XLIII. A STUDY OF CYSTINURIA IN TWO SISTERS.

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THE subjects of this investigation are sisters, one of whom, the younger, was first referred to the Birmingham Children's Hospital by Dr Burns of Redditch for the investigation of albuminuria. She was admitted and on examination was found to have cystinuria. A subsequent examination of a specimen of urine from the sister revealed cystine crystals and she, also, was admitted for investigation and observations on metabolism.

Family history.

Cystinuria is one of a group of disorders which have been aptly described by Sir Archibald Garrod in the picturesque phrase "inborn errors of metabolism". An enquiry was instituted, therefore, into the occurrence of the derangement in other members of the family, and with the co-operation of the patients' doctor and parents many specimens of urine were collected from each of seventeen blood relatives, at intervals of several weeks, and examined by us for cystine. The tests applied were:

(1) Microscopical examination of sediment: (a) from the fresh specimen, (b) after acidifying with dilute acetic acid and standing for one week, and (c) from acidified specimens concentrated by evaporation.

(2) The Sullivan reaction as modified by Brand *et al.* [1930].

(3) The cyanide-nitroprusside reaction described by the same authors.

These investigations revealed well-marked cystinuria in one other relative, whose position in the genealogical tree is shown in Fig. 1. The positions of the two

Fig. 1. Genealogical tree.

♂ ♀ male and female normals respectively; ● female cystinurics.

subjects of this investigation are indicated by the encircled figures, while crosses denote the existence of members who were not examined. In addition to the discovery of one other definite cystinuric it was found that the urine of several other members of the family gave faintly positive results with one or other of the chemical tests for cystine, and a few showed one or two hexagonal crystals after prolonged microscopical examination of an acidified specimen of urine concentrated by evaporation.

These findings, together with the results of tests for albumin and estimations of total amino-acids, appear in Table I. It will be seen that the presence of traces of cystine in the urine of one individual was not constant and that specimens in which, on microscopy, two or three hexagonal crystals were found did not always yield positive chemical tests and *vice versa*.

Various writers [Loewy and Neuberg, 1907; Lewis and Simon, 1902] have suggested that the degree of cystinuria may vary considerably from time to time and even disappear temporarily. At first we were tempted to believe that these other members of the family were occasional or mild cystinurics and that the union of two such individuals had resulted in two florid examples of the metabolic error. We do not feel justified, however, in advancing this thesis on such slender evidence. There was no consanguinity of parentage in any of the families and no family history of stone or of nephritis known to the parents of the two children investigated.

Clinical history.

W.B. Aged 12 years. First child in family of two. Admitted to Birmingham Children's Hospital on April 26th, 1932.

History. Quite well until 6 months previously; since then vaguely unwell; easily tired, frequently complaining of backache referred to both loins and relieved by lying down. For 3 months prior to admission scalding micturition but no increased frequency of micturition or other urinary symptoms. Occasional headaches for many years.

Examination. A tall slender girl: weight 5 stone 6 pounds: nutrition moderately good and general appearance one of health. Mentality normal. No abnormality of cardiovascular, respiratory, alimentary or nervous systems. B.P. 125/80. Kidneys neither enlarged nor tender.

Urine. S.G. 1030. Reaction slightly alkaline to litmus. Albumin: light cloud. Sugar: nil.

Deposit. Cystine crystals in large numbers in specimens which were allowed to stand, both before and after acidifying with acetic acid. Sullivan and nitroprusside tests both strongly positive for cystine.

X-ray of renal tract—no evidence of stone.

<i>Blood.</i>	Urea	(fasting)	30.9 mg./100 ml.
	Non-protein-nitrogen	"	30.3 "
	Uric acid	"	2.81 "
	Amino-acid-nitrogen	"	11.48 "
	Chlorides	"	459 "
	Cholesterol	"	182 "
	Creatinine	"	1.26 "

Urea concentration test (Calvert's) showed normal renal function:

15 g. urea given.

Specimen A. Volume 500 ml.: Urea 2.32 g./100 ml.

Specimen B. Volume 930 ml.: Urea 0.39 g./100 ml.

J.B. Aged 10 years. Second child in family. Admitted to Birmingham Children's Hospital, April 6th, 1932.

