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KIDNEY AND BLADDER CALCULI IN SPONTANEOUSLY HYPERTENSIVE RATS

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Summary.—Naturally occurring kidney stones are rare in animals. The Japanese strains of spontaneously hypertensive rats (SHR) are normotensive at birth but develop high blood pressure, hyperglycaemia and hyperlipidaemia as they mature. The SHR strain is prone to develop kidney stones. A unique sub-strain of SHR has been developed in which some animals develop hypothalamic obesity concomitantly with their rising blood pressure, *i.e.* Obese/SHR. The Obese/SHR characteristically develop microscopic kidney stones which become detached at an early stage of formation, migrate to the bladder, and grow by concretion into huge, rounded calculi. The stone nidus starts as a subepithelial cyst-like focus containing oedema, colloidal acidic mucoprotein, and red and white blood cells suspended on a delicate network of fibrils. The nidi grow by concretion of an admixture of calcium and acidic protein in a lamellar arrangement. The disparate morphogenesis and anatomic location of kidney stones in Obese as opposed to non-obese/SHR suggest that calculus formation may be governed by specific differences in genetic programming. The incidence of kidney stones parallels the severity and chronicity of the hypertension in SHR, non-obese and Obese/SHR, and the Cushingoid habitus in the Obese/SHR.

THE JAPANESE have developed a strain of rats which are normotensive at birth but their blood pressure begins to rise acutely at 4–5 weeks of age, reaching 190–240 mmHg by the time they are 4 months old (Okamoto and Aoki, 1963). These spontaneously hypertensive rats (SHR) develop peripheral vasoconstriction and myocardial haemodynamic changes which mimic essential hypertension in humans. Despite intensive research into the pathogenesis of this spontaneous hypertension, the central aetiology remains a mystery. In addition to the popular SHR sub-strain, we have a unique sub-strain variant, *i.e.* the obese SHR (Obese/SHR) (Wexler, Iams and McMurtry, 1980). In parallel with their rapidly rising blood pressure, these rats develop a voracious appetite at 4–5 weeks of age and they develop massive obesity, attaining weights of 700–900 g. In this report, we describe our finding of a high incidence of kidney stones in the various sub-strains of SHR. The incidence and severity of the naturally occurring kidney stones parallels the severity and chronicity of the spontaneous hypertension in SHR.

MATERIALS AND METHODS

The regular sub-strain of SHR, the non-obese SHR, and the Obese/SHR were kindly provided by Dr Carl T. Hansen, Animal Genetics Division, National Institutes of Health, Bethesda, Maryland. From this original stock, we have succeeded in raising several generations of SHR and Obese/SHR by inbreeding. The genes for the expression of the abnormally high blood pressure in SHR have remained intact and undiminished. Only 25% of the young born to Obese/SHR parents develop obesity; the remaining young retain their high blood pressure but are lean, *i.e.* non-obese/SHR. The findings

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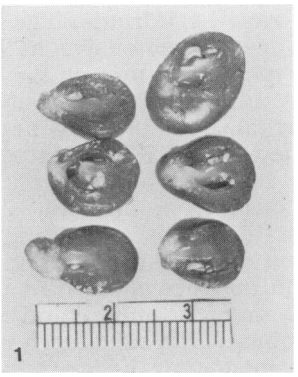
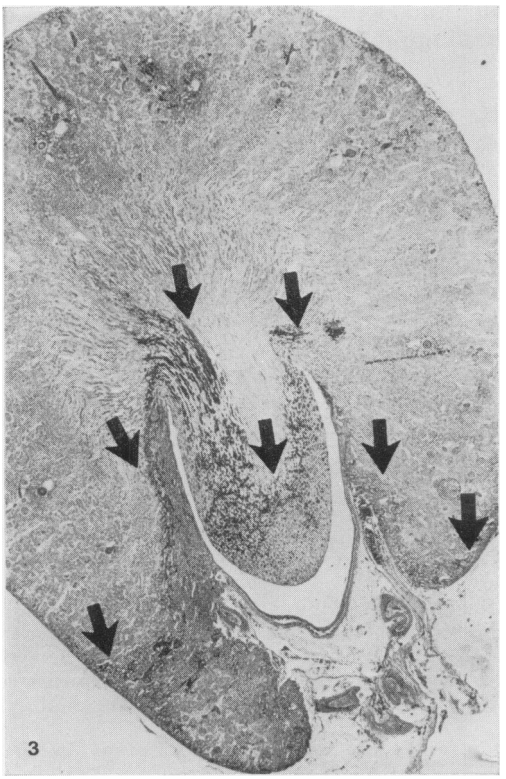
described here are based on 5 years' experience raising these SHR sub-strains, and necropsy of thousands of SHR, hundreds of non-obese/SHR, and 186 and 167 female Obese/SHR.

