of turkeys fed 0.1% 2-amino-5-nitrothiazole for only 2 weeks was greatly retarded whereas nithiazide in a feed concentration of 0.1% had little effect on growth of young turkeys.

The feeding of nithiazide at 0.025% concentration in the ration 1 day before or 3 days after infection with *H. meleagridis* was highly effective in preventing mortality from enterohepatitis. Furthermore, the feeding of 0.05% nithiazide 3 days after infection was completely effective in preventing mortality from infectious enterohepatitis which killed 73% to 83% of untreated controls.

When treatment was not started until 5 days after infection and continued for only 9 days, nithiazide in feed concentration of 0.025% was relatively ineffective. Under these conditions, a feed concentration of 0.05% was effective in preventing mortality during medication; however, the 9-day period of medication was insufficient and relapses occurred in 37% of the poults. When the concentration of nithiazide was increased to 0.1% or 0.2% and the period of feeding was extended to 14 days, mortality from enterohepatitis was effectively controlled even when medication was delayed until the 7th day after infection. The use of 0.01% to 0.04% nithiazide in the water, beginning 3 days or 7 days after infection, effectively prevented mortality from enterohepatitis. Mortality from enterohepatitis was also prevented with 0.04% nithiazide administered in the water 10 days after infection.

Summary. 1. The data presented show that nithiazide [1-ethyl-3-(5-nitro-2-thiazolyl) urea] was more potent and less toxic than 2-amino-5-nitrothiazole when fed to turkeys with enterohepatitis. 2. Nithiazide was most effective when administered prior to infection or beginning 3 to 7 days after infection. It prevented mortality from enterohepatitis produced either by oral exposure to cecal worm eggs or by rectal inoculation of *Histomonas meleagridis* cultures. Nithiazide may be administered in the feed or water and is equally effective in either form. 3. The therapeutic feeding of rations containing 0.05% nithiazide, starting 3 days after infection, prevented mortality from infectious enterohepatitis which was lethal for 73% to 83% of the untreated control turkeys.

1. Cuckler, A. C., Malanga, C. M., Basso, A. J., O'Neill, R. C., and Pfister, K., 3rd, 1956. Submitted for publication.

2. Balamuth, W., and Sandza, J. G., PROC. SOC. EXP. BIOL. AND MED., 1944, v67, 161.

3. Waletzky, E., Clark, J. H., and Marson, H. W., *Science*, 1950, v111, 720.

4. Cuckler, A. C., Porter, C. C., and Ott, W. H., *Poultry Sci.*, in press.

Received June 4, 1956. P.S.E.B.M., 1956, v92.

Inhibition of Experimental Nephrocalcinosis by Hypophysectomy.* (22521)

H. SELYE, P. BOIS, AND J. VENTURA.

Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada.

Excessive administration of various mono- or dibasic phosphate solutions causes extensive renal calcification in rats. In unadrenalectomized animals (sensitized for the nephrotoxic action of mineralocorticoids by unilateral nephrectomy), this nephrocalcinosis is aggravated by desoxycorticosterone acetate (DOCA) and inhibited by cortisol acetate (COLA)(1-3). From this it was concluded that the corticoids exert an important regulating influence upon the ability of the kidney to handle an excess of phosphate. Yet nephrocalcinosis can be induced by phosphate even after complete adrenalectomy(4). The question now arose whether pituitary hormones are necessary for induction of nephrocalcinosis by combined treatment with DOCA and an excess of phosphate. This appeared of interest, because nephrocalcinosis is so markedly enhanced by DOCA, a steroid whose other nephrotoxic actions (the produc-

* This work was performed with the aid of a Consolidated Grant from National Research Council of Canada.

TABLE I. Inhibition of Experimental Nephrocalcinosis by Hypophysectomy.

Group	No. of rats	Treatment	Renal lesions		
			Nephrocalcinosis	Hyalin casts	Cortical atrophy
1	10	Intact, $Na_2H\ PO_4$ p.o.	Severe (only in zona intermedia)	None	None
2	17	Hyp-X, " "	None	"	"
3	10	Intact, NaH_2PO_4 s.c.	Severe (throughout cortex)	Numerous	Intense
4	17	Hyp-X, " "	Trace	None	"

tion of malignant nephrosclerosis and proteinuria) are virtually abolished by hypophysectomy(5,6).