History. Always a delicate child though never suffering from any definite disease of any system. One year before admission commenced to complain of backache, was easily tired and had frequent frontal headaches. These symptoms continued. In November 1931 the child was noticed to be passing very little urine, was seen by Dr Burns of Redditch and found to have albuminuria. No history of haematuria or other urinary symptoms. She was thought to be abnormally thirsty at this time. Twelve months before admission the child had an acute febrile illness in which she vomited and developed a scarlatiniform rash but never peeled. The albuminuria persisted and the child was sent to hospital for investigation of renal function.

Examination. A tall slender girl—weight 4 stone 4 pounds—of healthy appearance, normal mentality and moderately good nutrition. Cardiovascular, respiratory and nervous systems normal. Many carious teeth present: alimentary tract otherwise normal. Kidneys neither enlarged nor tender.

Table I. *Investigation of cystinuria in blood relatives of patients.*

Hexagonal crystals in the urine																				
1st specimen			2nd specimen			3rd specimen			Sullivan's test*			Nitroprusside test*			Amino-acid-N* mg./100 ml.			Albumin		
specimen			specimen			specimen			2nd specimen			3rd specimen			2nd specimen				3rd specimen	
Relatives on Mother's side:																				
Mother, B. B.	2 or 3 crystals	-	-	-	+	+	-	-	+	+	2.8	6.4	Sl. haze							
Grandfather, J. A.	-	-	-	-	-	+	-	-	-	-	10.5	9.4	Sl. haze							
Grandmother, M. A.	-	-	-	-	+	+	-	-	+	+	10.0	11.7	-							
Aunt, G. T.	2 crystals	-	-	-	-	-	-	-	-	-	6.0	-	-							
Cousin, B. T.	-	-	-	-	-	-	-	-	-	-	8.75	-	-							
Cousin, I. T.	-	-	-	-	-	-	+	-	-	-	-	-	-							
Cousin, F. T.	-	-	-	-	-	-	+	+	-	-	12.2	-	-							
Relatives on Father's side:																				
Father, T. B.	-	-	-	-	+	+	-	-	-	-	14.5	14.4	-							
Grandfather	-	-	-	-	-	+	-	-	+	+	13.5	12.7	Sl. haze							
Grandmother	-	-	-	-	-	+	-	-	-	-	11.6	15.5	-							
Aunt, Al. B.	3 crystals	-	-	-	-	-	-	-	-	-	15.5	15.5	-							
Aunt, An. B.	-	-	-	-	+	+	+	+	+	+	7.24	11.7	-							
Uncle, E. B.	-	-	-	-	-	-	-	-	-	-	8.7	8.7	-							
Aunt, M. S.	4 crystals	-	-	-	-	-	+	+	+	+	8.7	8.7	-							
Cousin, J. S.	-	-	-	-	+	+	-	-	-	-	8.7	8.7	-							
Cousin, K. S.	3 crystals	-	-	-	+	+	+	+	+	+	14.7	14.7	-							
Cousin, M. S.	Many crystals	Many crystals	Many crystals	Many crystals	+	+	+	+	+	+	22.2	22.2	Haze							

* 1st specimen not tested.

+ denotes a trace colorimetrically.

+ + + denotes a definitely positive test.

Urine. On admission S.G. 1020. Reaction very slightly alkaline to litmus. Albumin: heavy haze. Sugar: nil.

Deposit. Cystine crystals in large numbers. Sullivan and nitroprusside tests for cystine both strongly positive.

X-ray of renal tract—repeated several times. On each occasion much colon gas was present but no stone was seen.

Blood. Wassermann reaction negative.

Urea	(fasting)	36.8	mg./100 ml.
Non-protein-nitrogen	„	36.5	„
Uric acid	„	2.15	„
Amino-acid-nitrogen	„	6.18	„
Cholesterol	„	170	„
Chlorides	„	482	„
Creatinine	„	1.27	„

A twenty-four hour specimen of urine—volume 373 ml.—collected when the child was having ordinary hospital diet showed:

Urea	1.72	g./100 ml.
Uric acid	0.051	„
Creatinine	0.098	„
Total nitrogen	1.60	„
Chlorides	0.61	„
Amino-acid-nitrogen	0.0297	„
p_H	7.05	„

Silver articles placed in contact with the skin of both children for periods of five days showed no discoloration, an observation which suggests that sulphur compounds were absent from the sweat of our patients. The urine of both children developed a greenish colour and gave off hydrogen sulphide and ammonia on standing.