Kidneys destined for histopathological examination were fixed in 10% neutral formalin and stained with haematoxylin and eosin, Hale's colloidal iron stain to demonstrate glycosaminoglycans, von Kossa's stain for calcium, PAS stain for glycoprotein, and alcian and toluidine blue for metachromasia.





RESULTS

Regular hypertensive SHR

SHR are prone to develop kidney stones which remain sequestered within the pelvis, where they grow in colonies, reaching grossly visible proportions (Fig. 1). Kidney stones are found with equal frequency in male and female SHR with an incidence as high as 38% based on gross and microscopic examination. Microscopically, the calculi begin as single swollen, oedematous cysts beneath the transitional epithelium of the renal pelvis. These cysts serve as nidi for calculus formation. The oedematous cyst-like space becomes filled with red and white blood cells enmeshed upon delicate fibrillary material. These elements are replaced by laminated concretions of an admixture of calcium and acidic protein (Fig. 2), which react strongly positive to the von Kossa stain. There is an unusual polar orientation (towards the hilus) of strongly metachromatic and Hale-stain-positive material within the tubules of the cortex and pyramid (Fig. 3).

Non-obese/SHR

The incidence of kidney stones in the non-obese/SHR is essentially the same as in the regular SHR sub-strain. Calcium–mucoproteinaceous and von-Kossa-positive-staining colonies are found entrapped within the renal pelvis (Fig. 4), where most calculi grow into grossly visible stones (Fig. 1). A few calculi break loose to become rounded ureteral stones.

Obese/SHR

Stone formation in Obese/SHR appears to parallel the severity of obesity since

FIG. 1.—Several transverse sections made through the left and right kidneys of a male SHR showing the grossly visible stones at various levels and diverse dimension.

FIG. 3.—Histochemical demonstration (Hale stain) of mucoproteinaceous and intensely metachromatic but PAS-stain-negative material (black in photo delineated by arrows) coursing through the tubules of the hilar and pyramidal portions of the kidneys of SHR. Hale stain ×13·5.

