that the amount of cholesterol coming out of solution with a given level of the plant sterols is a *direct* function of the level of cholesterol present. This is exactly opposite to what would be expected if cholesterol and the plant sterols were in competition for solubility sites. The data obtained are those expected if dissolved cholesterol were adsorbed on the solid plant sterols or if cholesterol and the plant sterols formed mixed crystals. It is not possible with the present data to decide between these two, or possibly other, alternatives. Mixed crystals of cholesterol and β-sitosterol have been observed by Davis when a mixture consisting of equimolar amounts of cholesterol and a β-sitosterol preparation is crystallized from methanol(5).

Summary. The effect of a plant sterols preparation on the solubility of cholesterol in various triglycerides at several levels of cholesterol concentration was studied. At cholesterol levels between one-half and full saturation cholesterol and the plant sterols preparation compete for solubility sites in the triglycerides. At cholesterol levels below one-half of saturation the dissolved plant sterols are without effect on the solubility of cholesterol but in the presence of excess, undissolved plant sterols preparation the concentration of cholesterol in solution is reduced, perhaps by adsorption on the undissolved plant sterols or by the formation of mixed crystals of cholesterol and the plant sterols. These studies furnish further evidence for the suggestion, previously made, that cholesterol at saturation in triglycerides is present in two states of dispersion; one form from one-half to full saturation that yields insoluble clathrates with appropriate dicarboxylic acid or imidazole and now appears to compete with the plant sterols preparation for solubility sites, and a second form from zero to one-half saturation that does not yield clathrates and does not appear to compete with the plant sterols.

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Urolithiasis in the Rat. V. *In vivo* Dissolution of Calculi.* (31511)

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Calculi formation in the urinary tract of a number of experimental animals can be induced by dietary means such as deficiencies of vit. A or B_6, magnesium or phosphate, excessive vit. D, or an imbalance between protein and mineral intakes(1). Over 50% of weanling rats fed diets with an imbalance of protein and mineral form uroliths within 2 or 3 weeks (2), but higher dietary protein levels protect against urolithiasis. The calculi-inducing properties of the imbalanced diet are thought to be related to its low content of precursors of acidic ions, particularly sulfate, and its high carbonate content. The present study was designed to ascertain whether rats with calculi formed in this manner could be freed of them by altering the dietary regimen.

Materials and methods. Male, weanling rats of the NMRI-D strain were used in all experiments. The basal calculogenic diet (15P4) had the following percentage com-

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TABLE I. Occurrence and Nature of Urinary Calculi Formed on Changing Diets.

Group	No. of rats	Weeks on experimental diet		No. of rats with calculi			
				Vesical*		Renal only	
		15P4	30P2	Hard	Soft	Hard	Soft
1	50	3	0	25	1	0	0
2	34	5	0	26	1	0	0
3	50	3	2	8	10	2	0

* Animals in this category had vesical calculi or vesical and renal calculi.

position: vitamin-test casein, 15.0; sucrose, 76.8; HMW salt mixture(3), 4.0; vitamin mixture,† 2.2; corn oil, 1.0; and linoleic acid, 1.0. It has been shown previously that both the protein and mineral levels are important in determining whether stones will form in the NMRI-D rat. The therapeutic diet (30P2) was formulated to raise the casein level to 30% and to lower the mineral content to 2%, with a concomitant reduction in the sucrose concentration. In Experiment 1, 21-day-old rats were fed the 15P4 diet for 3 weeks. Then some of the animals were sacrificed to determine the proportion of rats with calculi. The diet of some of the remaining rats was changed to the 30P2 diet and after 2 additional weeks all animals were sacrificed. The protein content of the stones was determined by the procedure of Lowry *et al*(4) and the hexose content by the anthrone reaction(5).

Results. NMRI-D rats fed the calculogenic diet (15P4) for 3 or 5 weeks developed calculi in the urinary tract (Table I). The calculi were similar to those seen in previous experiments both in composition and location. The predominant component was calcium citrate with a variety of other materials. The location of the stones was primarily in the urinary bladder with stones sometimes being seen also in the pelvis of the kidney. It was unusual, however, to find a stone in the kidney without calculi also being located in the bladder. For this reason the disease has some resemblance to the vesical calculus disease seen in children of Thailand, India, Pakistan, and Middle East countries. The stones were white, hard, oval in shape, and frequently several stones were clumped together in the bladder.

