CLXVIII. METABOLISM OF SULPHUR

VI. OXIDATION IN THE BODY OF THE SULPHUR-CONTAINING AMINO-ACIDS AND SOME OF THEIR PARTIALLY OXIDIZED DERIVATIVES[1]

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It has been shown repeatedly that the sulphur of *l*-cystine, *l*-cysteine and *l*-methionine is oxidized to sulphate when the free amino-acids are ingested by a normal person. Such experiments for the most part have been carried out with urine samples collected over periods of relatively long duration, i.e. several hours to 24 hr., a procedure which does not offer opportunity for comparison of the relative rates of oxidation of respective compounds or for the detection of minute amounts of the mother substance which may have escaped for brief periods into the urine.

Although considerable light is being thrown upon the probable course of oxidation of these compounds in the human organism by numerous recent studies of cystinuria, the question is pertinent as to how far the conclusions from such cases can be transferred to the normal subject. The following experiments with cystine, cysteine and methionine were therefore done in the hope of obtaining further insight into the metabolic relationships of these substances in the normal individual. In addition various oxidation products were employed similarly to determine, if possible, from their rate and degree of oxidation, whether they may be normal intermediates in the physiological oxidation of the three naturally occurring amino-acids.

Experimental procedures

Plan of the experiment. The various sulphur-containing compounds were ingested successively by a normal subject, the experiments being modelled upon the following plan:

6 p.m.–6 a.m.	Control period, A
6 a.m.–8 a.m.	Control period, B (meal)
8 a.m.	Ingestion of compound containing 345 mg. S
8.15 a.m.	Ingestion of compound containing 345 mg. S
8 a.m.–9 a.m.	Period 1
9 a.m.–10 a.m.	Period 2
10 a.m.–11 a.m.	Period 3
11 a.m.–1 p.m.	Period 4 (meal)
1 p.m.–3 p.m.	Period 5
3 p.m.–7 p.m.	Period 6
7 p.m.–12 midnight	Period 7 (meal)
12 midnight–6 a.m.	Period 8
6 a.m.–8 a.m.	Control period C (meal)

[1] Presented in part before the American Society of Biological Chemists, April 1935, and in part before the American Chemical Society, April 1937.

[2] Aided by the Robert McNeil Fellowship of the McNeil Laboratories, Inc.

[3] The author is indebted to Miss Anna Katherine Stimson for preparation of the charts and for other technical help.

The first five experiments were performed in duplicate, with an interval of 2 weeks between, but since there was no essential difference in the results the duplication was discontinued. Only one of each pair is reported. The compounds were: *l*-cystine (Merck), 2·58 g.; *l*-cysteine hydrochloride, 3·39 g., neutralized with $NaHCO_3$ immediately before using; *l*-methionine, 3·21 g., isolated from casein by the method of Pirie [1933]; *l*-cystinedisulphoxide, 2·93 g. (Merck), prepared according to Toennies & Lavine [1936]; *l*-cysteinesulphinic acid, 3·30 g., obtained from cystinedisulphoxide as described by Lavine [1936]; *l*-cysteic acid, 3·64 g., obtained according to Shinohara [1932]; *l*-α-dihydroxy-β-dithiodipropionic acid, 2·60 g., prepared as directed by Westerman & Rose [1928], purified in the form of the zinc salt and recovered with hydrogen sulphide; and *dl*-α-hydroxy-γ-methylthiolbutyric acid, 3·23 g., prepared as described by Akobe [1936]. Since it has been shown by Jackson & Block [1932–33] and others that *dl*-methionine, in contrast to *dl*-cystine, supports growth as well as the laevo-form, its metabolism (Eastman product) was included for purposes of comparison. In addition, entire control experiments were carried out from 8 a.m. one day until 8 a.m. the following day.

Attempts by Dr Toennies to isolate cysteinesulphenic acid in pure form proved unsuccessful on account of its extreme instability. Hence at his suggestion it was planned to utilize *S*-guanylthiocysteine, since, as shown by him [1937], it behaves in solution as if hydrolyzed to cysteinesulphenic acid and thiourea. Thiourea was ingested in a separate experiment as a control; 0·82 g. was administered, since it provides the molar equivalent of that theoretically available from the cysteine compound. But because of the toxicity displayed by thiourea, the experiment with *S*-guanyl-thio-cysteine was abandoned, in the hope that a similar cysteine compound could be obtained later without toxic properties.

Diet. Beginning 3 days before each experiment, the subject was placed on a constant, weighed diet of low protein and low sulphur contents (rice, sugar, lard, milk and fruit juices). The protein content was estimated as 33·0 g. (nitrogen, 5·36 g.) daily and the sulphur determined as 410 mg.

Methods. Total sulphur and total sulphates were determined as described in paper III of this series [Medes, 1937]. Inorganic sulphates were estimated by Folin's method [Folin, 1905–06], using 10 ml. of urine and carrying out the ashing and weighing in silica centrifuge tubes. Disulphide (cystine) determinations were carried out for the most part by the method of Medes & Padis [1936]. Total nitrogen was determined either by macro-Kjeldahl or by Pregl's micro-method and urea nitrogen according to Van Slyke & Cullen [1916].

Results

Control period (Fig. 1). The average hourly outputs of creatinine, total nitrogen, urea nitrogen, disulphide (cystine), total sulphur and total and inorganic sulphate were determined during a 24 hr. period from 6 a.m. to 6 a.m., following 3 days on the standard diet. As may be seen, all curves show little variation during the period except total and urea nitrogen, which fell gradually from average hourly excretions of 270 and 174 mg. respectively to 183 and 108 mg., indicating that the subject was still approaching nitrogen equilibrium. The output for the 24 hr. was 5434 mg., as against 5363 (estimated) in the diet. The average hourly output of sulphur was 17·65 mg., a total of 424 mg. during the 24 hr. period, or 103 % of the sulphur intake. There was a slight increase following meals, the highest average value, 19·55 mg., being attained during the period from 7 p.m.

to midnight, following the meal of highest sulphur content. Neutral sulphur averaged 2·73 mg. per hr. The slight variations could not be correlated with diurnal periods or with food intake. Ethereal sulphate also showed no significant variations from the average value of 1·0 mg. No SH was detected in the urine at any period. S—S values varied from 4·25 to 5·12 $M \times 10^{-6}$ (1·03–1·24 mg. as cystine) per hr., representing 0·27–0·33 mg. sulphur, or about 11 % of the neutral sulphur. Creatinine output varied from 40 to 49 mg. hourly during the different periods with a total of 1·01 g. The average value during the night was slightly lower than during the day, but the data are insufficient to show whether these variations are significant. No effect of food on creatinine excretion was demonstrated.

Period of cystine feeding (Fig. 2). By the close of the first hour following administration, the excretion of total sulphur had risen from an hourly level of 16·0 to 21·9 mg. The elevation continued until period 6, 8–12 hr. following ingestion, when an hourly average of 62·5 mg. during 4 hr. was excreted. The total output for the 24 hr. was 1040 mg., or 617 mg. above the sulphur of the control period and hence representing recovery of 90 % of the cystine sulphur. By the close of the 24 hr. period, however, the rate of excretion was still elevated (34·5 mg. per hr. from 6 to 8 a.m.) and did not fall to the control value until the period starting at 7 p.m. of the second day, about 35 hr. after administration. By this time 70 mg. of extra sulphur had been excreted, representing a total excretion of 100 % of the cystine sulphur. The average output of neutral sulphur per hr. was 2·78 mg. No test for sulphydryl in the urine was obtainable, and except for a slight rise during the hour of ingestion (i.e. from 5·0 to $8·2 \times 10^{-6}$ M of disulphide) the excretion of disulphide did not vary more than during the control day.

