosphatase of the red blood corpuscles. These findings are comparable to those of other investigators[8,9] for normal kidneys.

Discussion. In all cases reported[7,12-14] the proximal tubules always show a strong positive reaction, whereas the distal convolutions are generally negative. The glomeruli are usually entirely negative. Only the glomeruli of the cat kidney[12] shows a positive alkaline phosphatase reaction similar to that which we observed in the frog glomeruli.

The high concentration of alkaline phosphatase in the proximal tubules suggests the possible role of this enzyme in sugar reabsorption since it has been shown that these tubules are the site of glucose reabsorption in the normal kidney.[15] Then too, it is well known that phlorhizin inhibits alkaline glycerophosphatase and also prevents the active reabsorption of glucose in the amphibian kidney.[16] A scheme for such a glucose-reabsorbing mechanism has been presented for certain mammalian kidneys.[14]

Our observation of a complete lack of alkaline phosphatase in the tumorous portions of the affected frog kidney, and its marked depletion in the adjacent renal tissue, indicates that the neoplasm interferes in some way with the metabolism of the surrounding tissue even though there is no microscopic evidence of its infiltration.

It must be emphasized that our results are based solely on the use of sodium-beta-glycerophosphate as the phosphoric ester hydrolyzed by the phosphatase of the kidney. The work of Dempsey and Deane[17] indicates that certain regions of the duodenum contain substrate-specific phosphatases, which suggests the possibility that other phosphatases might very well be demonstrable in the neoplastic amphibian kidney if other substrates and different pH ranges were employed.

Summary. It has been demonstrated for the first time, histochemically, that the normal kidney of the frog, *Rana pipiens,* possesses a strong alkaline glycerophosphatase reaction

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in its proximal tubules, especially at their brush borders, whereas the distal convolutions are negative. The glomeruli show a moderate alkaline reaction, categorizing the frog in a class with the cat (the only other animal thus far investigated that gives this reaction). With regards to the neoplastic kidney, the tumor cells themselves are alkaline phosphatase negative with an accompanying depletion of the enzyme in adjacent non-tumorous portions of the renal tissue.

Both normal and tumorous kidneys are essentially negative for acid glycerophosphatase. The significance of these results is discussed.

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Depressing Effect of Inositol on Serum Cholesterol and Lipid Phosphorus in Diabetics.* (17437)

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The problem of the relation of serum cholesterol levels to human atherosclerosis is much debated. An agent capable of depressing serum cholesterol levels in hypercholesteremic individuals would offer a new approach to its study. There is some evidence to show that inositol is such an agent.

McHenry and Patterson[1] suggested that inositol has a specific effect on cholesterol metabolism; they pointed out that it is an effective lipotropic agent particularly when large amounts of cholesterol are present in the liver. Herrmann[2] found that inositol depressed blood levels of cholesterol and choles-

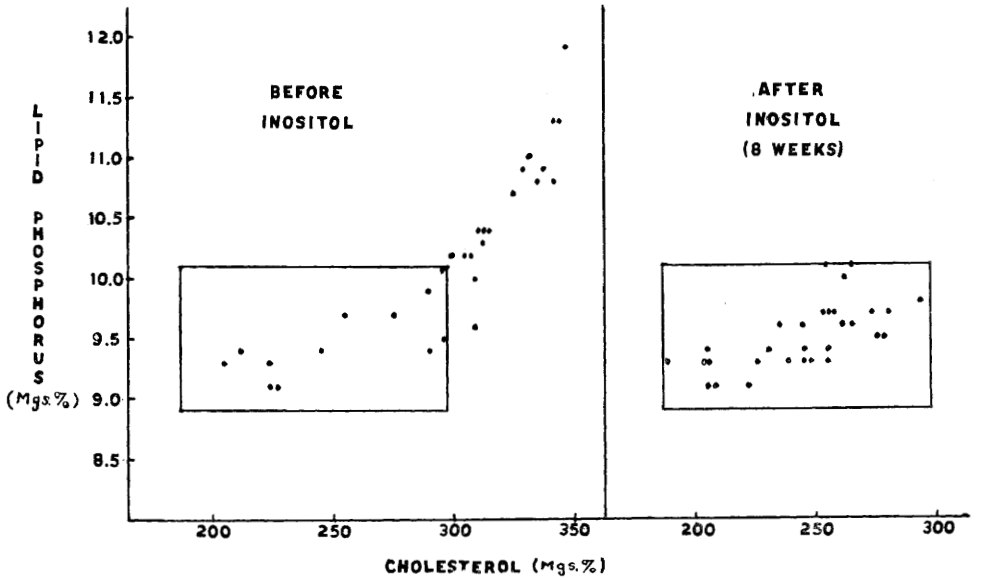

FIG. 1.

Values of serum total cholesterol and lipid phosphorus for 30 diabetic patients before and 8 weeks after inositol. Enclosed areas represent range of normal.

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