Two observations were made which might be of considerable significance: (1) Many of the rats which first received the 15P4 diet and then were changed to the 30P2 diet still showed foreign bodies in the bladder; however, the nature of the bodies was quite distinct from the calculi normally observed. Instead of being hard and white, the bodies were rubbery, flexible, and of a slightly yellow hue. These are thought to be the remains of the matrices of dissolving calculi. The term "matrix" calculi or "matrix" stone has been applied to such bodies found in man. (2) Some of the rats whose diet had been changed still showed hard stones in the kidney. This is interpreted as indicating the greater ease of dissolution of vesical calculi.

Of 50 rats fed the 15P4 diet for 3 weeks just over 50% had either vesical or a combination of vesical and renal calculi. All but one of these animals had calculi of the usual variety. One rat had a calculus which was neither hard, nor as soft as the rubbery bodies observed in the experiment. None of the rats of this group had renal calculi without bladder stones also being present. Fifty rats were fed the calculogenic diet for 3 weeks, then the protective diet (30P2) for 2 weeks additional. Of these animals only 8 showed the typical hard calculus formations, 10 had "matrix" bodies in the bladder, and 2 rats had hard calculi in the pelvis of the kidney but not elsewhere in the urinary tract. Twenty-six of the 34 rats fed the 15P4 diet for a 5-week period developed calculi of the usual variety and one showed some "matrix" stones. Two duplications of Experiment 1 gave essentially the same results. In one of the duplicate runs an additional group was fed diets in the following sequence: calculogenic (3 weeks) — protective (2 weeks) — calculogenic (3 weeks). The purpose of this was to determine if the "matrix" bodies would recalcify.

† The vitamin mixture provided the following per 100 g of diet: Vit. A, 1980 Units; Vit. D, 220 Units; mg quantities of alpha tocopherol, 11.0; ascorbic acid, 100; inositol, 11.0; choline chloride, 165; menadione, 5.0; p-aminobenzoic acid, 11.0; niacin, 9.9; riboflavin, 2.2; pyridoxine HCl, 2.2; thiamine HCl, 2.2; calcium pantothenate, 6.6; and μg quantities of biotin, 44.0; folic acid, 198; and vit. B_{12}, 3.0.

TABLE II. Protein and Hexose Content of Rat Calculi.

Type of stone	Protein % dry wt	Hexose % dry wt
Hard calculi	2-5	.01-.06
"Matrix" calculi	32-75	.40-.50

Unexpectedly, "matrix" bodies were still found in the bladder of some animals, but about 25% of the rats were found to have hard calculi in the renal pelvis with no stones in the bladder. In our experience this is an unusually large proportion of rats to have stones only in the renal area.

A study of the nature of the rubbery foreign bodies found in the present study was undertaken (Table II). The hard calculi usually found in the bladder of the NMRI-D rat fed the 15P4 diet contain 60% or more of calcium citrate. The "matrix" calculi contained little or no citrate. In a study of 9 "matrix" calculi the protein content varied from 32% to 75%. The hexose content of the few "matrix" calculi investigated varied from 0.40% to 0.50%. These values are considerably higher than the protein and hexose contents of normal, hard calculi.

Discussion. The term urolithiasis is used to indicate stone formation in any part of the urinary tract. However, it is important to recognize that vesical lithiasis in man has characteristics quite distinct from renal lithiasis and the two situations probably have different etiological patterns. In many countries of the Far East and Middle East, vesical lithiasis is a serious medical problem affecting many thousands of children yearly. In many respects the lithiasis observed in the NMRI-D rat, fed diets with an imbalance between protein and mineral, resembles the vesical lithiasis reported in children. One major difference is that the stones in the NMRI-D rat are predominantly calcium citrate, whereas, in many areas where vesical lithiasis occurs in children, the stones contain calcium oxalate and urates along with other minor constituents. In spite of the differences, the rat provides a test system which may be used as a model for exhaustive investigation of the disease.