Presence in the urine of tyrosine, leucine, putrescine and cadaverine.

The urine of both patients was examined repeatedly for the presence of leucine and tyrosine and for the diamines cadaverine and putrescine. Specimens were concentrated by evaporation *in vacuo* and the following tests performed:

Examination of the concentrate for crystals of leucine and tyrosine.

Tests with Millon's reagent.

Treatment of the concentrate with β -naphthalenesulphonyl chloride (method of Abderhalden and Shittenhelm [1905]).

Benzoylation method of Baumann and Udransky [1889].

Phenyl isocyanate method of Loewy and Neuberg [1904].

These tests failed to reveal the above amino-acids and diamines.

Test for the presence of a precursor of cystine in fresh specimens of urine.

A perusal of the literature on cystinuria reveals frequent references to the presence in the urine of cystine precursors, which usually break down quite rapidly with the liberation of free cystine, especially after the addition of dilute acetic acid. These may, however, from time to time prove more stable and resist the action of the somewhat hypothetical urinary ferments which are supposed to bring about the necessary chemical changes. Wallis and Garrod [quoted by Garrod, 1923] reported a case in which cystine could not be found by the Gaskell method, and which showed cystine crystals in the urine only after six months' standing. Garrod remarks "the inference cannot be avoided that under certain conditions cystinurics excrete a sulphur compound which is not free cystine but a compound or unoxidised derivative thereof".

From time to time various workers [Klemperer and Jacoby, 1915; Looney *et al.*, 1923] have reported a diminution in the cystine excretion (as determined colorimetrically) after the prolonged administration of alkali without significant changes in the neutral sulphur values, and these findings have been regarded as further evidence in favour of the excretion in freshly voided urine of a cystine complex and as an explanation of the phenomenon of intermittent cystinuria. This subject receives careful consideration by Brand *et al.* [1930] who, in their observations on a cystinuric, found that the freshly voided urine of their patient gave a negative Sullivan reaction, but after standing for 24 hours the brilliant red colour indicating a positive reaction had developed and this colour continued to increase in intensity for a further 24 hours before fading. In a similar experiment we have observed an almost exactly similar phenomenon in both of our patients. The red colour commenced to develop at the end of the first hour, deepened gradually during the next 48 hours and then slowly faded.

Metabolism experiments.

The source of the cystine excreted in the urine, whether of endogenous or exogenous origin, and the effect of diet on the amount of this excretion have been the points studied by various workers. The investigations here recorded were undertaken in order to test the effect of various diets on the excretion of urinary cystine, in an endeavour to discover a diet sufficient for the requirements of these growing children which was least productive of cystinuria and therefore less likely to lead to stone formation; simultaneously an attempt was made to locate the source of the cystine.

The feeding experiments carried out were as follows:

- (1) Ordinary hospital diet.
- (2) Ordinary hospital diet *plus* pure cystine.
- (3) Low cystine, medium protein diet.
- (4) High cystine, medium protein diet.
- (5) High cystine, high protein diet.
- (6) Ordinary hospital diet *plus* pure cystine for estimation of faecal loss of cystine.

Method. The experiments were carried out concurrently on the two patients and on a normal control—a boy aged 12 years, who had recovered from pneumonia a few weeks earlier and appeared to be in good health at the time of the investigation. Throughout the experimental period all these children were allowed to play in the ward and garden each day. All urine passed was collected in 24-hour specimens under toluene and subjected to the following estimations. Total nitrogen (micro-Kjeldahl method using Koch and McMeekin's modification of Nessler's solution, as quoted by Peters and Van Slyke [1932], ammonia (Folin's aeration method), urea (urease), uric acid [Folin, 1930], creatinine [Folin, 1914], amino-acid-nitrogen [Folin, 1922], cystine (Looney's method using Folin's [1930] uric acid reagents), sulphur in all forms [Fiske, 1921] and p_H (electrometrically, using the quinhydrone electrode or by indicators).

A summary of the results obtained is shown in Figs. 3, 4 and 5.

