TABLE I. Infective Titer and Total Nitrogen Content of Infected 20% Chick-Embryo Tissue Suspensions Before and After Adsorption with Attaclay SF.*

Trial No.	Infective titer ($-\log LD_{50}$) Before	After	Total N (mg/ml) Before	After	% N reduction
1	7.7	6.9	.93	.20	79
2	6.8	6.6	1.01	.24	76
3	8	7.2	1.11	.29	74
4	6.5	6.6	1.36	.45	67
5	5.5	5.2	1.07	.27	75
6	6.2	6.6	1.39	.40	71
7	6.6	7	1.13	.29	74

* 20 ml of dry packed clay used for each 100 ml of tissue suspension.

infectivity and the nitrogen content of the suspensions is summarized in Table I. The data show that it is possible by this method to remove about 75% of the nitrogen content of a crude virus suspension without serious loss of infectivity. It should be pointed out that the figure expressing the infective titer of a virus preparation is inherently of a low order of accuracy ($\pm$ 0.3 log unit). The apparent losses of infectivity in Trials 1 and 3 do not appear significant when considered in context with the apparent gains in Trials 6 and 7. Some of the infective particles are undoubtedly lost in the adsorption process and in the subsequent centrifugation. However, the loss is usually not sufficient to result in a significant decrease in infective titer.

The simplicity and convenience of the technic described suggest that it may have a useful application as a preliminary step in virus purification and in the preparation of vaccines and diagnostic antigens.

Summary. Japanese encephalitis virus in 20% suspensions of infected chick-embryo tissues may be partially purified by adsorption of inert material on particles of Attaclay SF, a naturally-occurring sorptive clay of exceedingly small pore diameter. Removal of about 75% of the nitrogen content of a crude virus suspension may be accomplished without a prohibitive loss of infectivity.

The technical assistance of Marina S. Madden and Milton J. Francis is gratefully acknowledged by the authors.

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Excretion of Amino Acids in Cystinuria. (19189)

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The precise nature of the metabolic disturbance responsible for cystinuria remains a matter of conjecture. At one time the disease was considered by many to be linked exclusively to aberrations in the metabolism of the sulfur containing amino acids. Numerous reports have appeared, however, indicating that amino acids other than cystine may be excreted in abnormal amounts by the cystinuric. References to the early literature on the subject may be found in Garrod(1). More recently, Yeh, Frankl, Dunn, Parker, Hughes and Gyorgy(2) employed microbiological assay to analyze the urine of an 8-year-old cystinuric girl and observed a consistent and unusually high level of lysine and arginine excretion. Since then Dent(3) and Dent and Rose(4) have employed paper chromatography to investigate the amino acid content of a number of cystinuric urines. Although the paper chromatographic method is not quantitative, Dent and Rose were led to

the conclusion that lysine, not cystine, was the amino acid excreted in largest amounts in cystinuria, and that the quantity of arginine excreted was also markedly elevated in most cases. It became of interest, therefore, to examine the amino acid content of cystinuric urines employing the recently developed quantitative chromatographic technics which employ columns of the ion exchange resin Dowex-50(5).

Cases. The cases studied had all been diagnosed previously as cystinurics. It is a pleasure to acknowledge with thanks the help of Dr. Robert F. Watson of the New York Hospital, Dr. George A. Perera of College of Physicians and Surgeons, Columbia University, and Dr. Frank L. Horsfall, Jr., of the Rockefeller Institute, in securing the urine specimens. A brief summary of the pertinent aspects of the case histories follows: *Case 1.* A 29-year-old, married, white male, weight 150 lbs. Has suffered intermittently from bilateral renal colic, passage of stones, and surgical removal of stones since the age of 17. *Case 2.* A 58-year-old, married, white female, weight 192 lbs. The patient has had intermittent renal colic and has passed stones. Right nephrectomy was performed at age 31. *Case 3.* A 29-year-old, single, white female, weight 133 lbs; suffered intermittently from renal colic; passed a right renal calculus 3 years ago. *Case 4.* A 49-year-old, single, white male, weight 202 lbs; suffered from left renal colic; passed a stone at age 36. *Case 5.* A 31-year-old, married, white female, weight 115 lbs. Has a long history of renal complaints including bilateral calculi and pyelonephritis.

