CXXVII. METABOLISM OF SULPHUR

V. THE REPLACEABILITY OF *l*-CYSTINE IN THE DIETS OF RATS WITH SOME PARTIALLY OXIDIZED DERIVATIVES^{1,2}

BY MARY ADELIA BENNETT

From the Lankenau Hospital Research Institute, Philadelphia

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RECENTLY several partially oxidized products of *l*-cystine have become available for biochemical study. These compounds possibly play an important role in the intermediary metabolism of cystine. Since the amino-acid cystine is essential for growth, it is of interest to find out to what extent these partially oxidized compounds can replace it. The partially oxidized sulphur groups considered in the present paper are (—SO—SO—), (—SO₂H) and (—SOH). The (—SO—SO—) was used in the form of *l*-cystinedisulphoxide, the (SO₂H) as *l*-cystinesulphinic acid, and the (—SOH) in the form of *S*-(guanyltio)-cysteine. 2HCl, which as shown by Toennies [1937] hydrolyses to thiourea and *l*-cystinesulphenic acid. As controls for the latter, thiourea and dithioformamidine were employed. As an additional control, highly purified *l*-cystine was also used.

PREPARATION OF COMPOUNDS

The purified cystine was prepared in the following manner: 80 g. of *l*-cystine (Merck) were dissolved in 1330 ml. *N* HCl, filtered and diluted with water to 10,000 ml.; 1240 ml. *N* NH₄OH were added to bring the solution to pH 2.2–3.0 with thymol blue and bromophenol blue as indicators. The cystine was filtered off after 1 hr. and washed with water, alcohol and ether. About 83% was recovered. Ten recrystallizations were made using the same proportions of reagents. The final yield of cystine was 20 g., 25% of the original amount.

The *l*-cystinedisulphoxide was generously prepared by Merck and Co., Rahway, N.J., according to the method of Toennies & Lavine [1936]. The thiourea was also their product.

The *l*-cystinesulphinic acid was prepared by the alkaline decomposition of *l*-cystinedisulphoxide [Lavine, 1936]. The sulphinic acid present in solution was determined by KI-HCl reduction and titration with Na₂S₂O₃. The cystine present in solution was determined by the colorimetric method of Shinohara [1935] and was found to amount to 0.3–0.4 mg. per rat daily. Half of the control animals were fed this amount of *l*-cystine per day, but no effect on growth could be detected.

The *S*-(guanyltio)-cysteine. 2HCl was prepared by Dr Toennies [1937]. The author is also indebted to him for the dithioformamidine. 2HCl, synthesized by him from thiourea by the method of Böeseken [1936].

EXPERIMENTAL

Young albino rats, Wistar strain, were used as experimental animals. All were kept on Dyer & du Vigneaud's [1936] cystine-deficient diet, containing 6.0% casein and 16.0% milk vitamin concentrate. In all experiments the basal diet was freshly prepared every 4 or 5 days and kept in the refrigerator.

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The special compounds were fed individually in small pieces of butter, the control animals receiving the same amount of butter. These special diets were mixed with the butter once a week and kept in individual covered glass dishes in the refrigerator. The chemicals for the third experiment were mixed with butter and immediately placed at -10° to prevent decomposition, since the dithioformamidine and the *S*-(guanyltio)-cysteine are unstable when neutral. The special diets were continued for 2 weeks, the animals being weighed every other day and the average weight of the group plotted.

Fig. 1.

Fig. 1. Replaceability of *l*-cystine in the diets of albino rats by *l*-cystinedisulphoxide.

Fig. 2.

Fig. 2. Replaceability of *l*-cystine in the diets of albino rats with *l*-cysteinesulphinic acid.

In the first experiment 32 rats from 6 litters, about 1 month old, were divided into 4 comparable groups of 8 animals each, 6 males and 2 females. After 8 days on the cystine-deficient diet group A was continued without cystine supplement, group B received in addition 20 mg. of *l*-cystine per day, group C 20 mg. of recrystallized *l*-cystine and group D was supplemented with *l*-cystinedisulphoxide containing the sulphur equivalent of the cystine fed, 22.6 mg. daily. Fig. 1 shows the growth curve obtained.

In the second experiment 26 rats from 4 litters, about 1 month old, were divided into 3 groups of 8, 9 and 9 animals respectively; 5 females in each group, the rest males. After 8 days on the cystine-deficient diet group A was continued on the same, group B was fed 20 mg. of *l*-cystine daily, group C was given the sulphinic acid containing the sulphur equivalent of the cystine, 25.5 mg. daily for 14 days. Fig. 2 gives the results.