When this work was completed and the figures for the various periods analysed, it was found that there were well-marked daily variations in the output of various urinary constituents, particularly in total nitrogen, uric acid, creatinine, cystine and neutral sulphur. These diurnal variations were all in the same direction so that the graphs for each showed great similarity. Further, these daily variations coincided in the three children, suggesting that the same factor was operating in all three, and as this could not be of a dietetic nature it was thought to be the result of changes in metabolism associated with differing

amounts of exercise. Consequently we readmitted the younger child, J.B., for investigation of this point.

Effect of exercise. The patient was given a generous varied diet containing similar foodstuffs in the same amounts each day and supplying approximately 86 g. of protein and 2000 Calories daily. In addition to fluids taken with meals she was given 4 pints of lemonade daily, to ensure a diuresis and consequent rapid elimination of metabolic products.

The metabolism period commenced at 9 a.m. after a preliminary period of 21 hours in bed on the diet. During the first 48 hours the child remained at rest in bed. For the first 9 hours of the next 24-hour period she played around the ward without indulging in any strenuous games (indicated in Fig. 2 as "light exercise"). On the following day she was made to run around the garden and to skip until tired (indicated as "moderate exercise"). After a further period of complete rest in bed she was again exercised and the metabolism period was completed with 48 hours in bed. Fig. 2 shows the excretion of various waste

Fig. 2. J.B. Analyses of urine to demonstrate the effect of exercise.

A, at rest in bed; B, light exercise; C, moderate exercise.

products during this 8-day period. The first 24 hours yielded high total nitrogen and low sulphur values, and we regret that the child was not given a longer time to become stabilised before the urine was collected. The urea-nitrogen figures remained at a very constant level over the whole period, indicating the constant nitrogen intake. Light exercise was associated with increased excretion of cystine and neutral sulphur but did not affect the uric acid or creatinine figures. Moderate exercise resulted in a greatly increased excretion of uric acid, creatinine, cystine and neutral sulphur, so that the graphs for the output of these various bodies are almost identical. The figures for total nitrogen tend to show similar variations. The increased uric acid and creatinine excretions must result from an

increased tissue metabolism associated with exercise and it seems highly probable that the increased cystine excretion was also of endogenous origin. In the investigation of a case of cystinuria, Brand *et al.* [1930] noted an increased creatinine excretion on the day when their patient had 2 hours' exercise (hand-ball) and mentioned that Alsberg and Folin [1905] also discovered an increased excretion of creatinine associated with exercise. They did not comment on the fact that associated with this increased creatinine excretion there was a well-marked increase in the output of cystine in their patient. Robson [1929] also noted well-marked daily fluctuations in another case, in spite of careful control of the protein intake, which he was unable to explain. He did not mention whether his patient was in bed or not throughout the metabolism period and it may be that exercise was the factor responsible for his results.

The importance of the exercise factor was not appreciated at the time when our feeding experiments were carried out and the children were not confined to bed. The exercise taken, although varying a little from day to day, was never strenuous and, in our opinion, was not sufficient to affect seriously the average figures for a 5-day period.

Feeding experiments.

Experiment 1. Hospital diet with a medium protein and low cystine content. After a preliminary period of 2 weeks on this diet the metabolism of nitrogen and sulphur was followed over a 5-day period and it was then noticed that the albuminuria which was present in both cystinurics on admission to Hospital had almost disappeared. The cystine excretion at this time was low and a remarkable feature of the investigation was the increased albuminuria which occurred later when the patients were having a high protein diet and the output of cystine was increased. This phenomenon was observed in both children, the degree of albuminuria reflecting quite accurately the degree of cystinuria. The control child was always albumin-free. Orthostatic tests carried out on the cystinurics were negative.

Another striking feature was the high p_H of the urine of both cystinurics throughout the whole of the metabolism period. The 24-hour specimens of urine were collected under toluene, kept under exactly the same conditions, and the p_H was estimated at the earliest possible opportunity. The figures for the control child varied between 5.2 and 7.3, with an average of about 6 for the whole period, and the ammonia values were low. The urines of the cystinurics were much more alkaline, occasionally reaching figures as high as p_H 9. The highest figures corresponded with the peaks in the graph for ammonium compounds, although even when the ammonia values were low and corresponded with those of the control child, p_H values of between 7 and 8 were frequently found; further, the alkalinity of any specimen from the cystinurics increased considerably on standing, although fresh specimens of urine examined from time to time showed this same tendency to alkalinity. We are unable fully to explain this phenomenon. The children were observed over a long period of time, their urinary tracts appeared to be free from infection, they were not given medicines or other preparations which might have contained alkalis, and the experiment was adequately controlled. This peculiarity may have been of very great practical importance in preventing calculus formation in the two children.

