

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 struc-

ture 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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with polycythemia, who were out-patients, controlled every few month by therapy.

*Methods.* The components of interest in a 24-hour urine sample were first adsorbed on a charcoal column and then separated on a Dowex-1 column. Prior to its use, the charcoal (500 g of Norite A, Fischer Scientific Co.) was washed by heating in 4 l of 2 N formic acid for one hour at 100°. After collection in a large Buchner funnel, it was washed with distilled water, and the filter cake resuspended in 4 l of a mixture of water:ethanol:conc. ammonia (by vol. 60:30:20), heated to the boiling point, filtered, washed with distilled water, and dried overnight at 140°.

Freshly collected 24-hour specimens of human urine were adjusted to pH 3 with 6 N HCl. After acidification, the urine was left in the cold at 2° for several hours, and the precipitate, which consisted mainly of uric acid, was removed by filtration and discarded. The components of interest were then adsorbed on a column of 40 g of charcoal. Use was made of a large column 4.5 cm in diameter fitted with a sintered glass disc. As a support for the charcoal, a coarse grade of celite (#545, Johns Manville) was poured into the column to a depth of about one cm, and charcoal was carefully layered above the celite. The column was not allowed to run dry during this packing and subsequent procedures. Best adsorption and faster flow rates are achieved when only part (about 5 g) of the charcoal is used to form a layer on the celite and the remainder is added to the urine before pouring it onto the column. To increase the flow rate, an air pressure of about 15 cm Hg has been applied to the top of the column. After adsorption is complete, the column is washed with distilled water until free of Cl<sup>-</sup> ions, and the adsorbed material eluted from the column with 1.5 l of 10% pyridine in water. Concentration of the effluent and subsequent ion exchange column separation was carried out as described earlier(7). The absorbancies of the fractions were read at 260 m $\mu$  and 290 m $\mu$  in 1:20 diluted aliquots, in which the pH was adjusted to 2 and 12 with HCl and NaOH. Accordingly, in the figures representing column

separations, the observed absorbancies were multiplied by 20, to account for the 1:20 dilution of the fractions subjected to spectral measurements.

In a search for additional urine components, the fractions comprising each of the other peaks, obtained in the Dowex separation, were desalted by the use of charcoal and further analyzed by paper chromatography in butanol-water(8), as described for the peak of 5-ribosyluracil(7).

*Results.* As can be observed in Fig. 1, 2 and 4, ten peaks were obtained, none of which was homogenous when analyzed by paper chromatography in butanol-water. Most of the peaks have been studied in some detail, as follows:

Peak I has been separated into 5 distinct UV absorbing bands without further identification.†

Peak III has also been separated into 5 components through the use of butanol-water. The two components with the highest R<sub>f</sub> values gave spectra identical with N-methyl-4-pyridone-5-carboxamide and N-methyl-2-pyridone-5-carboxamide(10), major end products of nicotinic acid metabolism. The component with the lowest mobility gave a spectrum almost identical to cytidine.

Peak V consists of 5-ribosyluracil and in most of the diseased cases has not the high degree of purity as found in the urines of normal persons. However, all of the compounds found so far to contaminate 5-ribosyluracil in peak V in various diseases show virtually no absorbancy at 290 m $\mu$  (pH 12) when separated in butanol-water from 5-ribosyluracil, and their UV spectra recorded. Hence the summation of the absorbancies at 290 m $\mu$  (pH 12) yields good quantitative values for 5-ribosyluracil as verified by its isolation from paper.

Peak VI was separated into uracil and 3 unidentified components.

Peak VIII consisted mainly of hypoxanthine. Two unidentified compounds were also present.

*Polycythemia:* The urines of 2 patients

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† More details about the isolation of components and their UV spectra can be found in (9).

with Polycythemia Vera have been subjected to the column separation procedure. The results for one of these patients is shown in Fig. 1. In contrast to the situation which obtains in the urines from normal subjects(7), the spectral qualities of the solutes in peak V indicate considerable inhomogeneity. The 5-ribosyluracil estimated from the absorbancies at 290  $m\mu$  (pH 12) of the solutes in peak V, in both cases amounted to 63 and 93 mg/24 hr, respectively, and identical values were obtained after the butanol-water separation of 5-ribosyluracil.