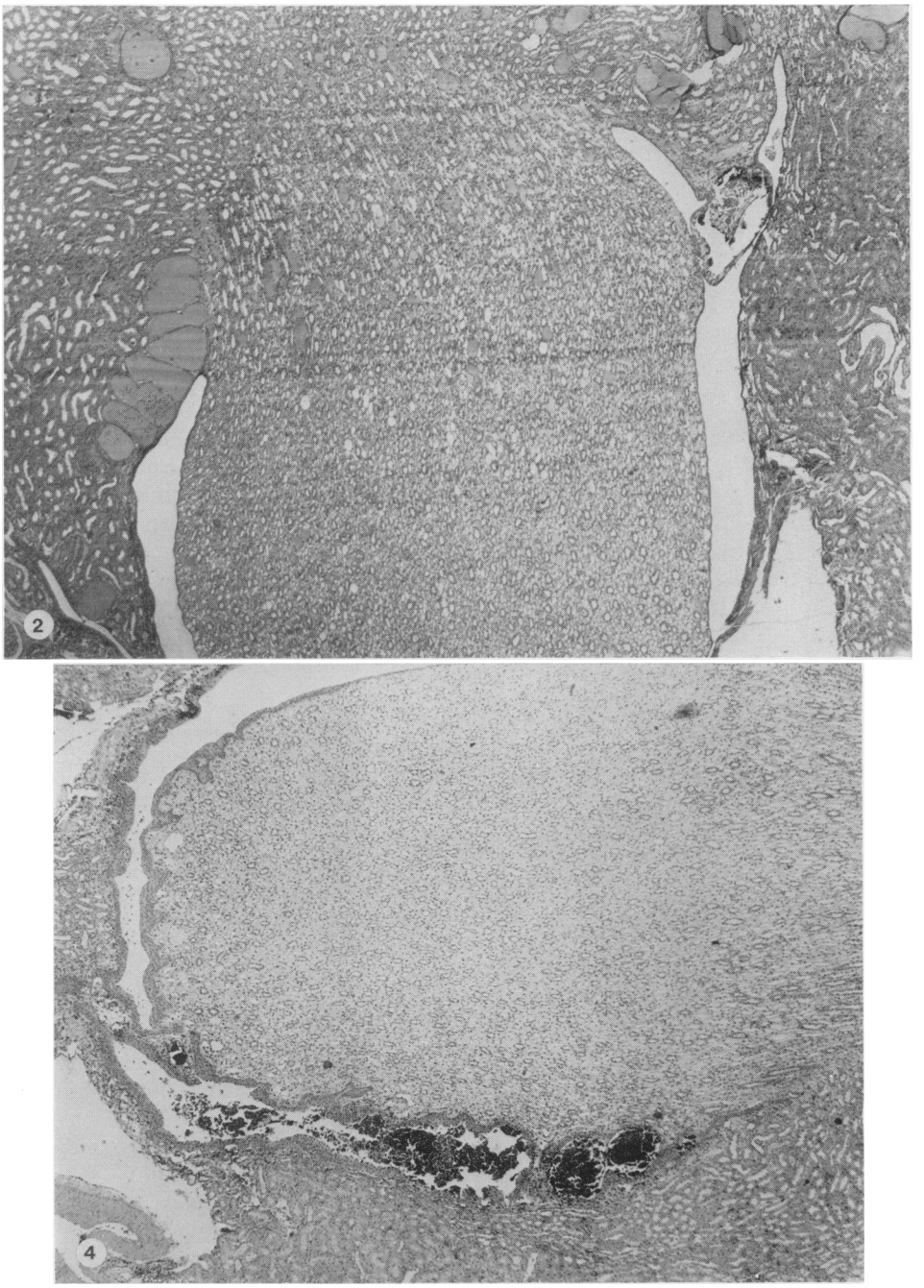


FIG. 2.—Kidney stone growing in the most proximal portion of the pyramido–pelvic junction. The central core (black-coloured detritus) stains strongly positive for haematoxylin and is an admixture of calcium and acid mucoprotein. H. & E. ×33.

FIG. 4.—Colonies of kidney stones which tend to remain within the pelvis of a male, non-obese SHR. H. & E. ×18.

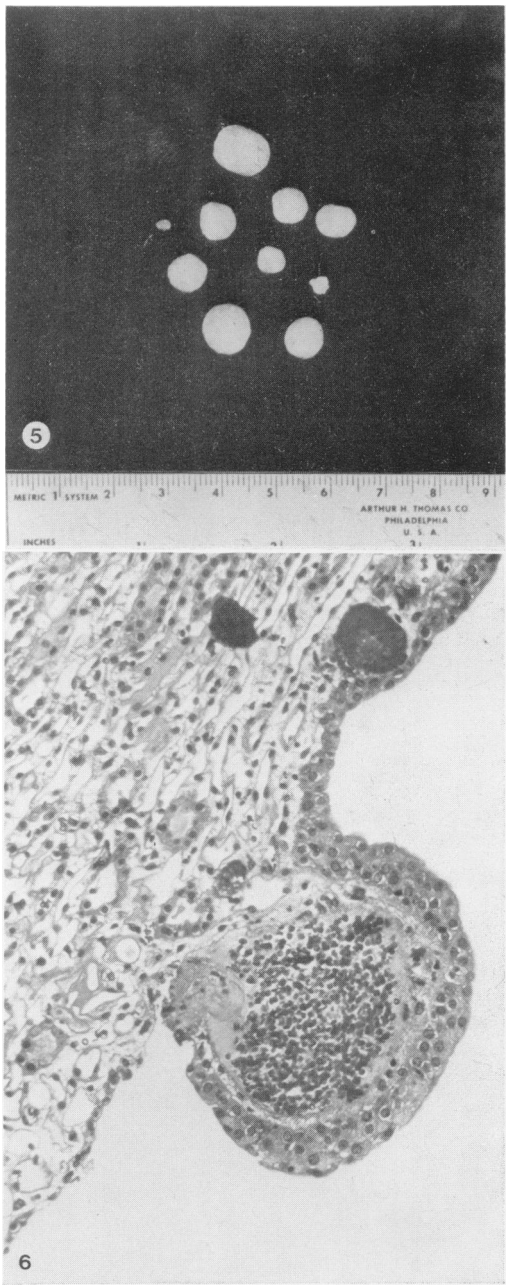


FIG. 5.—Vesicle stones found in the bladder of a male, Obese/SHR. (*cf.* Fig. 6)

FIG. 6.—High-power view of a kidney stone nidus in an Obese/SHR. The nidus consists of a papillomatous outgrowth capped by transitional epithelial cells. The cyst-like core is filled with oedema and a fibrinous mucoprotein colloid complex which is covered later with a calcium–mucoproteinaceous admixture. H. & E. × 100.