The total nitrogen excreted during the 24 hr. after ingestion was 6059 mg., an increase of 625 mg. above the control level and 325 mg. above that of the control and cystine nitrogen combined. Urea nitrogen was also elevated, an output of 3808 mg. occurring during the 24 hr. of the experimental day as against 3244 mg. on the control day. The excretion of this excess nitrogen was distributed for the most part over the periods extending from 1 p.m. to midnight. Creatinine output, 0·98 g., was unaffected.

Period of cysteine ingestion (Fig. 3). The oxidation of cysteine occurred with much greater rapidity, the rise in the excretion of total sulphur being from 16·5 to 52·7 mg. during the hour of ingestion and reaching 104 mg. the following hour. The peak, 132 mg., was attained during the third hour and by the end of 24 hr. the sulphur excretion had returned approximately to the basal level, with a recovery of 668 mg., or 97 % of the ingested cysteine sulphur. This was recovered almost entirely as sulphate, the neutral sulphur being changed only from 66 to 71 mg. during the 24 hr. period. The excretion of disulphide was increased from 111×10^{-6} M of S—S to 178×10^{-6} M, the latter equalling 42·9 mg. cystine. This disulphide would contain 11·5 mg. sulphur, an increase of 4·4 mg. above the disulphide sulphur of the control day (7·1 mg.). All the excess neutral sulphur, therefore, was excreted as disulphide. In contrast to its immediate brief appearance in the cystine-ingestion experiment, here no elevation of disulphide occurred until the third hour following ingestion, and the increased output continued for the succeeding four periods.

The curve of total nitrogen excretion, as well as of urea nitrogen, followed closely that in the cystine experiment, the output of each being approximately the same, 6027 total nitrogen and 3745 urea nitrogen. Creatinine excretion, 1·01 g., was unaltered.

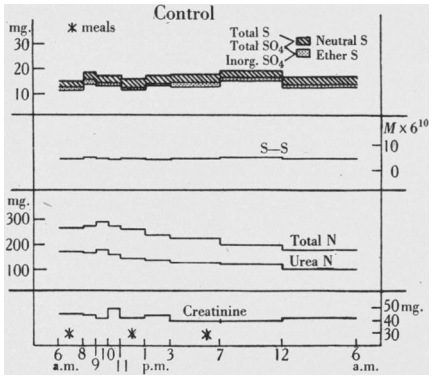

Fig. 1.

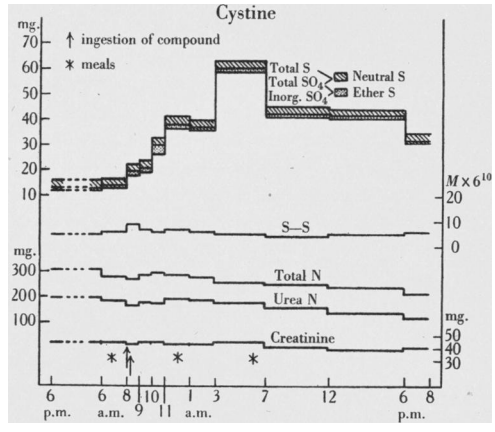

Fig. 2.

Fig. 1. Metabolism of sulphur and nitrogen of a normal human subject during a 24 hr. control period. The figures at the base represent the times at which urine was collected.

Fig. 2. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 2·58 g. *l*-cystine (690 mg. sulphur). The figures at the base represent times at which urine was collected. Two control periods before ingestion, one of 12 hr. and one of 2 hr., are also given.

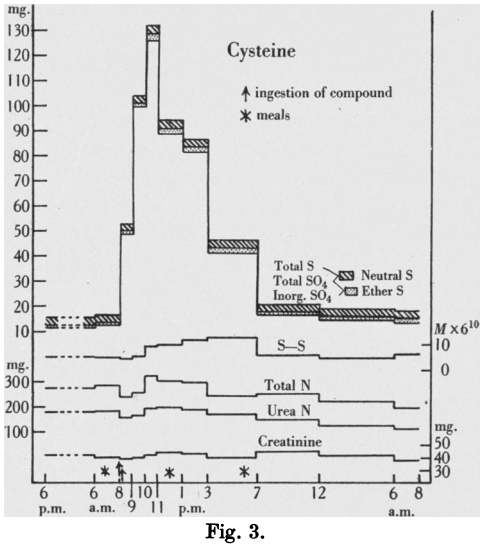

Fig. 3.

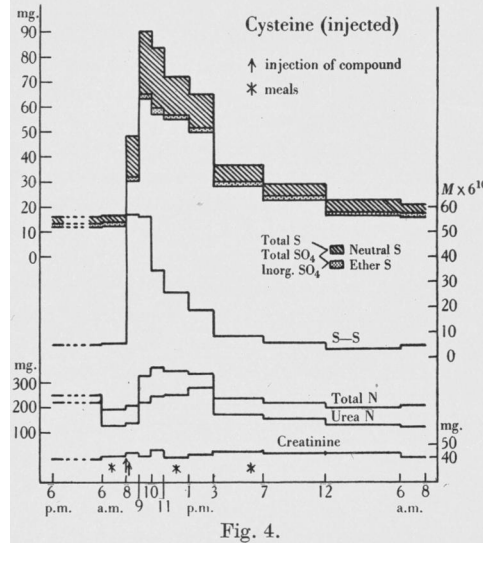

Fig. 4.

Fig. 3. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 3·39 g. of *l*-cysteine hydrochloride (690 mg. sulphur) neutralized. The figures at the base represent times at which urine was collected. Two control periods before ingestion, one of 12 hr. and one of 2 hr., are included.

Fig. 4. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following injection of 3·39 g. *l*-cysteine hydrochloride (690 mg. sulphur) neutralized. The figures at the base represent times at which urine was collected. Two control periods before injection, one of 12 hr. and one of 2 hr., are given.

Period of cysteine injection (Fig. 4). This experiment was similar in all respects to the ingestion experiment, except that intravenous injection of cysteine into the median basilic vein was substituted. It was hoped to eliminate the problem of different rates of absorption of the various compounds through the intestinal wall by this procedure, and hence more rapid oxidation and excretion were anticipated. As may be seen from the figure, a lower degree of oxidation and delayed excretion resulted, indicating that the amount employed was too great to be handled normally by the body and since the entire metabolic picture was altered, no further work along this line was pursued for the present.

During the 24 hr. following injection, a total of 964 mg. of sulphur was excreted, representing 540 mg. above the sulphur of the control day, or 78 % of the cysteine sulphur (690 mg.). Of the 540 mg., only 393 mg. were excreted as sulphate (751 mg., total sulphate sulphur minus 258 mg. representing the control day) or 73 % of the excreted cysteine sulphur. The neutral sulphur output was 210 mg. as contrasted with 71 mg. in the cysteine feeding experiment, and 66 mg. on the control day, an output of 144 mg. of extra neutral sulphur. The excretion of disulphide also rose immediately and in general followed a curve of the same type as was pursued by the neutral sulphur. A total of $3{\cdot}1 \times 10^{-4}$ *M* of disulphide was determined in the urine, the equivalent of 20·6 mg. of sulphur, 13·5 mg. above that of the control day. The disulphide sulphur, therefore, accounts for only about one-tenth of the extra neutral sulphur. No sulphydryl was detectable in the urine. Possibly partially oxidized intermediates which would not respond to either test were excreted but were not identified.