Methods. The urine samples chromatographed were withdrawn from 24-hour specimens. The subjects were leading a normal life at home during the period of the urine collection, and no attempt was made to control their diet. The bottle furnished each individual for collection of the urine contained 6 cc of chloroform and 6 cc of toluene as a preservative. The specimens all arrived in the laboratory within a few hours of the close of the collection period, and were stored thereafter at 4°C. The first chromatograms (the 100 cm columns referred to below) were usually run immediately. The 15 cm columns were run 1 to 2 weeks later. Two urine samples (Cases 3 and 4) deposited a precipitate before samples could be withdrawn for the 100 cm columns. These specimens were stirred vigorously and 25 cc poured off rapidly into a graduate and enough N HCl (about 2.5 cc, predetermined on a separate aliquot) was added to bring the pH to 2.3 to 2.5. Although this procedure served to dissolve most of the precipitate, it is possible that some cystine, if it came out of solution, did not redissolve. The cystine figures for Cases 3 and 4 may be slightly low on this account. Prior to chromatography on the 100 cm columns, all urine samples (about 3 cc in volume) were brought to pH 2.3 to 2.5 with N HCl. The chromatograms were run in the manner recommended(5) for urine, no desalting being required. In order to increase the resolution in the basic amino acid range, a phosphate buffer of pH 7.5 (prepared by mixing 200 cc of 0.1 M Na_2HPO_4 and 15 cc of 0.2 M $NaH_2PO_4 \cdot H_2O$) was interposed for 180 cc between the pH 6.7 and pH 8.3 buffers. The pH 8.3, 9.2, and 11.0 buffers each were run for 100 cc. For the quantitative determination of the basic amino acids, 1 cc of urine was chromatographed on the 15 cm columns of Dowex-50 recommended heretofore(5). At the close of each of these latter chromatograms, fractions of the 0.2 N NaOH employed to clean out the columns were collected and analyzed by the ninhydrin method. In this manner it was established that only very small amounts of tenaciously held basic substances remained on the columns. A single 100 cm and a single 15 cm chromatogram were run on each sample of urine.

Results. Those amino acids found in grossly abnormal amounts in cystinuric urine are given in Table I. It is clear that arginine, lysine, cystine, and also ornithine are excreted by cystinurics in 50 to 100 fold the amounts found for normal subjects. In addition, the isoleucine level in cystinuric urine is elevated by about twofold, whereas taurine is excreted in unusually small quantities, about ⅓ or less of the normal. By virtue of the chromatographic method of analysis employed, it is possible to state that the quantities of all the

TABLE I. Excretion of Amino Acids by Five Cystinurics.

Amino acid	Excretion in g per diem					Normal range*	Relative molar excretion Ornithine chosen as 1				
	Case 1	2	3	4	5		Case 1	2	3	4	5
Ornithine‡§	.42	.18	.36	.50	.42	None	1	1	1	1	1
Cystine	.97	.42	.82†	.74†	.70	.01 -.03	1.3	1.3	1.3†	.83†	.91
Arginine	1.24	.55	.92	.77	.67	0 -.02	2.3	2.3	2	1.2	1.2
Lysine‡	2.30	1	1.98	2.38	1.35	.01 -.05	4.9	5	5.1	4.4	2.9
Isoleucine	.057	.038	.043	.065	.064	.015-.03					
Taurine	.05	.008	.062	.086	.052	.1-.3					

* Unpublished data.

† These values may be low.

‡ Ornithine and lysine yield completely separated but neighboring peaks on the 100 cm Dowex-50 columns. Although quantitative recoveries of the basic amino acids are not obtained under these conditions, the relative amounts of ornithine and lysine can be determined. A value for the sum, ornithine + lysine, can be obtained from the short 15 cm column which, however, does not separate these two amino acids. Combining the data from the 15 cm and 100 cm columns, it is readily possible to calculate the amount of both ornithine and lysine present.

§ Ornithine was also identified on the 100 cm columns by analyzing alternate fractions of the ornithine peaks by the color reaction of Chinard in the manner already indicated(6).

other ninhydrin positive substances in these cystinuric urines are within the normal range, and furthermore, that no unusual ninhydrin positive substances (cadaverine or putrescine, for example)(1,4) occur in appreciable amounts. In one instance (Case 1), a second sample of urine collected some weeks later gave results agreeing to within ± 5 to 20% with those given in Table I. Upon hydrolysis of a single urine sample (Case 1) with boiling 6 N HCl for 16 hours, the values for arginine, ornithine, lysine, taurine, and isoleucine agreed to ± 10% with those obtained before hydrolysis. The cystine figure after hydrolysis was substantially lower, doubtless as a result of the destruction of cystine during hydrolysis.

