METABOLIC STUDY OF AN ALKAPTONURIC INFANT

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Alkaptonuria is an extremely rare condition, which is due to a defect in protein metabolism. The most probable explanation is that it is due to the absence of the particular enzyme which normally effects a particular stage in the complete destruction of tyrosine and phenyl-alanine. Alkapton is the intermediate product beyond which metabolism can no further go, and it is excreted as such in the urine. The defect is congenital and it is inherited, although the line of inheritance is difficult to trace. Transmission is seldom direct from parent to child, but several children in one family may be affected. Consanguinity between parents seems to have some responsibility for its occurrence. It is probably a Mendelian recessive characteristic. It is more common in males than in females. It may give rise to pigmentation of the cartilages and to arthritis.

Very little has been written on the subject since the publication of Sir Archibald Garrod's¹ book on 'Inborn Errors of Metabolism' in 1923. There he recounts the development of the knowledge of this metabolic disorder since the discovery in 1858 by Bödeker of a second reducing substance in the urine of a diabetic. He called it alkapton. In 1891, Wolkow and Baumann showed that the reducing substance was homogentisic acid, with the empirical formula $C_8H_8O_4$. This has been confirmed by several other workers, and its constitution definitely proved to be that of hydroquinone-acetic acid, with the following formula :—

Homogentisic acid is derived from those proteins which are rich in tyrosin and phenyl-alanine. The administration by mouth of tyrosin or phenyl-alanine, or of proteins rich in these substances, to an alkaptonuric, leads to an increased output of alkapton in the urine. During starvation the amount

of alkapton in the urine diminishes but does not disappear entirely. Some is derived, it is therefore deduced, from body protein also. On a more or less average mixed diet, the amount of alkapton in the urine bears a constant relationship to the total amount of nitrogen excreted in the urine. From the investigations made by different workers on different alkaptonurics, this ratio has been found to be roughly as 40: 100. But Gross and Allard, whose patient's diet contained a large quantity of milk, found the ratio to be 60-70: 100. Most of the cases investigated have been adults, but in 1911 Katsch³ published a full report on the nitrogen metabolism in an alkaptonuric child 3 years of age. His findings correspond to those of other workers, but with this exception: during a starvation period of three days, the homogentisic acid output fell, until on the third day it disappeared completely from the urine. There was, during this period, a corresponding rise in the output of acetone. A similar result was obtained during a period in which a diet of carbohydrate and fat with little protein, was given. Katsch assumes that in his case the homogentisic acid is further oxidized to acetone. That this may be so, there is some confirmation from Embden's experiment, in which he shows that if homogentisic acid is perfused through a normal liver it is converted to aceto-acetic acid.

Two questions seem to require answers:—

(1) Is homogentisic acid an intermediate product of normal metabolism, or is an alternative path in the metabolism of the aromatic-protein complex followed in an alkaptonuric?

(2) Does the degree of inability to metabolize the aromatic-protein complex vary; or, in other words, is the error maximal?

That it is an intermediate product of normal metabolism is supported by the fact that normal individuals can ingest homogentisic acid without producing alkaptonuria. It is also supported by Katsch's findings; but these, obviously, confute the suggestion that the error is maximal.

Dakin⁵ especially holds to the view that the formation of homogentisic acid is abnormal, and that a path other than normal has been followed in the metabolic process. He seems to have proved, at least, that alternative paths exist.

Garrod supports the intermediate product theory, and holds to the view that the error is maximal, basing his opinion on the constancy of the ratio of homogentisic acid to nitrogen; but he says: 'If in alkaptonuria the error were complete and maximal, all subjects of that anomaly, when fed upon a given standard diet, should excrete equal quantities of homogentisic acid, and changes in quantities consumed should leave the ratio of homogentisic acid to nitrogen undisturbed. . . . It would be necessary to place a number of alkaptonurics upon a standard diet, in which not only are the proteins the same, but in which also the several proteins are in uniform proportions. These conditions would be best fulfilled by milk alone.' Further he says: 'If it can be shown that, on absolutely the same diet, the homogentisic acid to nitrogen ratio is far above the average in some cases . . . it will be proved that the uniformity of the homogentisic acid to nitrogen ratio does not indicate complete conversion of tyrosin and phenyl-alanine into homogentisic acid,

We shall be driven to conclude that the katabolism of some part of these aromatic fractions of proteins of the food and tissues habitually follows some other path than that through homogentisic acid.'