No marked differences between the excretions of total nitrogen and urea nitrogen were observable in this experiment and in the experiment with ingested cysteine, indicating that the cystine was probably deaminated and the nitrogen excreted as urea. Creatinine excretion was unchanged (1·03 g.).

Period of l-*methionine ingestion* (Fig. 5). The curve of excretion of sulphur following the ingestion of *l*-methionine resembled closely that of cysteine, except that the metabolic processes were slightly slower. The rise during the first 3 hr. was less and the subsequent fall was retarded, periods 3, 4 and 5 presenting a nearly level plateau. The base line was not reached until the eighth period. A total of 1145 mg. of sulphur was excreted in the 24 hr., 721 mg. above that of the control day or 105 % of the methionine sulphur ingested. It was excreted entirely in the form of sulphate, except for a slight rise in the neutral sulphur in period 5, when an average of 3·34 mg. per hr. was excreted during the 2 hr. period. Disulphide excretion was not elevated.

The curves for the excretion of total nitrogen, urea nitrogen and creatinine closely resembled that following cysteine ingestion and the total output of each was not significantly different. Creatinine output was 1·02 g.

Period of dl-*methionine ingestion* (Fig. 6). The oxidation of *dl*-methionine was slightly more retarded than that of *l*-methionine, the peak of excretion occurring during period 5 (1–3 p.m.). A total output of 1082 mg. was determined representing 658 mg. in excess of that excreted on the control day, or 95 % of the methionine sulphur. The output of neutral sulphur was approximately the same as that on the control day, in spite of slight elevations of neutral sulphur during the fourth and fifth periods (3·01 and 2·95 mg. respectively). The output of disulphide rose somewhat during the fifth to the eighth periods, with a total output for the 24 hr. of $1{\cdot}49 \times 10^{-4}$ *M* S—S (35·8 mg. as cystine = 9·5 mg. S) as compared with $1{\cdot}11 \times 10^{-4}$ *M* S—S (26·8 mg. as cystine = 7·1 mg. S) excreted on

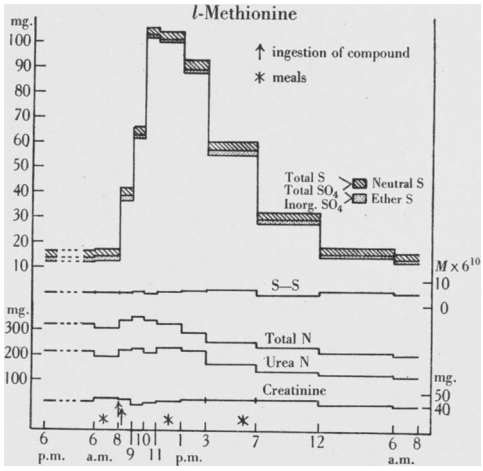

Fig. 5.

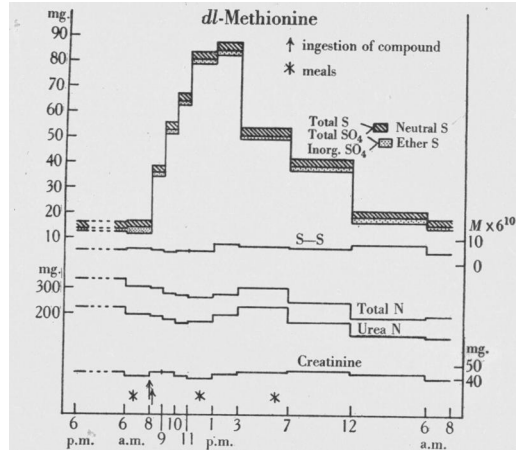

Fig. 6.

Fig. 5. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 3·21 g. *l*-methionine. The figures at the base represent times at which urine was collected. Two controls before ingestion, one of 12 hr. and one of 2 hr. are also given.

Fig. 6. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 3·21 g. *dl*-methionine. The figures at the base represent times at which urine was collected. Two control periods, one of 12 hr. and one of 2 hr., are also given.

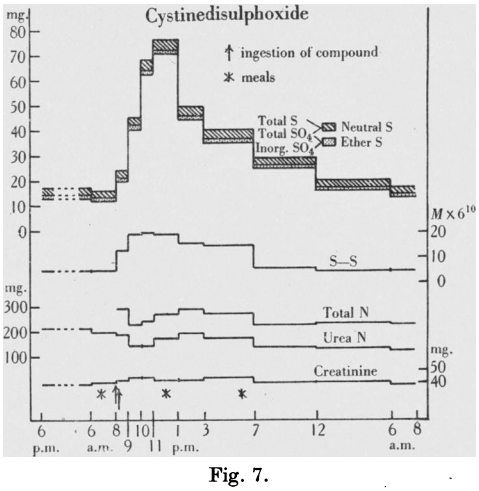

Fig. 7.

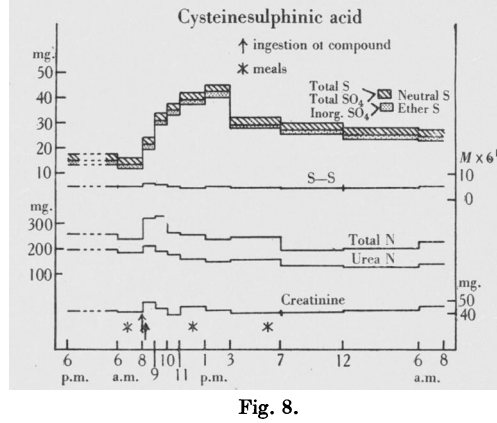

Fig. 8.

Fig. 7. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 2·93 g. *l*-cystinedisulphoxide. The figures at the base represent times at which urine was collected. Two control periods preceding ingestion, one of 12 hr. and one of 2 hr., are given.

Fig. 8. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 3·30 g. *l*-cysteinesulphinic acid (690 mg. sulphur). The figures at the base represent times at which urine was collected. Two control periods preceding ingestion, one of 12 hr. and one of 2 hr., are also given.

the control day. This slight excess of 2·4 mg. was not detectable in the neutral sulphur output, being within the limits of error of the methods employed.

The curve of nitrogen excretion indicated a delayed deamination, although the amount excreted during the 24 hr. was essentially the same as that following administration of *l*-methionine. The peaks of excretion of both total nitrogen and urea nitrogen occurred during period 6, from 7 to 12 p.m. and the rates were still slightly elevated at the end of the 24 hr. period. Creatinine again was unaffected (1·03 g.).

