Effect of Corticoids on the Resistance of the Kidney to an Excess of Phosphates[1]

HANS SELYE AND PIERRE BOIS

From the Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

ABSTRACT

In unilaterally nephrectomized rats, the substitution of 1.2% NaH_2PO_4 for drinking water rapidly produced marked calcium deposition in the pars intermedia of the kidney. This effect of the phosphate solution is enhanced by concurrent treatment with desoxycorticosterone acetate (DCA) and inhibited by cortisol acetate (COLA). The possible rôle of corticoids in the production of clinical nephrocalcinosis and renal calculus formation is briefly discussed.

EARLIER experiments (1, 2) had shown that oral administration of various mono- or dibasic phosphate solutions causes extensive renal calcification in rats. This change is particularly severe in animals simultaneously treated with high doses of desoxycorticosterone acetate (DCA), after conditioning for the nephrosclerotic actions of this mineralocorticoid by unilateral nephrectomy and a high sodium-intake. In view of the manifold, and frequently antagonistic, interactions between mineralo- and glucocorticoids, the question arose whether cortisol acetate (COLA) would influence this singular effect of DCA on the ability of the kidney to handle a great excess of inorganic phosphate.

METHODS

The right kidney was removed, through a subcostal incision, in 40 female Sprague-Dawley rats, having an initial body-weight of 103 gm. (range: 95–110 gm). Next day, the animals were subdivided into four equal groups of 10 rats each, and treated with 2 mg/day of DCA and/or COLA microcrystals (administered subcutaneously in 0.2 ml aqueous vehicle), as indicated in table 1. At the same time, a 1.2% aqueous solution of NaH_2PO_4 was substituted for drinking water. Only this solution and 'Purina fox chow' were made available during the rest of the period of observation. The experiment was terminated on the 12th day by killing the animals with chloroform.

The adrenals, kidney, heart, pancreas, stomach and lung were removed for histologic study, half of the specimens being fixed in Susa solution (for subsequent staining with hematoxylin and eosin) and half in neutral formalin (for the histochemical demonstration of calcium deposits with Kossa's silver nitrate technique).

Received for publication April 13, 1956.

[1] These investigations were supported by a grant from the Gustavus and Louise Pfeiffer Research Foundation.

RESULTS

Macroscopic inspection of the cut surface of the kidney revealed a chalky white deposit, sharply limited to the region designated by Peter (3) as the "inner stripe of the outer zone of the medulla," and by Heidenhain (4) as "pars intermedia." This stratum consists exclusively of unbranching collecting tubules and loops of Henle. The whitish areas were most conspicuous in the rats receiving DCA alone (*group 2*). In some individuals, the white spots were equally distributed throughout the pars intermedia while, in others, they were more or less selectively localized along the outer and/or inner edge of this zone. Upon naked-eye inspection, no calcifications were seen outside the pars intermedia in the kidney, nor in any other organ.

Table 1 lists the mean degree of tissue-calcification in the kidney, using a scale of 0 to ++++, as judged by microscopic observation of tissues stained with the Kossa technique. Histologically, no calcifications could be detected in the stomach, lung, pancreas or adrenals of any animal, irrespective of the hormone-treatment. The heart was also notably free of histochemically detectable calcium deposits, except for a few dust-like granules in the occasional inflammatory gran-

TABLE 1. EFFECT OF CORTICOIDS ON RENAL CALCIFICATION IN PHOSPHATE-TREATED RATS

Group No.	Hormone Treatment	Gain in Body Weight (%)	Fluid Intake, ml*	Calcification in Kidney
1	None	+35	32	++
2	DCA	+ 7	31	++++
3	COLA	−17	31	+
4	DCA + COLA	−18	41	++

* Mean of last 3 days.

ulomatous foci, which had developed under the influence of DCA in two rats.

Table 1 shows that the substitution of the phosphate solution for ordinary drinking water did not considerably interfere with the growth-rate of the rats receiving no hormone treatment (*group 1*), as judged by their increase in body-weight. DCA depressed growth slightly, while COLA, given alone or in combination with DCA, produced a marked loss in body-weight, as could be expected in view of the high dosage at which this anti-anabolic hormone was administered.