Discussion. Dent and Rose(4) have proposed that the diagnosis of cystinuria be limited to include only those individuals who excrete large quantities of cystine, lysine, and arginine. The data reported in this communication suggest that the pattern of the amino acid excretion in cystinuria is even more definitive, and is characterized by a very excessive excretion of cystine, lysine, arginine and ornithine, a moderately excessive isoleucine excretion, and a markedly diminished output of taurine.

The data given in the right hand half of Table I show that the relative molar amounts of cystine, arginine, ornithine, and lysine excreted are quite similar from one subject to the next. The similarity of these figures is all the more striking in view of the heterogeneous nature of the case material, and the fact that no attempt at dietary control whatsoever was attempted at any time during or before the period when the urine specimens were collected. Dent and Rose(4) believe that cystinuria is the result of a physiological defect in the kidney tubule, and consists in a failure to reabsorb some part of the basic amino acids and cystine. Previous metabolic studies with the sulfur amino acids(7-9), coupled with the observations reported here, would appear to be more compatible with the working hypothesis that cystinuria is a metabolic defect, enzymatic in nature, which affects simultaneously at some site or sites (probably the kidney) some phase of the metabolism of arginine, ornithine, lysine, and the sulfur amino acids, and perhaps also isoleucine and taurine.

Summary. The amino acid content of the urine of five cystinurics has been determined employing chromatography on columns of the ion exchange resin, Dowex-50. In every case the amount of arginine, lysine, ornithine, and cystine was found to be from 50 to 100 fold the normal level. The quantity of isoleucine was about twice the normal, whereas the taurine output was diminished to about ⅓ or less of normal. All other ninhydrin positive constituents were in the normal range. It is particularly striking that the relative molar

excretion of ornithine, cystine, arginine, and lysine, which was found to be about 1:1:2:4, is quite similar from one subject to the next despite the haphazard nature of the case material and the absence of any dietary control. The results suggest the hypothesis that cystinuria involves an enzymatic defect which affects simultaneously at some site or sites (probably the kidney) some phase of the metabolism of arginine, lysine, ornithine, and the sulfur amino acids, and perhaps also isoleucine and taurine.

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The Screening of Antiulcer Drugs by a Quantal Procedure. (19190)

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The pyloric ligation method of Shay(1) which has won universal acceptance has made it possible to solve many problems of the pathogenesis of gastric ulcer(2-4). The Shay rat, however, has been used only occasionally for evaluating the antiulcer effect of drugs: antacids(2,3), Thephorin(6), tetraethylammonium(7), phenothiazine derivatives(8), and urinary extracts(4,9).

Moreover, studies of the activity of atropine have been quite contradictory. Protection has been noted with 250 mg/kg subcutaneously (2); 10 mg kg(6); 1.5-3 mg/kg(10); whereas, no protection has been observed with 100 mg kg(8). A systematic investigation of compounds reported clinically effective in peptic ulcer therefore seems warranted. For that purpose a quantal procedure has been devised.

Material. Twelve drugs were tested. A. Anticholinergic agents: 1. atropine (sulfate) USP; 2. trihexyphenidyl, *Artane*. B. Pure ganglionic blocking agents: 1. pentamethonium bromide; 2. hexamethonium bromide; 3. tetraethylammonium bromide; 4. tetraethylammonium chloride. C. Ganglionic blocking-anticholinergic agents: 1. B-diethylaminoethyl-xanthene-9-carboxylate methobromide, Methantheline Bromide, *Banthine;* 2. 10 - (B - diethylaminoethyl) - phenothiazine (hydrochloride) *Diparcol*. D. Antihistaminic agents: 1. tripelennamine hydrochloride USP, *Pyribenzamine;* 2. diphenhydramine hydrochloride USP, *Benadryl;* 3. phenindamine hydrogen tartrate, *Thephorin;* 4. Chlorothen Citrate, *Tagathen*. 580 male albino Wistar rats maintained on a diet of Wayne "Rat Blox" were used; those weighing 180-250 g were starved for 72 hours before the ligation, while younger rats weighing 125-180 g were starved for 48 hours only. *Method.* The original Shay rat method(1) was used. However, whereas previous authors used as criteria the decrease of gastric acidity and volume, together with the pepsin concentration and a system of scoring the severity of ulcerations, the test selected in our assays concerns only the appearance of ulcerative lesions in the rumen of the rat ob-