truly corpulent Obese/SHR have a 76% incidence of stones. To date, no grossly-visible stones have been encountered in the kidneys or ureters of Obese/SHR; all of the stones have been found exclusively within the bladder (Fig. 5). In many instances, the bladder stones are of such large proportions that the bladders of Obese/SHR are distended 6–7 times their normal size. The only suggestion of kidney stone formation found in Obese/SHR have been microscopic foci (Fig. 6). These papillomatous out-growths consist of oedematous cysts filled with red and white blood cells (early stage) entrapped upon a matrix of fibrin and colloidal mucoprotein. The colloidal–cellular matrix is replaced later by a calcium–protein admixture and surrounded by a distended mantle of transitional epithelium. These nidi also stain strongly positive with the von Kossa reaction. The process of nidus formation and growth appears to be identical in all of the sub-strains of SHR.

DISCUSSION

It is generally agreed that spontaneous stone formation is rare in rats (Marshall *et al.*, 1955; Vermeulen and Goetz, 1954). The appearance of kidney stones in animals having hypertension, hyperglycaemia, hyperlipidaemia, arteriosclerosis, and other degenerative diseases is suggestive evidence that kidney-stone formation may be the manifestation of a fundamental derangement of body metabolism rather than a disease in itself. Several investigators have offered evidence in support of this proposition and contend that urolithiasis reflects body endocrine or metabolic derangement (Boyce and King, 1959; Burkland, 1954; Burkland and Rosenberg, 1955).

We have established that neither diet, serum calcium, urinary pH, stone-forming bacteria, urinary citrate, nor abnormal parathyroid function can be correlated with the pathogenesis of stone formation in our several experimental models of renal calculi (Wexler, 1963*a*, *b*). The best

correlation we can establish is that urolithiasis becomes progressively more severe and frequent in parallel with progressively worsening hyperadrenocorticism and its attendant spectrum of Cushingoid degenerative changes as described in spawning slamon, repeatedly bred rats, and genetically hypertensive SHR (Wexler, 1976; Iams, McMurtry and Wexler, 1979; Wexler *et al.*, 1980). We suggest that in all of these experimental modesl the common denominator of hyperadrenocorticism creates a physical–chemical milieu which favours the formation of kidney stones. It is well known that adrenal and gonadal steroids have profound effects on citric-acid binding of calcium, calcium metabolism *per se*, and connective tissue metabolism in general. Adrenal steroids such as aldosterone and deoxycorticosterone, *i.e.* mineralocorticoids, cause increased depolymerization of ground substance which causes increased binding of calcium and prevents abnormal calcification, whereas the glucocorticoids such as corticosterone have opposite effects on connective-tissue metabolism (Rubin and Howard, 1950).

The intensely metachromatic and Hale-stain-positive (but PAS-stain-negative) material in the lower poles of the renal cortex and throughout the collecting tubules of the pelvis of SHR is intriguing. Histochemically, this would suggest that the material is acidic protein and not glycosaminoglycans. Acidic proteins are a major component of stone matrix and are found in the urine. The extra protein (acidic) may be pre-renal in origin and could be related to the hyperglycaemia in these hyperinsulinaemic and diabetic SHR. The naturally occurring kidney stones in SHR and breeder rats are composed chiefly of calcium, magnesium phosphate, and hydroxyapatite (Wexler, 1963*a*,*b*). Boyce and Garvey (1956) have shown that the matrix of stones containing calcium apatite stain consistently metachromatic and the degree of metachromasia with toluidine (or alcian) blue in the matrices of human stones is proportional to the amount of calcium phosphate present. It is of interest that acid mucoproteins are also alleged to be associated exclusively with calcium phosphate stones and not with calcium-oxalate or ammonium-urate stones (Sobel, 1955). Bills, Eisenberg and Pallante (1971) have shown that the von Kossa silver staining reaction is indicative of calcium phosphate molecularly integrated with certain open-chain organic acids, *e.g.* citric acid. When citric acid becomes exposed to light, it serves as the reducing agent for the silver stain.

Although the morphogenesis of stone formation in spawning salmon, breeder rats, and SHR is essentially identical, *i.e.* starting as sub-epithelial nidi and growing by lamellar concretions of a calcium–mucoproteinaceous admixture, it is provocative that the polyploid nidi of Obese/SHR separate early, migrate, and grow exclusively within the bladder. In contrast, the kidney stones in non-obese/SHR form, grow, and are retained within the kidney proper. This would indicate that although the sub-strains of SHR are genetically programmed to be prone to calculus formation, the precise morphogenesis of the stone is under the *aegis* of specific genetic transcription and hormonal and/or metabolic direction.

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