CASE REPORT.

Gordon M., born on 29th August 1928, and first seen in January 1929, is the first child of healthy parents, who are not consanguineous, and who do not know of the existence of the anomaly under discussion in any of their antecedents. His mother stated that the child's urine had been very dark at intervals of several days since birth. She also stated, with conviction, that the child had attacks of abdominal pain at those times when the urine passed was not so dark. She brought with her a napkin stained a deep chocolate colour, and she had the wisdom to bring the child unwashed. He looked perfectly healthy and well-developed, presenting no abnormality except a deep brown staining of the thighs and lower abdomen. A specimen of urine obtained gave a positive reaction to tests for alkapton.

He has been in hospital on two occasions during which were made the investigations whose results are set out below: from 26th February until 23rd March, when his weight varied from 12 lb. 10 oz. to 12 lb. 14 oz.; and from 22nd May to 26th June, when his weight varied from 13 lb. 12 oz. to 13 lb. 8 oz. He attended the out-patient department in the interval. He was in very good health until the first week in May, when he became peevish, looked pale and unwell, and his mother complained that he had attacks of pain. This state continued for about four weeks, and no cause was found except that he was cutting teeth. Some of his pain may have been associated with micturition.

The urine varied in colour, sometimes dark when passed, sometimes dark on standing, but at no time, except during starvation, was alkapton absent. By 'dark' is meant a pinkish, smoky brown colour, which gave place rarely to a deep chocolate colour. On many occasions the appearance of the urine did not suggest any abnormality to the uninitiated, and it is quite easy to understand why a life-long history of the abnormality is not always obtained. His Wassermann reaction was negative.

METABOLIC INVESTIGATIONS.

The investigations were made during seven periods, and to explain each period in turn seems to be the simplest plan.

Period 1. March 7—13. Diet=Milk, 24 oz.—For seven consecutive days he was fed on milk only. The urine was collected continuously and removed every 3 hours, and the total nitrogen and total alkapton excretion in 24 hours estimated. The presence or absence of acetone was noted daily (Table 1).

TABLE 1. MARCH 7—13.

In grammes	7th	8th	9th	10th	11th	12th	13th	Average
Total N. in 24 hr.	1.33	1.53	1.67	1.89	1.30	1.17	1.43	
Total Homogent. Ac. in 24 hr. ...	.79	.75	1.01	1.07	.83	.75	.76	.85
Ratio $\frac{H.A.}{N.}$...	$\frac{59}{100}$	$\frac{49}{100}$	$\frac{73}{100}$	$\frac{56}{100}$	$\frac{64}{100}$	$\frac{64}{100}$	$\frac{53}{100}$	$\frac{60}{100}$
Acetone ...	—	—	—	+	trace	—	—	

Period 2. March 14—20. Diet=Milk, 30 oz. and egg yolk, 2 dr.—On March 14th, egg yolk was added to the milk, and the amount of milk was increased. Egg yolk is rich in tryosine, and we would expect that the output of alkapton would be increased. Estimations as before were made, after the child had been on this diet for three days (Table 2).

TABLE 2. MARCH 18—20.

In grammes	18th	19th	20th	Average
Total N. in 24 hours	1.40	1.83	2.46	
Total Homogent. Ac. in 24 hr. ...	.73	1.01	1.69	1.14 grms.
Ratio $\frac{\text{H.A.}}{\text{N.}}$	$\frac{52}{100}$	$\frac{55}{100}$	$\frac{69}{100}$	$\frac{59}{100}$
Acetone	trace	—	—	

The child was at home from 21st March to 22nd May, when his diet consisted of milk and carbohydrate. Potassium iodide, which is said to diminish the output of alkapton in the urine, was prescribed, but as his mother omitted to give it during the week before his re-admission to hospital, we repeated this experiment at a later date.

Period 3. May 22—31. Diet = Milk 30 oz.—After re-admission, the child was given milk only, and examinations made as before on the last three days of this period (Table 3).

TABLE 3. MAY 29—31.