Materials and methods. Fifty-four female Sprague-Dawley rats, having an average initial body-weight of 101 g (range: 92-111 g), were subdivided into 4 groups and treated as shown in Table I. The hypophysectomies in Groups 2 and 4 were performed through the parapharyngeal approach. On the day of the operation and on the following day all animals received 1 mg of COLA† subcutaneously, while 100 μg of DOCA† per day were administered in the same manner to the animals of all 4 groups throughout the experiment. The glucocorticoid was given during the immediate post-operative period only in order to raise the resistance of the hypophysectomized rats, but for uniformity all groups were similarly treated. On the other hand, since previous experiments had shown that glucorticoids inhibit nephrocalcinosis, only DOCA (which facilitates the production of this change) was administered during the rest of the observation period.

In Groups 1 and 2 an aqueous 1% Na_2HPO_4 solution was made available *ad lib,* as a drinking fluid. This salt is more effective in producing nephrocalcinosis than NaH_2PO_4, but also more irritating to tissues and, therefore, it does not lend itself as well to subcutaneous administration. Groups 3 and 4 were kept on ordinary tap water and received NaH_2PO_4 in the form of a 5% solution subcutaneously twice daily, the individual dose being gradually raised from 1 to 5 ml during the first 5 days and then maintained on the same level. Normally, it is impossible to administer such high concentrations and volumes of NaH_2PO_4, because multiple subcutaneous hemorrhages and skin-necroses ensue. We therefore used the "granuloma-pouch" technic(7), which makes it possible to give highly irritating solutions parenterally without causing significant local tissue-damage. For the purpose of the present experiment 25 ml of air was injected subcutaneously under the shaved skin of the back, this being immediately followed by the introduction of 1 ml of 5% NaH_2PO_4 into the air sac thus formed. The resulting local inflammation transforms the lining of the air sac into a thin but resistant granuloma which withstands the increasing concentrations of phosphate solution and yet permits salt absorption into the bloodstream. In this manner we could inject a total of 10 ml of 5% NaH_2PO_4 daily between the fifth and the tenth day after hypophysectomy. The experiment was terminated on the 11th day by killing the animals with chloroform. Immediately after autopsy one kidney was fixed in neutral formalin (for the histochemical demonstration of calcium deposits with Kossa's silver nitrate technic) and the other in Susa solution (for subsequent staining with the Periodic Acid Schiff, or "PAS," stain, to demonstrate hyalin, fibrinoid material).

Results. Upon naked-eye inspection of the cut surface, a chalky line of *calcium deposition* was clearly visible between the cortex and medulla of the kidney, in the "zona intermedia" [terminology of Heidenhain(8)], in all the rats of Group 1. The kidneys in Group 3 showed a more diffuse white discoloration—especially of their surface underneath the capsule—but no obvious change could be detected in Groups 2 and 4.

Table I lists the mean degree of nephrocalcinosis (assessed in a scale of 0 to

† The authors are indebted to Pfizer Laboratories for generous supplies of cortisol acetate ("Cortril") and to Schering Corp. for desoxycorticosterone acetate ("Cortate").

+++), as judged by microscopic observation of tissues stained with the Kossa technic. It should be added that even histochemically virtually no calcification could be detected in the outer cortex of the animals of Group 1, although marked calcification occurred in the zona intermedia of the kidney, where it stimulated the secondary formation of granulomatissue with giant cells. In Group 3, calcium deposits were seen in the proximal convoluted