The fluid-intake fell roughly within normal limits in all four groups: evidently, variations in the degree of renal calcification can therefore not be ascribed to major differences in the amount of phosphate solution ingested.

Calcification in the kidney was by far most pronounced in *group 2* (treated with DCA alone) and least pronounced in *group 3* (receiving only COLA). It was intermediate and about equally severe in *group 1* (the controls) and in *group 4* (rats receiving both DCA and COLA). Histochemically demonstrable calcium deposition cannot be expressed in a strictly quantitative manner, but individual variation was very slight and there can be no doubt that DCA enhanced and COLA inhibited renal calcification, as compared with the untreated controls and the rats which received both types of steroids.

Careful histologic study of the kidneys revealed furthermore that the calcium deposits were, in fact, almost exclusively localized in the pars intermedia, as suggested by macroscopic inspection. Still, occasional calcified cylinders were also seen in the papilla and, quite exceptionally, in convoluted tubules of the cortex. Since the calcium deposits damaged the epithelium, it was usually impossible to identify the affected segment of the nephron. However, judged by very early stages of the process, it appears that calcium deposition occurs with great selectivity in the thick portions of Henle's loops. After the epithelium is severely damaged by incrustation with calcium, polynuclear syncytia of the 'foreign-body giant-cell' type are formed around the calcified, chalky debris (cf. fig. 1).

DISCUSSION

We have seen pathologic calcium deposition in the kidneys of rats as part of the general syndrome of metastatic calcification, following the administration of irradiated ergosterol preparations (5) and after treatment with large doses of parathyroid extract (6), while Rabl (7) made similar observations in mice, alternately fed with acid and alkaline diets. In all these cases, however, the renal calcification was only part of a generalized calcinosis, affecting many organs, particularly the stomach, lung and heart. It is noteworthy therefore that a rather selective calcification in the pars intermedia of the kidney can be so rapidly produced by the simple device of substituting a dilute phosphate solution for drinking water.

In many respects glucocorticoids, such as COLA, and mineralocorticoids, such as DCA, antagonize each other. This is so, for instance, as regards their influence upon inflammation, lymphatic-tissue development and body-weight, under the usual experimental conditions. Curiously, in rats sensitized by unilateral nephrectomy and an excess of NaCl, the nephrosclerotic action of DCA is further aggravated by COLA (8). The fact that the ability of the kidney to handle an excess of phosphates is diminished by DCA and improved by COLA shows that, even in kidney function, the two types of steroids can be mutually antagonistic, at least in some respects.

Clinical observations have shown that renal calcification may occur in patients as a consequence of derangements in chloride metabolism, due to excessive vomiting or diarrhea (9). Nephrocalcinosis has also been induced experimentally in cats, by ligature of the pylorus combined with the administration of dextrose