In grammes	29th	30th*	31st	Average (29th & 31st)
Total N. in 24 hours	1.27	1.08+	1.93	
Total Homogent. Ac. in 24 hr. ...	.5	.35+	.60	.55
Ratio $\frac{\text{H.A.}}{\text{N.}}$	$\frac{38.9}{100}$	$\frac{32.8}{100}$	$\frac{30}{100}$	$\frac{34}{100}$
Acetone	—	—	—	

*Some urine lost.

Period 4. June 1—6. Diet = Milk, 30 oz. and yolk of 1 egg.—Egg yolk was now added to the diet, but no alteration was made in the quantity of the milk. After three days, specimens were again collected and similar estimations made (Table 4).

TABLE 4. JUNE 4—6.

In grammes	4th	5th	6th	Average
Total N. in 24 hr.	2.5	2.58	2.6	
Total Homogent. Ac. in 24 hr. ...	.71	1.16	1.15	1.01
Ratio $\frac{\text{H.A.}}{\text{N.}}$	$\frac{28.2}{100}$	$\frac{45.1}{100}$	$\frac{48.1}{100}$	$\frac{40.5}{100}$
Acetone	trace	trace	—	

As already stated, before the time of his re-admission up to this point, the child was not so well as previously, and he lost weight. He was peevish and looked pale and out of sorts. There was no fever, and no explanation found besides the fact that he had cut his third, and had almost cut his fourth tooth. From May 31 to June 6, the stools were large, although there was not a frank diarrhoea,

Period 5. Diet=Water.—In order to see whether in our case, as in Katsch's case, alkapton would be replaced by acetone during starvation, water only with a small quantity of sugar was given for 36 hours. Urine was collected continuously and removed every three hours. The presence or absence of alkapton in each specimen was determined by the addition of a few drops of ammonia to 1 c.cm. of urine. If alkapton is present a brown colour is at once obtained, and the depth of the colour is an indication of the amount of alkapton present. We noted a gradual diminution in colour until in the 11th specimen it was entirely absent. No urine was passed in the last three hours. At the end of 36 hours starvation, milk was again given, and within three hours the deep colour of the urine returned when ammonia was added. Other estimations were made as before (Table 5).

TABLE 5. JUNE 6—8.

In grammes	6th—7th	7th	7th—8th
	Water 24 hr.	Water 12 hr.	Milk 12 hr.
Total N. in 24 hrs.	1.31	.23	.59
Total Homogent. Ac. in 24 hr....	.02	.007	.29
Ratio $\frac{H.A.}{N.}$	$\frac{1.7}{100}$	$\frac{3.1}{100}$	$\frac{49.4}{100}$
Acetone	+	trace	

Katsch found that homogentisic acid was absent from the urine in the third day of starvation in his child 3 years of age. In our case, homogentisic acid disappeared by the middle of the second day. We did not find, however, that the output of acetone in the urine or in the breath was any greater at this time than at any other time. Had we not examined the urine at frequent intervals, the fact that homogentisic acid was absent after 30 hours' starvation would have been missed. A striking feature at this point is the prompt return of homogentisic acid to the urine on the administration of a homogentisic-acid-producing food.

Period 6. June 8—14. Diet=Milk 32 oz.—The child was now in much better health, and was again given a diet of milk. After three days, the total urine output was collected on three successive days, and the same estimations repeated (Table 6).

TABLE 6. JUNE 12—14.

In grammes	12th	13th	14th	Average
Total N. in 24 hr.	2.66	2.60	3.13	
Total Homogent. Ac. in 24 hr. ...	1.53	1.38	1.73	1.55
Ratio $\frac{H.A.}{N.}$	$\frac{58}{100}$	$\frac{54}{100}$	$\frac{55}{100}$	$\frac{56}{100}$
Acetone	trace	—	—	

Period 7. June 15—25. Diet=Milk, 32 oz. Potassium iodide $1\frac{1}{2}$ gr.—To decide the possible responsibility of potassium iodide for the low figures obtained during Periods 3 and 4 the child was now given $1\frac{1}{2}$ gr. daily for a week, and on the same diet, estimations were again made for comparison with those in Table 6.

TABLE 7. JUNE 24 AND 25.

In grammes	24th	25th	Average
Total N. in 24 hr.	2.32	2.79	
Total Homogent. Ac. in 24 hr. ...	1.27	1.82	1.54
Ratio $\frac{\text{H.A.}}{\text{N.}}$	$\frac{55}{100}$	$\frac{65}{100}$	$\frac{60}{100}$

CONCLUSIONS.