Period of cystinedisulphoxide ingestion (Fig. 7). The rise of sulphur excretion following ingestion of cystinedisulphoxide occurred with much greater rapidity than after that of cystine and less than following oral administration of either cysteine or *l*-methionine. The peak occurred during the fourth period, an average of 76 mg. sulphur being excreted hourly between 11 a.m. and 1 p.m. The total output of sulphur during the 24 hr. following ingestion was 860 mg., or 438 mg. in excess of that on the control day, representing 63 % of the sulphur of the disulphoxide. This sulphur was almost completely oxidized, the neutral sulphur being 75 mg. for the day, as against 66 mg. on the control day. The slight increases of neutral sulphur occurred during periods 3–5, when the outputs equalled 3·80, 3·83, 3·77 and 3·78 mg. respectively. Excretion of disulphide was increased during all periods through the sixth, or until midnight of the day of ingestion. The total output was $2{\cdot}43 \times 10^{-4}$ *M* of S—S, or 58 mg. computed as cystine. This is the equivalent of 15·6 mg. S, which is 8·5 mg. in excess of that excreted on the control day and hence approximately accounts for the additional neutral sulphur. Since disulphoxide affects the uric acid reagent, efforts were made to identify the compound excreted. Iodimetric tests carried out by Dr Lavine indicated that it was not the sulphoxide. Moreover, the characteristic behaviour of cystinedisulphoxide with phospho-18-tungstic acid, i.e. the slow, prolonged development of colour, did not occur with these specimens, a further indication that the compound excreted was not cystinedisulphoxide. The curves of urinary nitrogen and creatinine (0·99 g.) were not characteristically changed from those following administration of the other amino-acids.

Period of sulphinic acid ingestion (Fig. 8). The oxidation of sulphur of sulphinic acid was much slower than that of any of the previously employed compounds. The curve of excretion was still elevated through the final period of the 24 hr. during which an hourly average of 28 mg. of sulphur appeared. The total excretion of sulphur during 24 hr. was 774 mg., 350 mg. above that of the control day, or 51 % of the sulphinic acid sulphur. This was totally oxidized to sulphate, the output of neutral sulphur being 67 mg., a figure identical within experimental error with that of the control day. Total disulphide (S—S) excretion was $1{\cdot}12 \times 10^{-4}$ *M* or 27 mg. computed as cystine, a figure also unchanged from that of the control.

The nitrogen excretion was also delayed. The total nitrogen was 5639 mg., 205 mg. in excess of that of the control, or 67 % of the sulphinic acid nitrogen. Creatinine output was unchanged (1·01 g.).

Period of cysteic acid ingestion (Fig. 9). The extremely low ability of the body to convert the completely oxidized sulphur of cysteic acid into inorganic sulphate is demonstrated in the accompanying figure. The total output of sulphur for the 24 hr. period was 582 mg., 159 mg. above that of the control day, or 23 % of the cysteic acid sulphur. A total of 138 mg. of neutral sulphur, 72 mg. in excess of the control neutral sulphur, was put out during the same time. This is the equivalent of 382 mg. of cysteic acid, 10·5 % of that ingested. In other words, approximately half of the recovered cysteic acid sulphur was excreted in organic form and about half in the form of sulphate.

The rates of excretion of these two forms of sulphur differed widely. The period of maximum elevation of the neutral sulphur was between 11 a.m. and 1 p.m. of the first day, during which time it was excreted at the rate of 9·8 mg. per hr., an average increase of 7·1 mg. above the control and the equivalent of 37·5 mg. of cysteic acid. The average output of neutral sulphur from the beginning of the experiment until midnight was 6·5 mg. per hr., after which it fell to 4·9 mg. per hr. between midnight and 8 a.m. and was continued at the average of 4·1 mg. per hr. between 8 a.m. and 12 midnight.

On the other hand, during the early portion of the experimental period extra sulphate formation was very slight. Up to midnight the average hourly excretion of total sulphate was 16·2 mg. as compared with 15·4 during the same period of the control. About midnight the sulphate excretion began to rise and averaged 19·4, 22·7, 24·0 and 27·3 mg. per hr. during the periods from midnight to 6 a.m., from 6 to 8 a.m., from 8 a.m. to noon and from noon to 6 p.m. respectively. Unfortunately the determinations were not carried further. The output of disulphide during 24 hr. was $1{\cdot}07 \times 10^{-4}$ *M* S—S or 25·6 mg. computed as cystine (control, 26·8 mg. cystine).

The total nitrogen output was 5784 mg., of which 3488 mg. were excreted as urea nitrogen. In other words, all the nitrogen of the cysteic acid was recovered, although urea nitrogen accounts for only about 80 % of it. Creatinine, as in the other experiments, was unaffected (0·99 g.).

Period of α-dihydroxy-β-dithiodipropionic acid ingestion (Fig. 10). This compound was prepared by the method of Westerman & Rose [1928], precipitated as the zinc salt and recovered with H_2S. Sulphur was determined both in the zinc salt and in the recovered acid and the results agreed within 1 % of the theoretical. Disulphide determinations (Shinohara's method) also gave theoretical values. Tests for nitrogen were negative. Neither the phenylhydrazine nor the fuchsine test for aldehyde could be elicited. This hydroxy-analogue of cystine also proved to be a compound handled with difficulty by the body. By the close of the usual 24 hr. period, 593 mg. of sulphur had been excreted, 171 mg. above that of the control, 25 % of the sulphur of the ingested compound. By this time, the output, which averaged 17·4 mg. per hr. between 6 and 8 a.m., was again at the control level, 17·7 mg. per hr. The total sulphate output was 427 mg., 69 mg. above the control, or 10 % of the extra ingested sulphur. To sum up, of the 171 mg. of sulphur recovered, 60 % appeared as neutral sulphur and 40 % in the form of sulphate. The maximum output of both forms of sulphur was during the period from 9 to 10 a.m., and was followed by a gradual fall. The output of disulphide was $7{\cdot}12 \times 10^{-4}$ *M*, $6{\cdot}01 \times 10^{-4}$ *M* above the control, which would be the equivalent of 39 mg. excess sulphur, or about one-third of the extra neutral sulphur. The form in which this disulphide was excreted could not be definitely identified. That it was not cystine was demonstrated by tests by the Sullivan reaction. From previous knowledge of the behaviour of hydroxy-analogues of amino-acids in the body, it seems probable that the compound excreted was identical with that ingested.

The total nitrogen and urea nitrogen, 5588 and 3406 mg. respectively, were only slightly elevated as compared with those of the control day. Creatinine was 1·00 g.

Period of ingestion of α-hydroxy-γ-methylthiolbutyric acid (Fig. 11). In contrast to the behaviour of the hydroxy-analogue of cystine, the sulphur of which appeared in the urine to the extent of only about 25 %, 94 % of the sulphur of the corresponding derivative of methionine was recovered. Of the recovered sulphur, in the one case about 10 % was in the form of sulphate, in

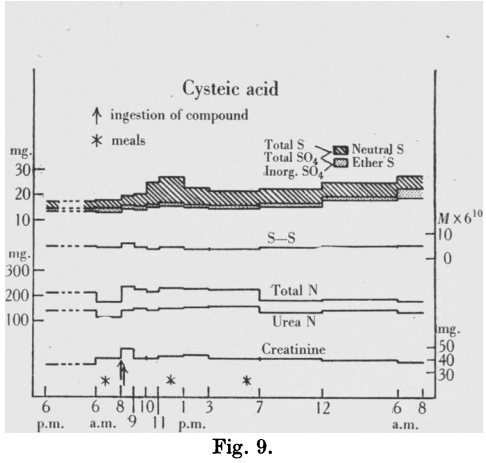

Fig. 9.