The excretions of nitrogen and urea are very similar in the cystinurics and the control. The sisters show high amino-acid-nitrogen and neutral sulphur values, as would be expected.

Experiment 2. Hospital diet plus cystine. To Diet No. 1 pure cystine (prepared from animal hair and supplied by B.D.H. Ltd.) was added in the following amounts:

1st day	1 g.	14 g.
2nd day	2 g.	
3rd day	4 g.	
4th day	7 g.	

On the 5th, 6th and 7th days no cystine was added.

The average figures for the first 5 days of this period, described here as the "cystine ingestion period", together with those for the following 2 days when the children were again on hospital diet only, are shown in the figures. In the case of

Fig. 3. J.B. Graphs showing urinary excretion of products of metabolism.

1. Exp. 1, hospital diet; 2. Exp. 2, cystine ingestion period; 3. hospital diet; 4. Exp. 3, medium protein, low cystine; 5. Exp. 4, medium protein, high cystine; 6. Exp. 5, high protein, high cystine.

W.B. (Fig. 4) the output of cystine was raised very slightly during the ingestion period and still higher figures were found on the 2 subsequent days, but this small increase is probably of no significance, especially as the figures for neutral sulphur were not increased. The output of inorganic sulphur was doubled during the ingestion period but returned to a level lower than the average for that period on the 2 following days. A calculation shows that about 60 % of the added cystine was metabolised during the 7 days. The fate of the residual 40 % formed

the object of an experiment carried out when all the dietetic investigations were completed, but which may conveniently be mentioned here. W.B. was given the weighed hospital diet and after a period of several days on this regime, in which to become stabilised, 4 g. of cystine were given by mouth. The increased output in the faeces, estimated colorimetrically, amounted to 1.43 g., representing 36 % of that ingested. It would appear, therefore, that cystine given as such was oxidised quantitatively to inorganic sulphate and that the mechanism of oxidation was not at fault.

Fig. 4. W.B. Graphs showing urinary excretion of products of metabolism.
1, 2, 3, etc. as in Fig. 3.

In the case of J.B. (Fig. 3) the output of cystine was slightly less than in Exp. No. 1 and only 47 % of the amount ingested was metabolised. The control (Fig. 5) oxidised 77 % of his ingested cystine and in addition showed a slight increase in neutral sulphur excretion. On 2 days his urine gave a slight coloration with the nitroprusside test for cystine.

Robson [1929], in a similar investigation of a male cystinuric aged 23 years, who was given 20 g. of cystine by mouth over a period of 4 days, found that 85 % of the ingested cystine was broken down and excreted as inorganic sulphate. The absence of any increase in urinary cystine during the ingestion period is in accord with the findings of most other investigators. Lewis and Lough [1929] calculated that the cystinuric investigated by them was able to oxidise about

64 % of the pure cystine ingested, the remainder being lost by failure of absorption or by destruction in the bowel by micro-organisms. Our results demonstrate the failure of absorption from the bowel but do not suggest any breakdown of cystine by micro-organisms.

Experiment 3. Low cystine, medium protein diet. After a few days' return to hospital diet the patients were given one consisting of one pound of apples and three pints of milk daily. This provides 5.8 g. protein, 0.6 g. sulphur (including, at most, 0.40 g. cystine) and 1390 Calories. After 3 preliminary days on the diet

Fig. 5. Control. Graphs showing urinary excretion of products of metabolism. 1, 2, 3, etc. as in Fig. 3.

urine was collected for a further period of 5 days. On this low cystine but medium protein intake the output of cystine was considerably raised in both cystinurics, resulting in a negative cystine balance of 0.8 g. per day in W.B. and 0.6 g. per day in J.B. Our results are in accord with those of Lewis and Lough [1929] who conducted a similar experiment, and additional evidence is furnished of the endogenous origin of a significant portion of the cystine excreted.