In the third experiment 40 rats from 5 litters, about 1 month old, were divided into 5 comparable groups of 8 animals, 4 males and 4 females in each group. After 8 days on a cystine-deficient diet, group A was continued without cystine supplement, group B was fed 20 mg. of *l*-cystine per day, group C was given thiourea; the first dose was 12.7 mg. daily, the amount containing 1 sulphur equivalent of the cystine fed. This dose proved too toxic for the rats, therefore the amount was halved on the second day, 6.4 mg. of thiourea being fed. The fourth group D was fed 1 sulphur equivalent of dithioformamidine.2HCl, 18.6 mg., which because of toxicity was also reduced to half the

dose, or 9.3 mg. after the first day. The final group E was fed 1 sulphur equivalent of *S*-(guanyltio)-cysteine. 2HCl, 22.3 mg. daily for 14 days. An equivalent of NaHCO₃, 14 mg., was added to the butter mixtures of each hydrochloride to

Fig. 3. Replaceability of *l*-cystine in the diets of albino rats with *l*-cysteinylsulphenic acid, theoretically produced by hydrolysis of *S*-(guanyltio)-cysteine. Thiourea and dithioformamidine are used as additional controls.

neutralize the acidity. The groups A, C, D and E were then fed 20 mg. of *l*-cystine daily, in addition to the basal and special diets, for 7 more days, in order to test the toxicity of the compounds being fed. Fig. 3 shows the results obtained.

DISCUSSION

Identical growth curves were obtained with *l*-cystine (Merck) and the highly purified *l*-cystine, showing that our control experiments with cystine were free from any contaminating substance.

Medes [1935] reported that *l*-cystinedisulphoxide was capable of replacing *l*-cystine in the diet of rats, but because of the high cystine content of her basal diet, the results were not clear-cut and hence it was deemed worth while to repeat the experiment. *l*-Cystinedisulphoxide in the present set of experiments produced growth, the slope of the curve being practically identical with that of the control animals receiving cystine. Therefore the reaction

probably occurs in the body. This would indicate that the dismutation, shown by Lavine [1936] to occur in solution, does not take place in the organism under the conditions of these experiments. According to that series of reactions only 1 equivalent of cystine is available from 3 equivalents of cystinedisulphoxide.

l-Cysteinylsulphenic acid produced no growth; the curve closely followed that of the control without cystine, indicating that $-\text{SO}_2\text{H}$ was not converted into $-\text{SH}$ or $-\text{S}-\text{S}-$. This is consistent with the prediction of Hammett [1930] that increase in the state of oxidation of SH means decrease in its reversible reduction capacity.

The —SOH derivative, *l*-cysteinesulphenic acid, was produced by feeding *S*-(guanylythio)-cysteine, which, according to Toennies [1937], hydrolyses in solution to give *l*-cysteinesulphenic acid, possibly according to the following set of reactions:

From the equation above it is possible to obtain only 33 % cystine. An alternative possibility may be expressed by the following equation, in which case cystine to the extent of 50 % would be available for growth:

The *R*-SOH compound produced an increase in weight, while the three control curves, in comparison, showed a gradual decrease. In spite of the evidence for the first group of reactions in solution, it is not certain that these occur in the organism. It is not possible from the growth curves of the animals to decide between these two possibilities, although the slight growth suggests the availability of the smaller amount.

Addition of *l*-cystine caused the groups containing the experimental substances to grow in approximately the same proportions, indicating that lack of growth was not due to toxic properties of the compounds fed but to absence of sufficient *l*-cystine from the diet.

SUMMARY

Identical growth curves were obtained with *l*-cystine (Merck) and with highly purified *l*-cystine added to a cystine-deficient diet of albino rats.

l-Cystinedisulphoxide, —SO—SO— group, proved capable of replacing *l*-cystine in the diet.

l-Cysteinesulphenic acid, —SO₂H group, produced no growth.

S-(Guanylythio)-cysteine.2HCl, which probably gives rise to *l*-cysteinesulphenic acid, —SOH group, resulted in a slight, but definite increase in growth.

The possible reactions involved are discussed.

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REFERENCES

- Böeseken (1936). *Proc. Acad. Sci. Amst.* **39**, 717.
 Dyer & du Vigneaud (1936). *J. biol. Chem.* **115**, 547.
 Hammett (1930). *Protoplasma*, **11**, 382.
 Lavine (1936). *J. biol. Chem.* **113**, 583.
 Medes (1935). *J. biol. Chem.* **109**, lxiv.
 Shinohara (1935). *J. biol. Chem.* **109**, 665.
 Toennies (1937). *J. biol. Chem.* (in Press: cf. *Proc. Amer. Soc. biol. Chem.*, April, 1937).
 — & Lavine (1936). *J. biol. Chem.* **113**, 571.