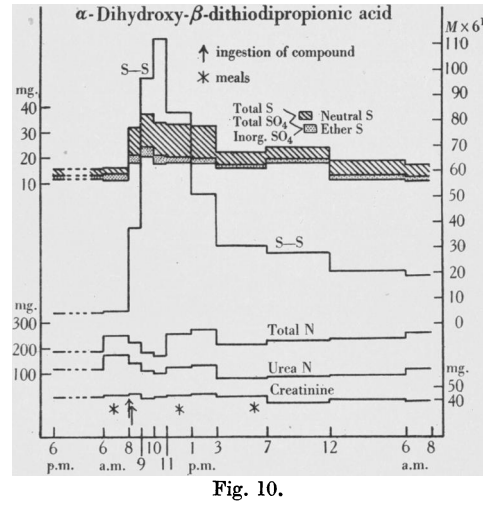

Fig. 10.

Fig. 9. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 3·64 g. *l*-cysteic acid (690 mg. sulphur). The figures at the base represent times at which urine was collected. Two control periods preceding ingestion, one of 12 hr. and one of 2 hr., are given.

Fig. 10. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 2·60 g. of *l*-α-dihydroxy-β-dithiodipropionic acid (690 mg. sulphur). The figures at the base represent times at which urine was collected. Two control periods, one of 12 hr. and one of 2 hr. preceding ingestion, are given.

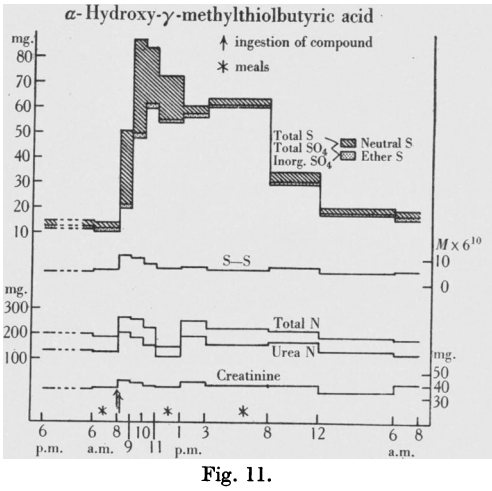

Fig. 11.

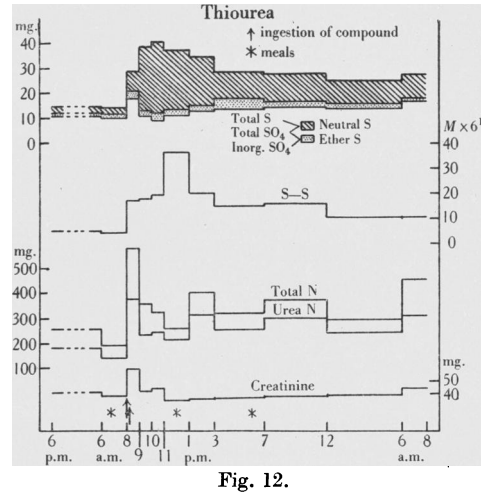

Fig. 12.

Fig. 11. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 3·23 g. *dl*-α-hydroxy-γ-methylthiolbutyric acid (690 mg. sulphur). The figures at the base represent hours at which urine was collected. Ingestion is preceded by two control periods of 12 and 2 hr. respectively.

Fig. 12. Metabolism of sulphur and nitrogen of a normal human subject for 24 hr. following ingestion of 0·82 g. thiourea (345 mg. sulphur). The figures at the base represent times at which urine was collected. Ingestion is preceded by two control periods of 12 and 2 hr. respectively.

the second case, 83 %. An increase in neutral sulphur following ingestion of the methionine derivative occurred during the first four periods covering 5 hr., with hourly averages of 29, 37, 22 and 17 mg. in the four periods respectively, after which there was a sudden drop to almost basal level. The sulphate excretion at this time began a rise to a new maximum, which occurred during the sixth period, closing at 7 p.m., 11 hr. after ingestion. The excretion of disulphide was elevated to approximately twice its normal level, 11·1 and $10{\cdot}2 \times 10^{-6}$ M per hr., during the first two periods, after which it fell almost to normal value. An increase in the Sullivan reaction indicated that the disulphide compound being excreted was cystine. However, this increase in disulphide would correspond to only 8 mg. of cystine, representing 2·2 mg. of neutral sulphur, or only about 2 % of the excess neutral sulphur. The remainder of the additional neutral sulphur was not identified, but judging by the rapidity with which it was excreted, may have been the unchanged hydroxy-compound.

The excretion of nitrogen was 5231 mg., an even lower output than during the control day. The urea nitrogen was approximately the same. Creatinine excretion was 975 mg.

Period of thiourea ingestion (Fig. 12). There was almost no evidence of oxidation of the sulphur of thiourea. The ingested compound apparently was excreted unchanged during a period of 48 hr., at the end of which time all determinations except total and neutral sulphur had returned to the base line. During the final period of the 48 hr., from 6 to 8 a.m. of the third day, organic sulphur averaged 3·67 mg. per hr. as against an hourly average of 2·74 on the control day. During this 48 hr., the extra sulphur excreted was 337 mg., almost the equivalent of the thiourea sulphur. Since this included 20 mg. of extra cystine sulphur, a small amount of thiourea, amounting to about 66 mg., was unaccounted for. As the organic sulphur was still slightly elevated, even more might have been recovered at a later period.

The amount of total sulphate excreted was about the same as during 2 days of the control period, except that in the first day following ingestion a rise of 24 mg. was observed followed by a depression of 26 mg. in the second 24 hr. During the entire period the inorganic output was lowered, the ethereal sulphate averaging 2·6 mg. per hr. during the first 24 hr. and 1·6 mg. during the second 24 hr., as against 1·0 mg. on the control day. These findings agree with those of Masudo [1910], who injected thiourea subcutaneously into rabbits and reported neutral sulphur and ethereal sulphates increased, while total sulphates were approximately unchanged.

A portion of the neutral sulphur was contributed by thiourea, which was determined qualitatively by the method of Grote [1931]. Attempts to modify the procedure for quantitative measurements proved unsuccessful. The test became strongly positive with the first urinary sample following the ingestion of thiourea and remained at approximately this intensity until the following morning, when it slowly decreased to a questionable positive in the sample collected from midnight of the second day to 6 a.m. the following morning (40–46 hr. after ingestion of the thiourea).

There was also an immediate rise in disulphide sulphur following ingestion of the compound. Tests by the Sullivan reaction indicated that it consisted largely of cystine. The total disulphide output during the first 24 hr. was $3{\cdot}84 \times 10^{-4}$ M, or $2{\cdot}73 \times 10^{-4}$ M above that of the control day, representing an additional output (computed as cystine) of 66 mg. cystine. By the close of the first 24 hr., the cystine output averaged $10{\cdot}3 \times 10^{-6}$ M per hr., which is approximately twice the control output. The excretion did not return to the

basal level until after another 12 hr. In order to ascertain whether this rise in cystine excretion represented a general amino-acid increase following a generalized protein destruction, amino-acids were determined (method of Northrop [1926] as modified by Van Slyke & Kirk [1933]) but no significant rise was found, and tests for tyrosine [Medes, 1932] were negative. The excretion of cystine must, therefore, have been a specific effect. The cystine did not appear to be combined with the thiourea, as shown by its response to the Sullivan test, as well as by the specific test that was elicited for thiourea. However, the possibility exists that a conjugated product of cysteine and thiourea may have been formed, which, because of its extreme instability, would hydrolyse to cysteinesulphenic acid (RSOH) and thiourea, the cysteinesulphenic acid ultimately dismuting to cystine and cysteinesulphinic acid.