In the case of W.B. there was a lessened excretion of total and neutral sulphur but approximately the same output of inorganic sulphate as on the basal diet. J.B. did not appear to absorb the milk protein as well as that of a mixed diet, indicated by a lessened urinary nitrogen output. At the same time poor absorption of protein cystine resulted in a diminished inorganic and total sulphur

excretion. On this diet, therefore, both patients appeared to be excreting very largely endogenous cystine, the low cystine intake being reflected in the low sulphur output.

That the excretion of endogenous cystine was stimulated by increased protein intake is confirmed by the results of feeding with a high protein diet.

Experiment 4. High cystine, medium protein diet. This consisted of one pint of milk, four eggs, half-a-pound of apples, four ounces of brown bread and one ounce of butter daily, and provided 53 g. of protein, 1271 Calories and 0.76 g. of sulphur—mostly cystine sulphur. The protein content was very similar to that of the previous diets. The average figures for the 5-day period show neither increased amino-acid nor cystine output. The control appeared to metabolise the added sulphur to inorganic sulphate, but either the cystinurics failed to absorb the extra cystine of the sulphur molecule, as the amino-acid itself or as some precursor, or, alternatively, they absorbed and stored it. In any case, the cystine output was not affected by the cystine intake.

The fall in the figures for uric acid and creatinine in Exps. 3 and 4 may be explained as the result of a vegetarian diet.

Experiment 5. The protein intake was doubled by adding eight ounces of cooked lean meat to Diet No. 4, the cystine content remaining practically at the same level. The cystine output was doubled in the two sisters and at the same time more sulphur was oxidised, as indicated by the rise in total and inorganic sulphur values. These rises were associated with similar increases in the figures for total nitrogen, urea, uric acid and creatinine. The results of this experiment are in conformity with those obtained by Robson [1929], who found that on a high protein diet the increased elimination of cystine was in almost direct proportion to the increased nitrogen excretion. We are unable to calculate the cystine intake on this diet, but it seems highly probable that there was again a negative cystine balance and that the output was of endogenous origin and increased by a high protein diet. Lewis and Lough [1929] reached a similar conclusion in a comparison of the effects of diets on varying cystine and protein values; these workers found that the excretion of cystine varied with the total nitrogen excretion and did not appear to be related to the cystine content of the diet.

It is generally recognised that diets rich in animal protein have a decided effect in stimulating tissue metabolism and raising the metabolic rate; this effect is thought to be due to the action of certain of the amino-acids resulting from such protein digestion which stimulate cellular activity. Further evidence on this point is furnished by our cases which show increases in uric acid and creatinine excretion more than twice as high as those obtained on the previous diet (No. 4) of half the protein value and therefore, partially at least, of endogenous origin, the result of increased tissue breakdown. The figures for neutral sulphur in the child W.B. showed no rise corresponding to the increase in the figures for cystine as estimated colorimetrically—in fact the graph for neutral sulphur throughout the metabolism period shows a steady fall. In the case of J.B. the graphs for neutral sulphur and cystine correspond more closely. The cystine-sulphur excretion as calculated from the cystine (estimated colorimetrically) was, on the whole, higher than the total neutral sulphur; an observation made by many previous workers, each of whom concluded that the colorimetric estimation tends to give unduly high readings. Estimation of cystine in the previous 24 hours' urine was carried out each day at approximately the same time; hence, at the time of the examination, the breaking down of any cystine precursors present in the freshly voided specimens should have proceeded to about the same extent. We therefore regard these figures as a more reliable

indication of cystine excretion than those for neutral sulphur, even though they may give inaccurate absolute values. The neutral sulphur figures for W.B. are probably inaccurate.

The undetermined nitrogen varied throughout the metabolism periods. Figures as high as 33 % were reached by W.B., 25 % by J.B. and 29 % by the control, and over the whole period the undetermined nitrogen was somewhat higher in the control child than in either cystinuric.

DISCUSSION.

The subjects of this investigation show many interesting features in common. Both were admitted with albuminuria which was not of the orthostatic type or associated with clinical evidence of renal insufficiency or other evidence of kidney damage, and which increased or decreased (to the point of disappearing) with increased and diminished output of urinary cystine. These observations are of great interest and may be of importance in view of the work of Cox *et al.* [1929], Newburgh *et al.* [1925], and Curtis and Newburgh [1926; 1927], who fed young rats on synthetic diets containing free cystine and produced in their animals an acute nephritis from which many of them died. Newburgh and Curtis [1927], by feeding rats on diets rich in caseinogen, beef protein and lactalbumin, were able to demonstrate the markedly nephropathogenic nature of the lactalbumin—a protein with a very high cystine content (4 %).