*Gout:* The 5 patients with uncomplicated gout were under drug therapy, which may account for the "normal" values (Table I) for

5-ribosyluracil, contrary to the reported(1) two-fold increase. The results of the column separation for one of these patients is shown in Fig. 2. Despite the large increase in peak V, the 5-ribosyluracil isolated by paper chromatography amounted to only 75.2 mg. The UV spectrum of one of the components of this peak is shown in Fig. 3; it is identical to that of 5-acetylamino-6-amino-3-methyluracil, identified by Fink *et al*(11) in the urine of a patient with cancer and considered to be a metabolite of purine degradation.

*Multiple myeloma:* The results of the column separation of the one case examined are presented in Fig. 4. The high degree of purity of peak V, to be expected from the

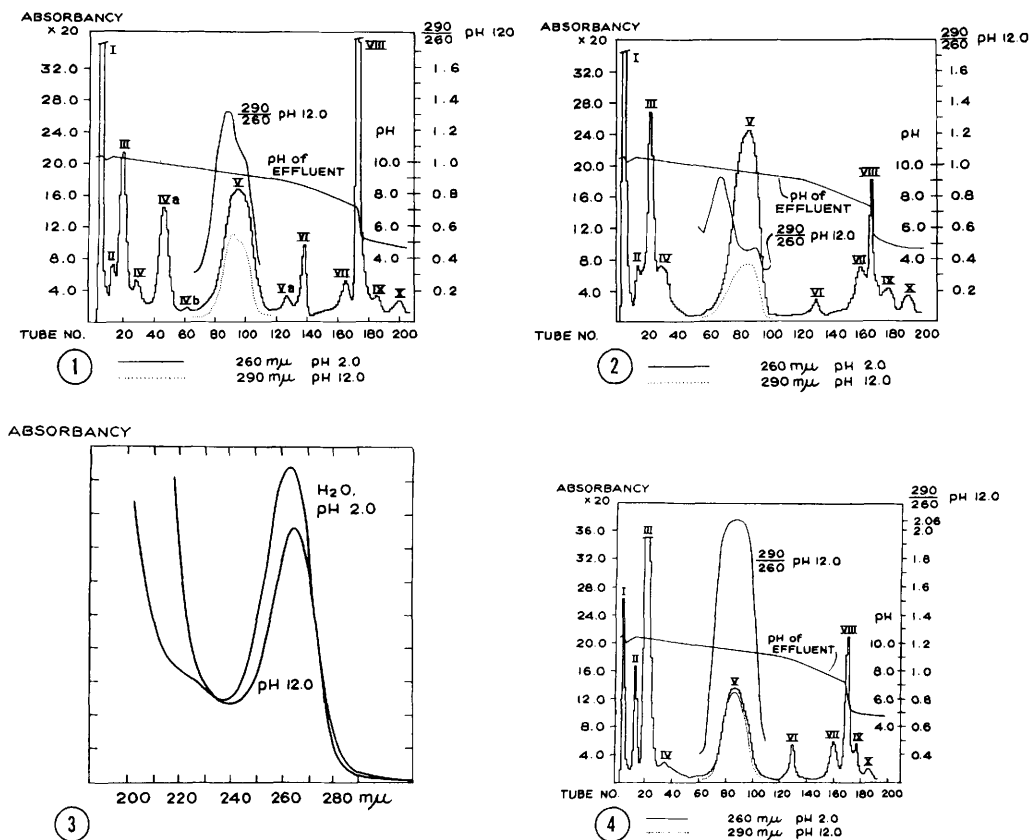


FIG. 1. Ion exchange separation of a 24 hr urine specimen of a polycythemic patient. (Dowex-1-Cl<sup>-</sup>, 200-400 mesh, 8 X, 3.8 cm<sup>2</sup> x 55 cm.)

FIG. 2. Ion exchange separation of a 24 hr urine specimen of a gouty patient. (The same conditions as in Fig. 1.)

FIG. 3. Ultraviolet absorption spectrum of a compound, isolated from peak V (Fig. 2) of a gouty patient. The spectrum was recorded on a Beckman DK-2 instrument.

FIG. 4. Ion exchange separation of a 24 hr urine specimen of a patient with Multiple Myeloma. (The same conditions as in Fig. 1.)

TABLE I. Amount of 5-Ribosyluracil/24 Hr Urine in Patients with Various Diseases.