When the child was in good health and having a diet of milk only, the daily output of homogentisic acid increased according to the total intake of milk: .75 to 1.07 grm. when having 24 oz., and 1.53 to 1.82 grm. when having 32 oz. The ratio to nitrogen remained practically constant, averaging in the first case 60 to 100, and in the second case 56 to 100.

While the child was unwell and on a milk diet, the output of homogentisic acid fell to .55 grm. daily, and its ratio to nitrogen fell to 34 to 100. That this was not due to the administration of potassium iodide may be accepted, for a similar fall was not repeated after a week's continuous administration of potassium iodide. The ratio then remained at 60 to 100. It is more probably an indication of the general metabolic upset due to teething, for it is associated with a loss in weight and with a fall in nitrogen retention, although the three things are not quite simultaneous.

In our first experiment with egg yolk, very little was given, and the result was further confused by the fact that the amount of milk was increased. In our second experiment, these mistakes were avoided, and the results obtained show a definite increase in the total daily output of homogentisic acid, and a rise in its ratio to total nitrogen. This is in agreement with the findings of previous workers.

During starvation, homogentisic acid disappeared from the urine after 30 hours. Katsch found that it disappeared in the case of a child of 3 years on the third day. In adults, alkapton is said to persist in the urine during starvation. May this not indicate the degree of retention in older patients rather than the production of alkapton from the body proteins? During starvation, also, we did not note a degree of acetonuria greater than at any other time.

FURTHER EXAMINATION OF THE URINE.

The nitrogen partition in the urine of normal infants is a subject on which opinions vary, but that the urea nitrogen is high seems to be a common finding. Abt⁶ quotes Camerer, Talbot, and Meyer and Rietschel, whose figures range from 60—83 per cent. of total nitrogen.

In alkaptonuria, Gibson and Howard¹ found low urea nitrogen, high ammonia nitrogen, high uric acid nitrogen, and high undetermined nitrogen. They quote Rayold and Warren as finding the urea below the average and

the uric acid diminished. Lusk, in his 'Science of Nutrition,' writes 'the distribution of the several nitrogenous constituents in alkaptonuria is said to remain normal.'

The figures which we give have been obtained from examination of the total urine on each of six days, while the child was having milk only. Accepting Abt's figures as normal, in our alkaptonuric the urea nitrogen is certainly low and the ammonia nitrogen is not high. The uric acid nitrogen is high, but the presence of homogentisic acid may have rendered this estimation inaccurate. The undetermined nitrogen is high.

TABLE 8. DIET=MILK.

	Volume c.cm.	Total N. in grm.	Urea N.	Ammon. N.	Uric acid N.
29th May	285	1.268	.356	.106	.151
30th „	225+	1.075+	.279+	.057+	.110+
31st „	427	1.931	.425	.137	.167
Average % of total N.			24.8%	7%	10%
12th June	641	2.658	1.069	.191	.317
13th „	780	2.593	.568	.417	.460
14th „	750	3.131	.738	.754	.540
Average % of total N.			28.6%	15.8%	15.7%

The relative rate of excretion of nitrogen and homogentisic acid is also disputed. Some workers have found that a fall in nitrogen excretion accompanies a rise in homogentisic acid excretion; while others have found that a rise in the one is accompanied by a rise in the other. Katsch found that during three days of protein-free diet, the nitrogen excretion rose while the homogentisic acid excretion fell, and when the protein was added to the diet, the nitrogen excretion fell while the homogentisic acid output rose. Reference to our tables shows that a rise in homogentisic acid is always accompanied by a rise in nitrogen in the urine, while a fall in homogentisic acid is accompanied by a fall in nitrogen. The greatest variation is shown in the starvation period: both fall, but the homogentisic acid falls much more than the nitrogen. This may be explained by the finding of Abderhalden and Block (quoted by Lusk²) that the addition of water to an otherwise constant diet caused an increase in the nitrogen of the urine, due to a washing-out of nitrogen end-products. There is, presumably, in this case, little or no retention of homogentisic acid in the tissues, for it disappeared from the urine after 30 hours' starvation. The return of homogentisic acid to the urine within 3 hours of the administration of milk, shows the rate of metabolism of the aromatic fraction of the protein and rate of excretion. The prompt return of the H.A. to N. ratio to that found immediately before 36 hours' starvation, suggests that metabolism of all protein fractions occurs simultaneously (Table 9).