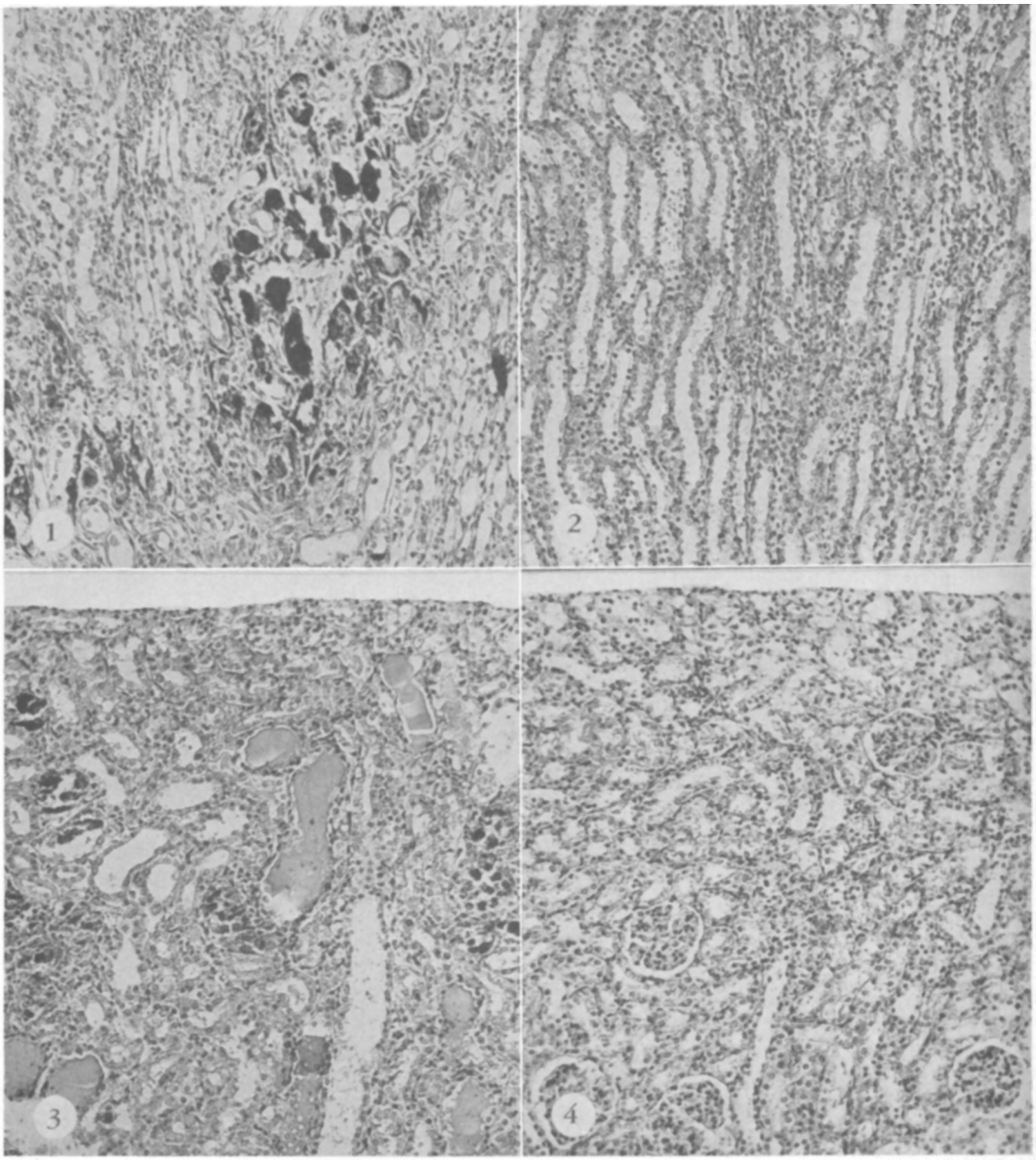

FIG. 1. Extensive calcium deposition with reactive inflammation and giant-cell formation in the "zona intermedia" of the kidney of a rat from Group 1 (Kossa stain × 100).

FIG. 2. "Zona intermedia" of the kidney of a rat from Group 2. Calcium deposition was completely prevented by hypophysectomy (Kossa stain × 100).

FIG. 3. Subcapsular renal cortex of an intact rat from Group 3. Diffuse calcium deposition and hyalin-cast formation (Kossa stain × 100).