The nitrogen output for 24 hr. was greatly increased, being 8·42 g., or 2·98 g. above the control and 2·85 g. in excess of the control plus the nitrogen of the thiourea. This excess could be accounted for entirely in the urea fraction. The total output of creatinine for the 24 hr. following administration was only 945 mg., an average of 39·4 mg. per hr., in spite of a rise to 60 mg. in the first hour following ingestion of thiourea. The gradual fall to 35 mg. during the fourth period of the first day and its subsequent slow return to normal at about the close of 24 hr. may have been associated with the symptoms of prostration experienced by the subject during that time.

Discussion

Rate of absorption. In discussing the oxidation of an ingested compound, the rate of its absorption through the intestinal wall must be considered. Andrews & Johnston [1933] and Stearns & Lewis [1930] attempted to evaluate this factor in the rate of oxidation of cystine. The former found from 25 to 37 and 68 to 79 % of *l*-cystine remaining in the isolated intestinal loops of dogs of 27·9 and 18·3 kg. body weight respectively, 4 hr. following introduction of 0·5 g. cystine. It may be significant that absorption was more rapid from the gut of the larger dog, probably because of the greater surface and blood supply. Stearns & Lewis employed two rabbits of 2·5 and 1·75 kg. weight. 6 hr. after feeding respectively 2 and 1 g. of cystine in the form of Na cystinate, 20 and 33 % were found remaining in the gastro-intestinal canal. In the four cases quoted above, the doses administered were 18, 27, 800 and 570 mg. cystine per kg. body weight. Since in three of these instances the percentage absorbed lay between 75 and 80, either rate of absorption is relatively independent of amount ingested or comparisons as to dosage and relative rates of absorption cannot be strictly drawn between the dog and the rabbit under the conditions of these two sets of experiments.

In the present experiment, the dosage was approximately 45 mg. per kg. body weight. About 8 % of the cystine sulphur ingested was excreted by the close of 4 hr., 15 % by the end of 6 hr., and 24 % by the end of 8 hr., a somewhat more rapid rate than that recorded by Stearns & Lewis for a rabbit of 2·4 kg. body weight, in which case (rabbit III, p. 99) about 15 % of the sulphur was excreted in the first 8 hr. period following ingestion of 0·812 g. In this case the dose equalled 338 mg. per kg. body weight. Here also it may be seen that in these two forms comparison between doses and rates of absorption cannot be drawn.

In an attempt to ascertain whether solubility of the compound plays an extensive role in the rate of absorption, the experiment was repeated with the same amount of cystine ingested as the lithium salt. Fig. 13 gives a comparison of the rates of excretion of the sulphur. As may be seen, there is a moderate shift to the left (curve *B*), 39 % being excreted by the close of 8 hr. as compared with

24 % in the previous case (curve *C*). The rate of oxidation is still much less than that of cysteine, the graph of which is superimposed in the same chart for purposes of comparison (curve *A*). The solubilities of lithium cystinate and cysteine are of approximately the same order and about as great as that of cysteic acid, whose absorption has been shown to occur with great rapidity [Andrews & Johnston, 1933]. Hence we may conclude that solubility of the compound ingested is a factor, though a minor one, in determining its rate of absorption.

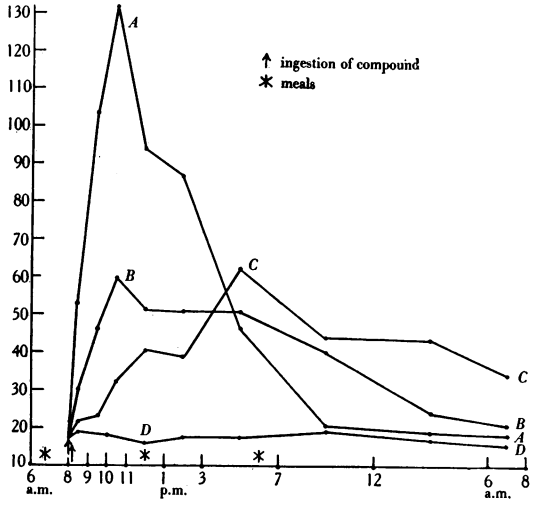

Fig. 13.

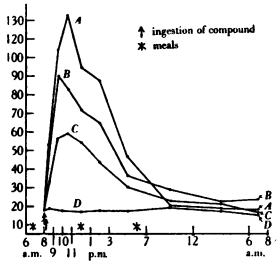

Fig. 14.

Fig. 13. Excretion of total sulphur following ingestion of 3·39 g. *l*-cysteine hydrochloride neutralized with $NaHCO_3$: (*A*), 2·71 g. lithium cystinate (*B*) and 2·58 g. *l*-cysteine (*C*), each containing 690 mg. sulphur (*D*) control.

Fig. 14. Excretion of total sulphur following administration of *l*-cysteine hydrochloride neutralized with $NaHCO_3$: (*A*), following ingestion of 3·39 g. (690 mg. sulphur); (*B*), following injection of 3·39 g. (690 mg. sulphur) and (*C*), following ingestion of 1·695 mg. (345 mg. sulphur) (*D*) control.

A second attempt to evaluate the factor of absorption was made by injecting cysteine in the same amount as was ingested in the previous experiment (Fig. 14). In this case only 78 % of the sulphur ingested was recovered (curve *B*) by the end of 24 hr. in contrast to the 97 % recovered in the ingestion experiment (curve *A*). When the cysteine was injected, 27 % of the sulphur recovered was in the form of organic sulphur, whereas less than 1 % was in this form following ingestion. That less sulphur is recovered and a greater percentage is excreted in organic form following injection has frequently been observed [Schmidt & Clark, 1922; Westerman & Rose, 1928; Stearns & Lewis, 1930]. These investigators explain this difference on the basis of a sudden flooding of the organism above its capacity to metabolize the cystine. Since in all these cases injection was into the peripheral circulation, the question as to the part played by the liver in preventing this overwhelming of the body cannot be evaluated until experiments are done injecting cystine under similar conditions into the mesenteric and peripheral veins. That exclusion of the liver is not the only cause of lessened ability to deal with cystine, is shown in the lithium cystinate experiment, in which case more rapid absorption was associated with a rise in the urinary organic sulphur.