Aberdalden [1903] described an infant, one of a family of cystinurics, who died of malnutrition at the age of 21 months. At autopsy the internal organs were found to contain crystalline deposits of cystine visible to the naked eye and in many ways similar to the deposits of sodium biurate in gout. The same writer [1922] observed kidney damage in rats ingesting free cystine and tyrosine and was able to demonstrate crystals of these amino-acids in the kidney tubules. It is difficult to reconcile these animal experiments with the results of feeding cystine to normal adults and to cases of cystinuria. Nevertheless, it seems probable that, in our patients, the excretion of the greater quantities of this abnormal urinary constituent, possibly associated with deposition of crystals in the renal tubules, was associated with some renal damage and a leak of protein into the kidney tubules. The urinary cystine in our patients we believe to be entirely endogenous. The exercise experiment demonstrates the importance of this factor in affecting the cystine output. Feeding isolated cystine showed that the amino-acid is completely oxidised to inorganic sulphate, even when administered in large quantities, a finding which is in agreement with those of other writers [Hele, 1909; Looney *et al.*, 1923; Robson, 1929]. Feeding with a diet which contained a high content of cystine sulphur in the natural foodstuff did not result in any greater output of cystine than when the children were given one with a low cystine value and similar protein content, but there is no evidence that the extra cystine of the diet was absorbed or in any way metabolised. We submit that the increased excretion of urinary cystine on a diet high in animal protein is of endogenous origin, the result of increased tissue metabolism due to the specific dynamic action of that protein and not derived from the cystine of the ingested protein molecule. The practical importance of this observation is obvious and our patients were sent home on a diet of low protein content, which they had taken conscientiously until they were seen again recently—two years after the original investigations were carried out. Both children were apparently quite well and free from any symptoms referable to the urinary tract. There was no evidence of stone formation or urinary infection.

With the co-operation of the parents, 24-hour specimens of urine were collected from each of the two girls when they were at home taking their usual diet and indulging in their normal activities. That from the elder child, W.B., was free from albumin and contained 9.45 g. of total nitrogen and 0.585 g. of cystine (colorimetric). The specimen from J.B. showed a faint haze of albumin (far less than when first seen two years previously), 5.1 g. of total nitrogen and 0.442 g. of cystine—all of them reasonably low figures.

The high p_H of the urine, which formed such a notable feature of this investigation, has already received consideration, and in view of this finding it was considered unnecessary to try the effect of administration of alkalis. For this reason also, many specimens of urine had first to be acidified with dilute acetic acid and allowed to stand before cystine crystals appeared, and the greater solubility of cystine in an alkaline solution may explain the fact that the subjects of this study, although passing relatively large amounts of the amino-acid, remain free from calculus formation.

SUMMARY.

Two cases of cystinuria occurring in sisters are recorded, together with the results of the examination for cystinuria in other members of the family.

With the aid of the Sullivan reaction, evidence is presented which indicates that in these patients a precursor of cystine was excreted into the urine, which later decomposed with the liberation of free cystine.

Other abnormal constituents—tyrosine, leucine, putrescine and cadaverine—were absent from the urine of these subjects.

Both passed urine with an abnormally high p_H and it is suggested that this may explain their freedom from stone.

Evidence is brought forward to show that the excretion of large amounts of cystine may bring about a certain amount of kidney damage, producing an albuminuria which varies with the amount of cystine excreted.

Cystine prepared from hair when administered by mouth did not produce an increased excretion of cystine in the urine but was oxidised quantitatively to sulphates. Much of it was unabsorbed and excreted in the stool.

Foodstuffs with a high cystine content did not cause increased cystinuria; the extra cystine may have remained unabsorbed or have been absorbed and stored but was neither oxidised to sulphate nor excreted in the urine as such.

The endogenous origin of some of the excreted cystine is demonstrated, and the effect of a high animal protein diet in increasing the cystinuria is explained as the result of a stimulation of tissue metabolism with liberation of more endogenous cystine.

We suggest that cases of cystinuria should be given a diet containing only small amounts of animal protein.

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