TABLE 9.

In grammes	Milk 24 hr. 5—6 June	Water 24 hr. 6—7 June	Water 12 hr. 7 June	Milk 12 hr. 7—8 June
Nitrogen ...	2.6	1.31	.23	.59
HA. ...	1.15	.02	.007	.29
HA. to N. ...	48.1 : 100	1.7 : 100	3.5 : 100	49.4 : 100

The acidity of the three hourly specimens of urine, 100 in all, was determined electrometrically, and it was found that the average pH range was from 5.43 to 6.7. On one occasion it was 7.16 and on one occasion 9.15. Lusk gives the normal range as 5.1—7.2. In alkaptonuria, then, the acidity is slightly greater than normal.

The depth of colour of the urine did not bear any constant relationship to its acidity, although the darkest specimen was that which had a pH of 9.15. The high acidity may be the reason for the dysuria, which certainly occurs from time to time.

PROTEIN METABOLISM.

Complete protein metabolism was studied during four periods of three days each—Period 1 (last 3 days), period 2, period 3, and period 4.

During periods 1 and 2, when the child was in good health, the average nitrogen intake was 4.02 gm., the average nitrogen absorbed was 93.75 per cent., and the average nitrogen retention 53.95 per cent.

During periods 3 and 4, during which time the child was unwell because of teething, the nitrogen retention fell to a low figure on the third day of Period 3, and remained low during the whole of period 4. The average nitrogen intake was 3.49 gm., the average nitrogen absorbed was 91.1 per cent., and the average nitrogen retention was 30.42 per cent. It is necessary to draw this distinction, in order to emphasize the point which we made concerning the low output of homogentisic acid during the two latter periods. The low total nitrogen retention, low homogentisic acid output, and loss in weight seem to be inter-related, although they are not entirely simultaneous (Tables 10 and 11).

During health, the nitrogen absorption and retention appear to be within normal limits, if compared with the results obtained in a hitherto unpublished study by one of us (E. M. H.) of the protein metabolism of a group of 23 babies fed on cow's milk. In them the average percentage absorption of nitrogen was found to be 88, with a range of 96—74, and the average percentage retention 49.7, with a range of 66—28.

TABLE 10.

		Nitrogen in grm. Av. total intake	Average total absorp- tion	Average total reten- tion	Average % absorp- tion	Average % reten- tion
In health	Period 1. 11—13 March ...	2.76	3.53	2.2	93.9%	58.6%
	Period 2. 18—20 March ...	4.27	4.03	2.105	93.6%	49.3%
Teething	Period 3. 29—31 May ...	3.54	3.05	1.54	91.4%	44.3%
	Period 4. 4—6 June ...	3.45	3.13	.57	90.8%	16.55%

TABLE 11.

Diet	Milk		Milk + egg yolk		
	30th May	31st May	4th June	5th June	6th June
HA. to N. ...	32.8 : 100	30 : 100	28.2 : 100	45.1 : 100	48.1 : 100
% N. retention	50.8	25.85	23.1	9.3	17.26
Weight ...		13 lb. 8 oz.			13 lb. 6 oz.

SUMMARY.

1. A case of alkaptonuria in an infant has been studied.
2. In health, on purely milk diet varied quantitatively, the homogentisic acid to nitrogen ratio is 55—60 : 100. This is very similar to the ratio found by Gross and Allard, whose patient, an adult, had a largely milk diet. It supports the 'maximal error' theory.
3. When the nitrogen retention fell, the homogentisic acid to nitrogen ratio fell to 34 : 100.
4. After 30 hours' starvation, homogentisic acid disappeared from the urine. Acetone did not take its place.
5. The output of homogentisic acid was not diminished by the administration of potassium iodide.
6. There is no evidence to show that homogentisic acid is other than an intermediate product of normal metabolism.
7. The nitrogen partition in the urine differs from the normal. Most notable is the low urea nitrogen.
8. The acidity of the urine is slightly higher than normal.
9. Total nitrogen absorption and retention is normal.

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