Amount of total sulphur recovered. In Table I are summarized the amounts of extra sulphur excreted, expressed both in mg. and in percentage of those ingested. It may be seen that, with the exception of the hydroxy-analogue of methionine, all those compounds whose recovery was nearly quantitative were the normally

Table I. *Recovery of total and neutral sulphur during 24 hr. following administration of various sulphur-containing compounds*

	Extra S ingested mg.	S recovered: Total S mg.	Total S % ingested	Neutral S mg.	Neutral S % ingested	Neutral S % recovered
Cysteine	690	668	97	6	1	1
Cystine	690	616	90	—	—	—
Cystinedisulphoxide	690	438	63	10	15	22
Cysteinesulphinic acid	690	350	51	—	—	—
Cysteic acid	690	159	23	72	10	46
Cysteine, injected	690	540	78	144	21	27
Lithium cystinate	690	514	75	12	2	2
Cysteine	345	331	96	—	—	—
Cysteinesulphinic acid	345	249	73	—	—	—
Cystinedisulphoxide	345	238	69	7	2	3
l-Methionine	690	721	105	—	—	—
dl-Methionine	690	658	95	—	—	—
α-Dihydroxy-β-dithiodipropionic acid	690	171	25	103	15	61
α-Hydroxy-γ-methylthiolbutyric acid	690	649	94	108	16	17
Thiourea	345	279	81	256	74	92

occurring forms, cysteine, cystine and methionine. In the series of partially oxidized derivatives, S—S, SO—SO, SO_2H and SO_3H, the recovery of sulphur during 24 hr. decreased with degree of oxidation of the compound administered: i.e. cysteine (SH), 97 %, cystine (S—S), 90 %; cystinedisulphoxide (SO—SO), 63 %; sulphinic acid (SO_2H), 51 % and cysteic acid (SO_3), 23 %. This diminishing recovery can scarcely be ascribed to failure of absorption, since all degrees of solubility exist in these compounds, from the extreme insolubility of cystinedisulphoxide to the high solubility and diffusibility of cysteic acid. Rather the conclusion seems justifiable that the partially oxidized sulphur compounds are oxidized to inorganic sulphate with greater difficulty than the amino-acids from which they are derived, and that they can scarcely be considered as in the direct line of physiological oxidation to inorganic sulphate. In normal metabolism leading to inorganic sulphate formation some other change probably occurs in the molecule during early stages of oxidation of the sulphur, thereby rendering the fragment more reactive. This does not preclude the possibility that the sulphur retained is utilized in some other metabolic process in the organism. Although it has not been demonstrated that any of the partially oxidized derivatives can contribute to the production of taurine, the possibility is not excluded, and the fact that the sulphur of each successively more highly oxidized compound is retained in higher proportions is suggestive.

Following ingestion of the hydroxy-analogues of cystine and methionine, the extra sulphur excreted was 25 % of that ingested with the former compound and 94 % with the latter. These results confirm the findings of previous investigators that from a metabolic standpoint these two compounds are not similarly related to the amino-acids from which they are derived. For instance, Akobe [1936] found that the hydroxy-analogue of methionine could replace methionine

in the diet of rats, whereas Westerman & Rose [1928] had demonstrated that the similar analogue of cystine could not substitute for cystine. Westerman & Rose, employing α-dihydroxy-β-dithiodipropionic acid, recovered 75 % of the sulphur after feeding 0·5 g. to a 1·8 kg. rabbit and 89 % after injecting the same amount into a 1·5 kg. rabbit, a recovery in marked contrast to the 25 % recovered in the present experiment, in which 2·60 g. were fed to a 60 kg. human subject.

Schmidt & Clark [1922] reported 40–52 % of the sulphur of cysteic acid excreted by dogs in the 24 hr. following ingestion. Their doses also were larger, 6·7 and 7·0 g. being fed to a 13·9 kg. dog and 5·8 g. to a 12·7 kg. dog. With this compound also, a greatly lessened recovery of sulphur was found in the human subject (23 % following ingestion of 3·65 g.).

The treatment of thiourea by the body is in marked contrast to that of the partially oxidized derivatives of the amino-acids, as in this case the excretion of sulphur failed to return to the basal level until approximately 100 % of the extra sulphur had been excreted (81 % at the close of the first 24 hr. and 98 % at the close of 48 hr.). 90 % of this extra sulphur was excreted as organic sulphur. Evidently no appreciable part of the sulphur of thiourea can be utilized by the organism. This prompt and complete expulsion of a non-physiological sulphur compound adds weight to the suggestion that the retained sulphur of the partially oxidized derivatives is utilized by the organism.

Rate of oxidation. Conclusions to be drawn as to rate of oxidation are less clear-cut, but by any criterion cysteine and methionine have shown themselves to be the most rapidly oxidized of any of the compounds ingested. In both cases nearly 100 % of the extra sulphur was excreted in oxidized form within 16 hr. after ingestion. With *dl*-methionine the rate was slightly slower, indicating that *d*-methionine may be metabolized somewhat less readily. The oxidation of cystine was considerably slower. This was demonstrated by the fact that following rapid introduction of cystine as the lithium salt the cysteine type of curve was not approached, but an even more retarded utilization occurred. The conclusion seems justifiable that cysteine, in spite of the rapid disappearance of sulphydryl from the blood stream following intravenous injection [Medes, unpublished] is not metabolized through the S—S form. The excretion of cystine rather than cysteine in all these cases remains to be explained.

Oxidation may in general be said to occur more slowly in the more oxidized derivatives. This is especially marked with cysteic acid, after ingestion of which the average hourly excretion of oxidized sulphur remained unchanged until midnight, 16 hr. after administration. At that time a slow rise in oxidized sulphur commenced and was continued throughout the following day. Although Schmidt & Clark [1922] conclude that the non-nitrogenous portion of the molecule is excreted unchanged, inspection of their data reveals a slight increase in sulphate excretion on the second day of some of their experiments. This delayed oxidation can scarcely be ascribed to action of intestinal bacteria, as cysteic acid is known to be absorbed very rapidly, and even though a small portion became converted into inorganic sulphate in the intestinal canal, 34 hr., the period of delay in these experiments, would hardly be required for its absorption and excretion.

Effect of variations in the amount ingested. In three instances the experiment was repeated using half the usual amount. In the case of cysteine, the percentage of sulphur recovered was approximately the same, 96 as compared with 97 %. In the case of cysteinesulphinic acid, 73 % was excreted in contrast to 51 % following ingestion of the usual dose. With cystinedisulphoxide the amount recovered increased from 63 to 69 % when the smaller amount was administered. Hence it seems that with the difficultly oxidizable compounds, ingestion of greater

amounts has essentially the same effect as rapid administration and is associated with decreased ability of the organism to handle them.

Degree of oxidation. In general it may be said that the compounds which underwent most rapid oxidation also resulted in the greatest output of sulphate sulphur (Fig. 15.) Neutral sulphur output (Fig. 16) increased in the order: cystine < cysteine < cystinedisulphoxide < cysteic acid. As may be observed, cysteinesulphinic acid did not fall in the series, but failed to produce any increase in excretion of neutral sulphur. According to Lavine [1937], this compound is one of the end-products of the dismutation of cystine: $2RSSR + 2H_2O \rightarrow 3RSH + R(SO_2)H$ in a medium in which RSH is removed from solution, the sulphinic acid undergoing no further dismutation. While it cannot be concluded that sulphinic acid is an intermediary in the metabolism of cystine, since its oxidation in the body takes place so slowly, it seems possible that this acid is more closely related to the normally occurring compound than is cysteic acid, i.e. more extensive disruption of the molecule may occur at the point corresponding to the sulphinic acid stage.

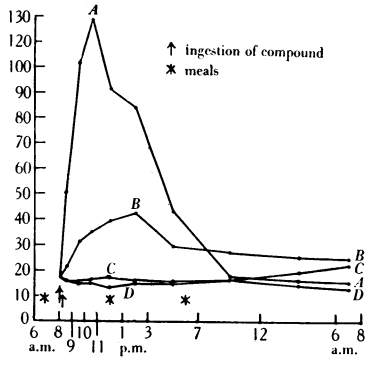

Fig. 15.