FIG. 4. Subcapsular renal cortex of a rat from Group 4. Hypophysectomy completely prevented both calcium deposition and hyalin-cast formation (Kossa stain × 100).

tubules throughout the entire width of the renal cortex; they were particularly numerous in the subcapsular region. On the other hand, in the hypophysectomized rats of Group 2, which drank phosphate solution, virtually no calcium deposits could be demonstrated, except for one rat, in which hypophysectomy proved to be incomplete. Among the hypophysectomized rats of Group 4, given phosphate solution subcutaneously, only 6 survived until the end of the experiment. Two of these showed very slight degrees of diffuse nephrocalcinosis, although no remnants of pituitary tissue could be detected at autopsy. All 15 remaining animals in this group (whether or not they survived until termination of the experiment) were free of detectable calcium deposits (Fig. 1-4).

Hyalin casts and precipitated proteinaceous material were plentiful in the renal tubules of the rats of Group 3, but were absent in all other groups.

A very unusual change in the structure of the kidneys was found in Groups 3 and 4. Here a marked *selective atrophy of the outer renal cortex* had developed under the influence of the subcutaneously administered phosphate. The epithelial cells of the proximal convoluted tubules became unusually small and their brush-border assumed an irregular lumpy appearance or disappeared completely. The cytoplasm tended to lose its staining ability and, in many cases, contained fine, hyalin, PAS-positive droplets. The nuclei in the proximal tubules did not appear to participate in this atrophy so that, in many nephrons, the entire lining looked somewhat like a "macula densa," consisting of aggregates of many nuclei, with but little, poorly staining, cytoplasm. The basement membranes around the atrophic tubules were thickened and strongly PAS-positive. Curiously, the capillary vessels throughout this involuting outer cortex were greatly dilated (Fig. 5-9).

Discussion. It is evident that, under our experimental conditions, in intact rats treated with DOCA the administration of phosphate in the drinking water produced the usual nephrocalcinosis, limited to the zona intermedia of the kidney, while subcutaneously introduced phosphate caused a more diffuse calcification of all parts of the kidney and especially of its subcapsular outer cortex. It is true that, in this particular experiment, Na_2HPO_4 was given *per os* and NaH_2PO_4 subcutaneously, but this could not account for the observed difference. Our earlier experiments on nephrocalcinosis had repeatedly shown that both the mono- and the dibasic phosphates cause calcification predominantly in the zona intermedia of the kidney (without cortical atrophy), when administered by mouth, and in the outer cortex (with cortical atrophy), when injected into the granuloma-pouch subcutaneously. Perhaps, in the latter event, the sudden flooding of the organism with large concentrations of phosphate is responsible for the diffuse renal damage, while when only moderate amounts are taken in gradually (*per os*), the nephron-segments specifically designed for the handling of excess phosphate are more selectively affected. In any event, it is clear that hypophysectomy greatly inhibits—and indeed usually abolishes—the nephrocalcinosis due to excess phosphate, irrespective of whether the subcutaneous or the oral route of administration is employed. This cannot be due merely to a decreased phosphate absorption. Daily measurement of the fluid-intake in Groups 1 and 2 showed it to be essentially the same in the intact and the hypophysectomized rats (usually about 35 ml/day). Furthermore, in Groups 3 and 4, at autopsy only an average of 5-8.8 ml of phosphate solution could be recovered from the granuloma-pouches (which corresponds to less than the amount injected in a single day), and chemical analyses invariably revealed a dilution, rather than a concentration, of phosphate in solutions thus introduced into subcutaneous air sacs.

In view of the fact that the hyalinosis of the kidney normally produced by DOCA-overdosage is prevented by hypophysectomy (5,6), it is interesting that the hyalin-cast formation, which resulted from the subcutaneous injection of phosphate, was likewise prevented in the absence of the pituitary (*cf.* Groups 3 and 4). On the other hand, the in-