In our early work with the NMRI-D rat we attempted to x-ray rats to determine whether they had calculi. We were unable to use this diagnostic technique because of the size of the animal and the relatively limited opacity of the citrate stones. It was necessary to sacrifice animals to determine whether they had developed stones and to ascertain the location and degree of stone formation. The same procedure has been used in the present experiment.

Rats fed the stone-forming diet for 3 weeks followed by the protective diet for 2 weeks showed fewer hard calculi than rats fed the 15P4 diet for either 3 or 5 weeks. This indicates that dissolution of calculi occurred during the period of feeding the 30P2 diet. Further evidence for the dissolution of preformed calculi comes from the finding of relatively soft, rubbery foreign bodies in the bladder of animals given the 30P2 diet after receiving the stone-producing diet for 3 weeks. These foreign bodies were considerably higher in protein and hexose content than the usual hard stones and it seems reasonable to assume that they are the result of the loss of the mineralized portion of the calculi. Several investigators have indicated that all urinary calculi contain an inorganic matrix distributed throughout the stone. Boyce and Garvey(6) reported that the average matrix content of 264 dehydrated calcigerous calculi was 2.5% by weight. Most of the 17 calculi assayed by Finlayson(7) contained less than 2% matrix. Boyce and King(8) observed that one poorly crystallized renal calculus had a matrix which accounted for 65% of the dry weight. It is thus apparent that the weight of the matrix of the stone can vary. They also studied the hexose content of the organic fraction of a variety of human stones and reported that the matrix of calcigerous calculi, "matrix" calculi, and uric acid calculi contained 9.6%, 8.9%, and 5.9% hexose, respectively. Uromucoid isolated from urine was reported to contain 10.7% hexose. These quantities of hexose are considerably higher than those found in our rat calculi. Investigations are under way to obtain more definitive information concerning the composition of the matrix of the rat calculi.

If it is assumed that the non-calcigerous

bodies found in our rats are the matrices of calculi, some conclusions concerning the dissolution of the calculi might be made. Several investigators have suggested the following sequence in stone formation:

stone salts + matrix + time for growth → stone

Our observations would suggest that in the dissolution of calculi the reverse procedure occurs and that the breakdown of the matrix probably occurs as a distinct process.

One further observation in this experiment deserves attention. Under the dietary regimen of 15P4 → 30P2 → 15P4 there was a tendency for the rats to develop renal stones without vesical calculi. This is of interest since it is known that in man, adults tend to form renal stones rather than vesical calculi which is predominantly a disease of young children. Further studies with the rat may indicate a similar tendency in this species.

Summary. Uroliths can be formed readily in the weanling NMRI-D rat by feeding a diet containing 15% casein and 4% HMW salt mixture. The calculi are found predominantly in the bladder, sometimes in the bladder and renal pelvis, but rarely in the kidney alone. Calculi formed by the above dietary means can be dissolved *in vivo* by changing the diet to include more protein and less mineral. It appears that the process of dissolution takes place in a sequence, involving first a demineralization and then dissolution of the matrix. Rats fed diets in the order of calculogenic—protective—calculogenic were found to have an unusually high occurrence of renal calculi without stones being found in the bladder. This may be related to the age of the animals.

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Studies on 5-Ribosyluracil in Man.* (31512)

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There have been many investigations on purine excretion in man, with attempts to correlate the results with the metabolism of nucleic acids in normal and various diseased states. But the results were never unambiguous. The other components of nucleic acids, the pyrimidines, are degraded and could not be used for the purpose of measuring nucleic acid metabolism by determining their specific metabolic end products in the urine.

However 5-ribosyluracil, the only minor RNA component that has the unusual structure of a C-C glycoside, has been found in human urine in rather large quantities(1-7). Our quantitative determinations in 24-hour urine samples indicated that the amount excreted is fairly constant, and feeding experiments demonstrated a complete lack of catabolism of 5-ribosyluracil in man(7). In this study, we will be concerned with the excretion of 5-ribosyluracil in patients suspected of abnormal RNA turnover, due to their disease states.

Experimental. Patients. All patients were kept on a diet free of meat and caffeine for at least one day prior to and on the day of collection. All were hospitalized except the two

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