Disease	Patient's		Before	After	Treatment
	Age	Sex	treatment	treatment	
			(mg)	(mg)	
Gout	52	♂		70.8	Colchicine
"	46	♂		50.2	"
"		♂		79.4	"
"		♂		73.8	"
"		♂		75.2	Anturane
" (?)	44	♂	163.0	140.0	Colchicine
Hodgkin's disease	26	♂	60.5	81.2	X-rays
" "	52	♂	65.2	84.5	Cytosan
" "		♂	93.8		
" "	18	♂	113.8		
" "	30	♀	57.2		
" "	20	♀	91.0		
" "	24	♂	45.6	71.1	Cytosan
" "	34	♀	58.6	117.0	"
Lymphocytic leukemia	66	♀	109.0		
" "	34	♂	163.5		
Polycythemia vera	52	♂	63.0		
" "	47	♂	93.0		
Multiple myeloma	56	♂	120.0		
Hyperthyroidism	24	♀		89.0	Propylthiouracil

magnitude of the 290/260 ration at pH 12, was established by paper purification; 120 g of 5-ribosyluracil were isolated.

*Hodgkin's Disease:* Table I presents the results of the urinary analysis of eight patients with Hodgkin's Disease. Although the values are both above and below the normal of 65-75 mg per day, in the untreated state, probably due to varying progress of the disease, there is an increased excretion following treatment by either X-ray or alkylating agents. Such treatment causes destruction of ribonucleic acids.

The reported(2) increase in excretion of 5-ribosyluracil in leukemia has been confirmed in 2 patients; values of 109 and 163.5 mg have been obtained. In one case of hyperthyroidism, 89 mg of 5-ribosyluracil was excreted in 24 hours.

*Discussion.* To overcome the troublesome presence of large quantities of inorganic salts and the large volume of a 24-hour urine sample, the published methods(1-5) for estimation of 5-ribosyluracil start with a small fraction of a 24-hour sample and use large columns of different ion exchangers. The results obtained for normal persons vary considerably, even on the same person on 2 subsequent days(3). The method(7) used

in this study removes inorganic salts during charcoal absorption, and concentrates the compounds of interest to a small volume prior to column separation. Under these conditions normal human urine yields a peak of 5-ribosyluracil that is quite homogenous, provided no coffee or tea is taken. Although in urines from diseased persons this peak is more contaminated, the summated absorbancies at 290 m $\mu$  (pH 12) still yield valid results for the amounts of 5-ribosyluracil. Paper chromatography was used each time to isolate the 5-ribosyluracil in a very pure state, and not for the sole purpose of its determination.

Although 5-ribosyluracil is not the end product of catabolism of most RNA pyrimidines, it certainly can be regarded as a representative of the catabolism of transfer RNA, because of its highly specific localization there(12-15) and of its demonstrated function in transfer RNA(16). The specific localization of this nucleoside simplifies the interpretation of its excretion rates, contrary to similar data obtained from excretion rates of uric acid. The latter represent the catabolism of all purines derived from RNA, DNA, and the soluble nucleotides and coenzymes with a high rate of turnover.

The results obtained in this study tend to

support this concept. In cases of malignant proliferation, the amount of excreted 5-ribosyluracil increases. The same is true after therapy that causes nucleic acid destruction (X-ray, Cytoxan). The latter results are consistent with similar data (17) on increased urinary 5-ribosyluracil excretion in rats following irradiation of the animals. We could not find patients with untreated, primary gout to confirm the reported(1) increase in the excretion of this nucleoside. But it may be worth noting that in one case of a patient with gout, complicated by other diseases of uncertain diagnosis, a single 2-mg dose of colchicine caused the amount of excreted 5-ribosyluracil to drop from 163 to 140 mg per 24 hours. This alkaloid, known for its inhibition of mitosis, and used in the therapy of gout, may therefore account for the "normal" values of excreted 5-ribosyluracil found by us in gout. The results of the study of excretion of 5-ribosyluracil in pathological conditions, as reported, give some indication of the potential value of such studies.

*Summary.* A method described for estimation and isolation of 5-ribosyluracil in 24-hour specimens of normal human urine (7) has been used to study its excretion in patients suspected of abnormal RNA turnover. In accord with the previously demonstrated lack of catabolism of 5-ribosyluracil in man(7), there is support for the view that the amount of excreted 5-ribosyluracil may be indicative of the turnover of transfer RNA, intimately associated with protein biosynthesis. With the use of this method, several as

yet unidentified components have been isolated from human urine and partially characterized.

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### Inhibition of the Stem-Cell Action of Erythropoietin by Estradiol.\* (31513)

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Previous observations have revealed that hematological values of females of various species, after the attainment of sexual maturity, are lower than those of males, and a comprehensive analysis of the data has

shown these differences are due to the pres-

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