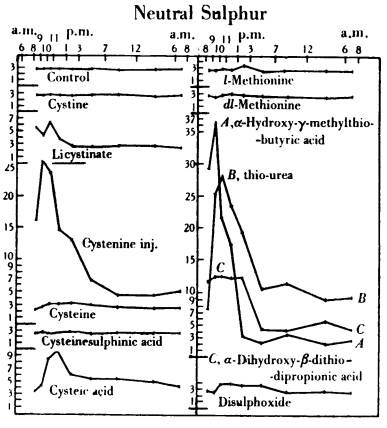

Fig. 16.

Fig. 15. Excretion of total sulphate following ingestion of (*A*), 3·39 g. *l*-cysteine, neutralized with Na_2CO_3; (*B*), 3·30 g. *l*-cysteinesulphinic acid neutralized; (*C*), 3·60 g. *l*-cysteic acid neutralized. All were the equivalent of 690 mg. sulphur (*D*) control.

Fig. 16. Excretion of neutral sulphur during 24 hr. of a control period and during 24 hr. periods following ingestion of various sulphur compounds containing 690 mg. of sulphur (thiourea 345 mg.). In the case of lithium cystinate, injected cysteine, ingested cysteine, methionine and cystinedisulphoxide, the additional neutral sulphur was identified as cystine, whereas following ingestion of thiourea and the hydroxy-analogues of methionine and cystine, the excess neutral sulphur appeared to be in the form of the compound ingested.

In Fig. 16 is also shown the effect of the rapid introduction of the experimental compounds into the body upon the neutral sulphur output following ingestion of lithium cystinate and injection of cysteine. How far the rate of entrance may be a factor in the degree of oxidation of all of the compounds is difficult to decide, i.e. would cysteic acid be metabolized without any increase of neutral sulphur if it were ingested sufficiently slowly?

Oxidation of cystinedisulphoxide. The oxidation of cystinedisulphoxide needs special consideration, since it has been shown [Bennett, 1937] that this compound is capable of replacing cystine in the diets of albino rats on a cystine-deficient diet. According to Lavine [1936], cystinedisulphoxide in solution

undergoes dismutation with the ultimate production of 4 molecules of sulphinic acid and 1 of cystine from 3 of cystinedisulphoxide. In the feeding experiments by Bennett, 1 molecule of the disulphoxide is apparently capable of replacing 1 molecule of cystine, hence under these conditions dismutation apparently does not occur, but a direct reduction of the disulphoxide to cystine. It is not certain, though, that such a course represents the path followed when the body is receiving sufficient cystine in the diet. According to Lavine the dismutative decomposition probably proceeds as follows:

Dismutation of cystinedisulphoxide

1	$2R(SO)_2R + 2H_2O \rightarrow$	$2R(SO)H +$	$2R(SO_2)H$
2	$2R(SO)H \rightarrow$	$RSH +$	$R(SO_2)H$
3	$2R(SO)_2R + 2H_2O \rightarrow$	$RSH +$	$3R(SO_2)H$
4	$R(SO)_2R + RSH \rightarrow$	$RSSR +$	$R(SO_2)H$
5	$3R(SO)_2R + 2H_2O \rightarrow$	$RSSR +$	$4R(SO_2)H$

If, however, the cysteine formed in equation 2 should be removed immediately from the reaction mixture, as may occur in the body where apparently it is oxidized with great rapidity, dismutation would proceed only to equation 3. Fig. 17 is a

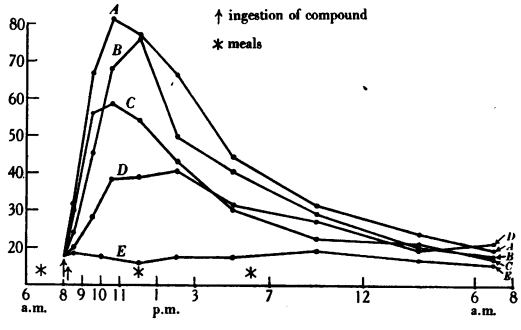

Fig. 17. Hypothetical behaviour of cystine in metabolism. (*B*) Excretion of total sulphur for 24 hr. following ingestion of cystine containing 690 mg. of sulphur. (*C*) Excretion of total sulphur following ingestion of one-half the molar equivalent of cysteine. (*D*) Excretion of total sulphur following ingestion of one-half the molar equivalent of cysteinesulphinic acid. (*E*) Control. (*A*) Composite curve of (*C*) plus (*D*), demonstrating general similarity of curve to (*B*). The greater height of the curve is due to the fact that when smaller amounts are ingested a greater proportion of the sulphur is recovered.

composite drawing in which curve *A* is obtained by addition of the total sulphur excreted when one-half the equivalent of cysteine was ingested, plus that when one-half the equivalent of cysteinesulphinic acid was administered. The close agreement of these two curves suggests that a reaction of this type may take place under the conditions of this experiment rather than a direct reduction of the disulphoxide to cystine.

Summary

The three naturally occurring amino-acids, *l*-cystine, *l*-cysteine and *l*-methionine, have been ingested by a normal individual under standardized conditions and the rate of excretion and degree of oxidation of the sulphur have been studied. A series of oxidized derivatives has been employed similarly, namely: *l*-cystinedisulphoxide, *l*-cysteinesulphinic acid, *l*-cysteic acid, *l*-α-dihydroxy-β-dithiodipropionic acid and *dl*-α-hydroxy-γ-methylthiolbutyric acid.

The sulphur of cysteine and methionine was recovered in the urine as inorganic sulphate approximately to the extent of 100% within 16 hr. after ingestion. Cystine was oxidized more slowly. When cystine was ingested as the highly soluble lithium salt the capacity of the body to oxidize it was enhanced and a greatly increased output of organic sulphur resulted. Nevertheless, the rate of oxidation was still much slower than that of cysteine. From these observations it was concluded that the oxidation of cysteine does not, under the conditions of this experiment, take place through cystine.

In the series of derivatives with oxidized sulphur, rate of oxidation to inorganic sulphate occurred in the following order: cysteine > cystine > cystine-disulphoxide > cysteinesulphinic acid > cysteic acid, indicating that this series does not represent the path of oxidation leading to inorganic sulphate formation, but that some other change, such as deamination, probably occurs early in the process.

Recovery of total sulphur also decreased in the same order, leading to the suggestion that the sulphur of the more highly oxidized members of this series may be more readily available for some other physiological function, such as taurine formation.

The sulphur of the hydroxy-analogue of methionine, α-hydroxy-γ-methylthiolbutyric acid, was recovered within 24 hr. to the extent of 95%, of which 83% was in the form of inorganic sulphate, whereas that of the similar analogue of cystine, α-dihydroxy-γ-dithiodipropionic acid, was recovered only to the extent of 25%, of which but 39% was oxidized. These results confirm the findings obtained by totally different experimental procedures, that these two hydroxy-analogues can scarcely bear similar metabolic relationships to the amino-acids from which they are derived.

The sulphur of thiourea was excreted to the extent of 100% by the close of 48 hr.; 92% of the recovered sulphur was in organic form. Along with thiourea there was an increase in the urinary cystine. The mechanism of this reaction is not yet elucidated.

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