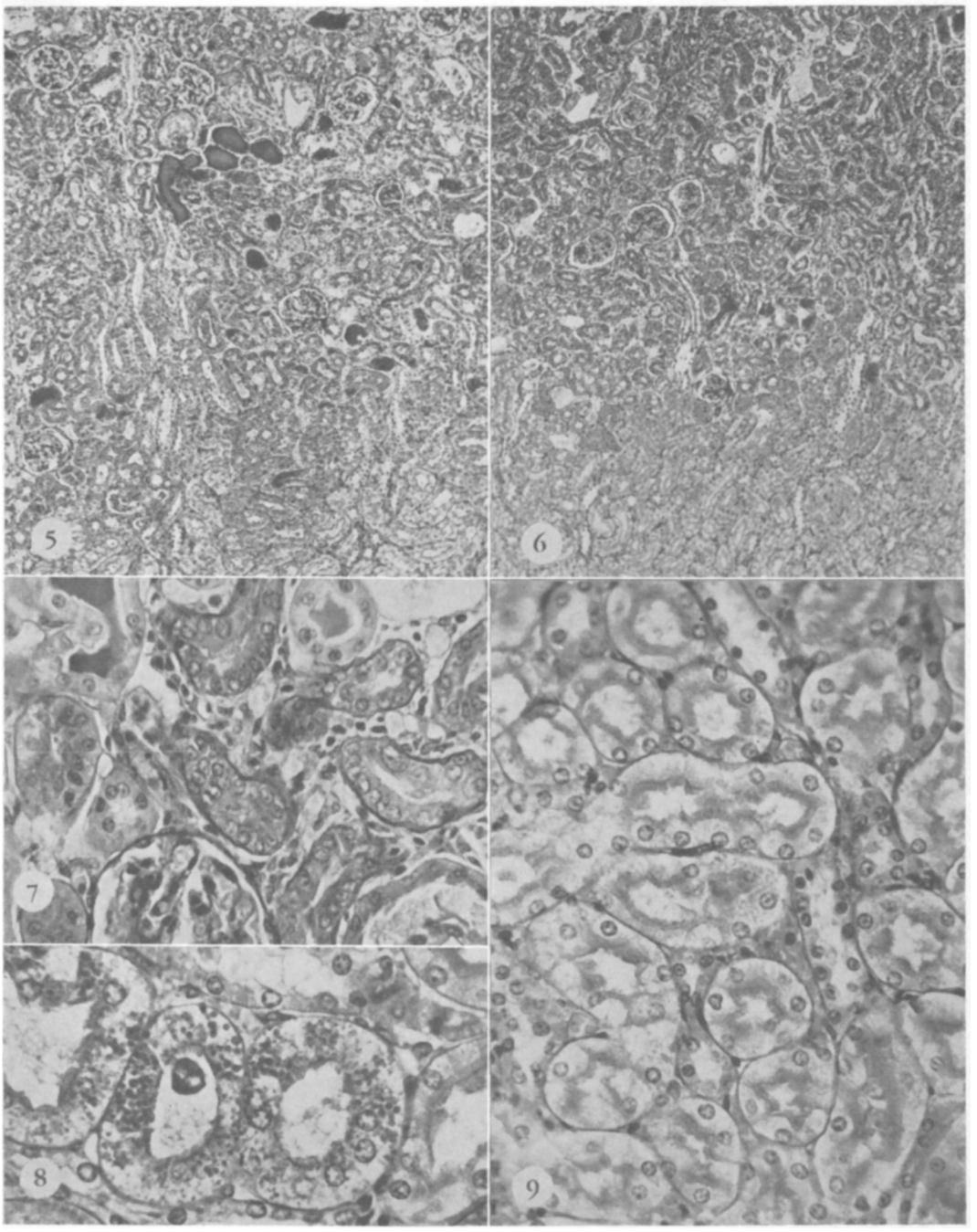

FIG. 5. Renal cortex of a rat from Group 3. The outer region, which contains the glomeruli, has undergone marked atrophy and disorganization of its pattern, due to connective-tissue proliferation. Many tubules are obstructed by dense casts, which are intensely stained with the Periodic Acid Schiff reagent (PAS stain × 75).

FIG. 6. Renal cortex of a rat from Group 4. Hypophysectomy has completely prevented hyalin-cast formation, but not the induction of cortical atrophy (PAS stain × 75).