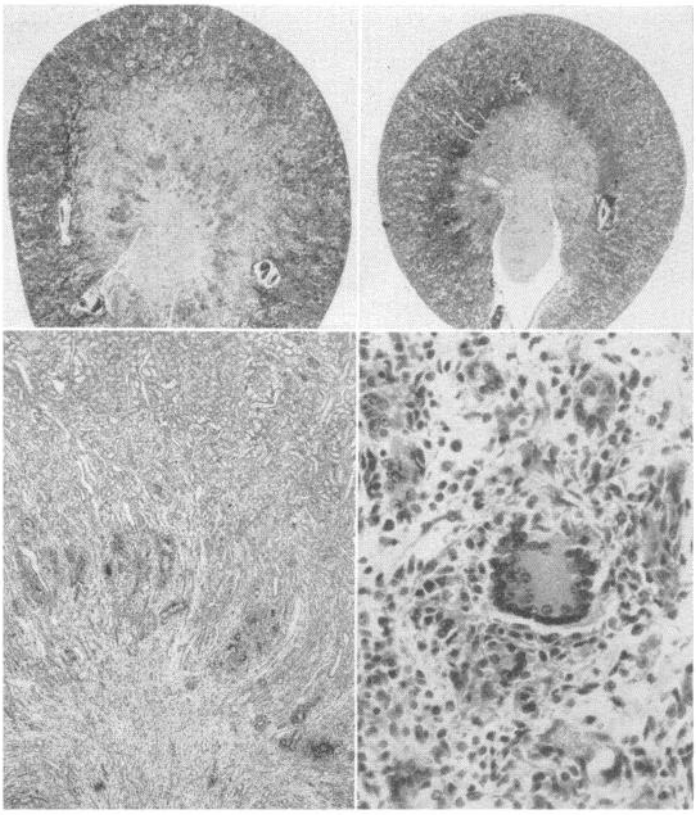

FIG. 1. Effect of corticoids upon phosphate-induced nephrocalcinosis. *Top left:* general view of cross-section through the kidney of a rat treated with DCA alone. Note the general distribution of the renal lesions caused by calcium deposition and secondary granuloma formation in the zona intermedia. *Top right:* kidney of a rat from *group 4* which, in addition to the same dose of DCA, also received COLA. The essentially normal zona intermedia is clearly visible at this low magnification as a gray semilune between the lighter papilla and the darker cortex. *Bottom left:* higher magnification of pars intermedia region from the kidney just above it. Here the calcified cylinders and the granulomatous nodules which surround them are more clearly discernible. *Bottom right:* polynuclear foreign-body giant cells apparently derived from tubular epithelium in the pars intermedia, as seen under higher magnification.

and sodium lactate to induce a chloride loss (10), as well as in rats, by merely keeping them on chloride-deficient diets (11). It had been suggested that here hypochloremic alkalosis is the immediate cause of calcium precipitation. Yet, intense nephrocalcinosis may also occur, due to hyperchloremic acidosis (12). Thus the physico-chemical basis of this change is still far from being clearly understood, but evidently derangements in chloride metabolism and in acid-base equilibrium play an important role. Mineralocorticoids influence both these factors through their effects upon sodium, chloride and potassium metabolism. It is highly probable, therefore, that these steroids secondarily also affect phosphate and calcium metabolism, although these latter actions have so far received comparatively little attention. Still, it may be significant that renal calcifications have also been noted in aldosteronism (13). Preliminary experiments (to be reported later) suggest that, in adrenalectomized rats, under otherwise similar conditions, the DCA-COLA antagonism, as regards renal calcification, is still more clear cut.

We feel that, in the nephrocalcinosis due to excess sodium phosphate, we have a simple function test which reveals the capacity of the kidney to deal with an excess of phosphate. It is already evident that the corticoids exert an important regulating influence upon this renal function. Further studies along these lines would be desirable, especially since these observations suggest that corticoids may also be involved in the formation of calcified renal calculi, which largely depends upon the solubility of calcium salts in the urinary tract.

The authors wish to thank the Schering Corporation for generous supplies of desoxycorticosterone acetate ('Cortate') and the Pfizer Laboratories for cortisol acetate ('Cortril').

REFERENCES

1. SELYE, H., J. MINTZBERG AND E. M. ROWLEY. *J. Pharmacol. & Exper. Therap.* 85: 42, 1945.
2. FRIEDMAN, S. M., C. L. FRIEDMAN AND J. R. POLLEY. *Am. J. Physiol.* 153: 226, 1948.

3. PETER, K. *Untersuchungen über Bau und Entwicklung der Niers*, vol. 1, VI, *Der Aufbau der Nierensubstanz*, Jena: Fischer, 1927, pp. 565–648.
4. HEIDENHAIN, M. *Synthetische Morphologie der Niere des Menschen.* Leiden: Brill, 1937.
5. SELYE, H. *Krankheitsforschung* 7: 289, 1928.
6. SELYE, H. *Endocrinology* 16: 547, 1932.
7. RABL, C. R. H. *Virchows Arch. path. Anat.* 245: 542, 1923.
8. SELYE, H. *Stress*. Montreal: Acta Inc., 1950.
9. ROHLAND, R. *Klin. Wchnschr.* 15: 825, 1936.
10. KERPEL-FRONIUS, E. AND R. MARTYN. *Klin Wchnschr.* 19: 440, 1940.
11. LOWENHAUPT, E. AND D. M. GREENBERG. *Arch. Path.* 42: 49, 1946.
12. ENGEL, W. J. *J. A. M. A.* 145: 288, 1951.
13. CONN, J. W. In: *Fifth Annual Report on Stress*, edited by H. SELYE and G. HEUSER. Montreal: Acta Inc., 1955/56.