FIG. 7. Representative field from the kidney shown in Fig. 5. Note atrophy of the tubules, thickening of basement membranes and proliferation of connective tissue, which appears to compress the tubules. Because of selective involution of the cytoplasm and virtual disappear-

ance of cell borders, "macula-densa-like" apparently syncytial structures arise, in which the nuclei are often actually enlarged and, in some places, deformed (PAS stain × 400).

FIG. 8. Higher magnification of another region of the kidney shown in Fig. 5. Here the PAS-positive, small, hyalin granules within the tubular epithelium are clearly visible. Note also that the brush-border tends to accumulate in clumps or is almost completely shed off (PAS stain × 720).

FIG. 9. Proximal convoluted tubules from the inner, not atrophic, stratum of the cortex shown in Fig. 5, 7 and 8. Unlike in the region shown in Fig. 7 (which is photographed at the same magnification), the tubules in this stratum (chiefly composed of spiral segments) are normal, the brush-border is well developed, the cytoplasm abundant and the stroma hardly detectable (PAS stain × 400).

tense and rather selective atrophy of the outer cortex was equally evident in the intact and in the hypophysectomized rats receiving subcutaneous NaH_2PO_4. Presumably, this change, unlike the nephrocalcinosis and the hyalin-cast formation, is not significantly dependent upon any of the pituitary hormones.

Summary. 1) Experiments on rats indicate that oral administration of excess sodium phosphate causes intense calcification in the "zona intermedia" of the kidney in intact but not in hypophysectomized rats. 2) The "granuloma-pouch" technic permits the subcutaneous administration of as much as 10 ml of 5% NaH_2PO_4 per rat daily. (Normally, this amount could not be given subcutaneously because of its topical necrotizing effect.) When so administered, the phosphate solution causes a generalized nephrocalcinosis throughout the kidney and this is also virtually abolished by hypophysectomy. Apparently, hypophyseal hormones play an important conditioning rôle in the pathogenesis of this kind of nephrocalcinosis. 3) Repeated injections of hypertonic phosphate solutions, into subcutaneous granuloma-pouches, cause the formation of numerous hyalin casts and protein-precipitates in the kidney and this change was likewise totally abolished by hypophysectomy. When thus administered, phosphate solutions can also induce a pronounced and highly selective atrophy of the entire outer renal cortex, but this change is essentially of the same severity in the presence and in the absence of the hypophysis.

1. Selye, H., Mintzberg, J., and Rowley, E. M., *J. Pharmacol. and Exp. Therap.*, 1945, v85, 42.

2. Friedman, S. M., Friedman, C. L., and Polley, J. R., *Am. J. Physiol.*, 1948, v153, 226.

3. Selye, H., and Bois, P., *ibid.*, in press.

4. ———, *Acta Endocrinol.*, in press.

5. Selye H., *J. Clin. Endocrinol.*, 1946, v6, 117.

6. Salgado, E., and Selye, H., *Rev. Canad. Biol.*, 1953, v12, 89.

7. Selye, H., *J.A.M.A.*, 1953, v152, 1207.

8. Heidenhain, M., *Synthetische Morphologie der Niere des Menschen.*, 1937, Brill, Leiden.

Received June 5, 1956. P.S.E.B.M., 1956, v92.

Mechanism of Heparin Protection Against a Histamine Releasor (48/80).* (22522)

R. D. HIGGINBOTHAM AND THOMAS F. DOUGHERTY.†
Department of Anatomy, University of Utah College of Medicine.

It was previously reported that fibroblasts ingest a variety of metachromatic substances which are deposited in their cytoplasm in granular forms(1,2). Since the recognition of mast cells is dependent primarily on a specific (metachromatic) staining of their cytoplasmic granules, rather than any definitive shape or appearance of the cell(3), the granulated fibroblasts are analogous to tissue mast

* Research supported by grants from the Department of the Army, No. DA-49-007-MD-130, and from Smith, Kline and French Research Foundation.

† Authors wish to acknowledge the technical assistance of Miss Martha Chamberlain, and express their appreciation to Dr. H. Wayne Gustaveson for the statistical analyses in this investigation.