groups in mixtures of homocysteine and cystine can be determined with the same accuracy although it is not possible to distinguish homocystine and cystine qualitatively by this method.

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Conversion of S-benzylglutathione to Benzylmercapturic Acid in the Rat.*

JACOB A. STEKOL.

From the Department of Chemistry, Fordham University, New York.

We reported recently that benzyl chloride, when administered to dogs, rabbits, and rats, yields in the urine of these animals N-acetyl-S-benzylcysteine. The same mercapturic acid was obtained from the urine of dogs, rabbits, and rats on feeding S-benzylcysteine to the animals.[1]

When S-benzylhomocysteine was administered to rabbits and rats N-acetyl-S-benzylhomocysteine was excreted in the urine.[2] This finding indicated that homocysteine is not the intermediate substance in the synthesis of benzylmercapturic acid from benzyl chloride *in vivo* and that benzylhomocysteine is not convertible to benzylcysteine in the animal body. The acetylation of S-benzyl-cysteine and S-benzylhomocysteine in the rat was confirmed.[3]

The study of the metabolism of the benzyl derivatives of sulfur-containing amino acids and their derivatives has now been extended to S-benzylglutathione. The experiments described here deal with the fate of S-benzylglutathione in the rat.

Experimental. Synthesis of S-benzylglutathione. 1.0 g of commercial glutathione was dissolved in about 30 cc of liquid ammonia and dry metallic sodium was added to the solution in small portions until a permanent blue coloration was obtained. An excess of benzyl chloride (0.5 cc) was then added dropwise, with shaking, and the ammonia was allowed to evaporate. The residue was then extracted with ether 3 times and then dissolved in water. Enough concentrated HCl was added to the solution to obtain a pH of 4 to 4.5, and

* Grateful acknowledgment is made to Mr. J. Alicino for the microanalytical work.

1 Stekol, J. A., *J. Biol. Chem.*, 1938, **124**, 129.

2 Stekol, J. A., *J. Biol. Chem.*, 1939, **128**, 199.

3 du Vigneaud, V., Wood, J. L., and Irish, O. J., *J. Biol. Chem.*, 1939, **129**, 171.

the reaction mixture was placed in a refrigerator overnight. The white granular precipitate was filtered, washed with cold water, then recrystallized twice from boiling water, filtered and dried *in vacuo* over H_2SO_4. The yield was 1.0 g, or about 80% of the theory. The analytical values of the substance are shown below.

	C	H	N	S	M.P.°C	
Found:	51.21	5.92	10.64	8.01	199-200	(uncorrected)
Calculated for $C_{17}H_{23}O_6N_3S$:	51.38	5.79	10.58	8.06		

Experiments with S-benzylglutathione. 0.4 to 0.5 g portions of S-benzylglutathione were fed mixed with food to each of 3 adult albino rats of 200 to 250 g weight. The urine was collected as was described previously.[1, 2] The urine of each rat was evaporated to a syrup *in vacuo* at 38-40°, acidified with HCl, and extracted with several portions of ethyl acetate. The extract was evaporated to dryness *in vacuo* at 38 to 40°, and the residue was dissolved in hot water. On cooling, crystalline material separated. It was centrifuged and recrystallized from hot water several times, filtered and dried *in vacuo* over H_2SO_4. The substance crystallizes from water in long needles. The analytical values are shown below.

	C	H	N	S	Acetyl	M.P.°C	
Found:	56.96	5.97	5.58	12.42	16.53	143-144	(uncorrected)
Calculated for $C_{12}H_{12}O_3NS$:	56.92	5.93	5.54	12.65	16.98		

One percent of the substance in 95% alcohol gave $[\alpha]_D^{26} = -42°$, and the melting point of 50% mixture of the substance with an authentic sample of N-acetyl-S-benzyl-*l*-cysteine remained unchanged.

The results reported above indicate the conversion of S-benzylglutathione to N-acetyl-S-benzylcysteine in the rat. It appears probable that S-benzylglutathione was hydrolyzed *in vivo* to yield S-benzylcysteine. The latter was then converted to the acetyl derivative, as could be expected from earlier work.[1, 2] As we suggested previously,[1, 2] the acetylation of S-benzylcysteine and S-benzylhomocysteine *in vivo* is probably preceded by oxidative deamination of the substances as postulated by the theory of Knoop.[4] This view is now supported by the experimental data of du Vigneaud, *et al.*[3]

It would be of interest to ascertain whether glutathione augments the synthesis of benzylmercapturic acid from benzyl chloride in the rat, particularly because our present data suggest the possibility of direct combination of benzyl chloride with glutathione *in vivo* prior

[4] Knoop, F., and Blanco, J. G., *Z. Physiol. Chem.*, 1925, **146**, 267.

to the formation of benzylmercapturic acid. It should be mentioned, however, that glutathione failed to augment the synthesis of p-bromophenyl and l-α-naphthalenemercapturic acids in the rat, suggesting the inference that glutathione is not involved directly with the synthesis of these mercapturic acids *in vivo*.[5]

Summary. 1. S-benzylglutathione was synthesized and fed to adult rats. N-acetyl-S-benzyl-*l*-cysteine was isolated from the urine of these animals and identified by analysis. 2. The results suggest that the benzylated tripeptide was hydrolyzed in the rat to yield S-benzylcysteine which was then acetylated *via* the mechanism proposed by Knoop.[4]

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Development of Vaccinia and Variola Viruses in Embryonated Eggs at 28°C.

JOHN B. NELSON. (Introduced by Carl TenBroeck.)

From the Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, N. J.

At a temperature of 28°C the embryo in fertile 10-day hen's eggs ceases to develop but may remain alive for 48 to 72 hours, showing active movement on removal. By the 4th day, however, the embryo is usually inactive and autolytic changes may be apparent in the chorioallantoic membrane. It seemed of interest to determine whether certain viruses which are readily propagated in fertile eggs at temperatures favoring a normal development of the embryo could also be maintained at 28°C.

Accordingly, observations were made on the behavior of vaccinia and variola viruses in 10-day embryonated eggs held at a constant temperature of 28°C for 2 to 4 days in a humidified incubator. The chorioallantois was retracted from the shell membrane by suction and small unmeasured amounts of the respective virus suspensions in saline were introduced through a window in the shell. The eggs were generally opened on the 3rd day and the chorioallantoic membrane was removed for microscopic examination.

Vaccinia virus originally derived from the New York City Board of Health strain was established on the chorioallantoic membrane of embryonated eggs incubated at 28°C and maintained in 2 series of

[5] Stekol, J. A., *J. Biol. Chem.*, 1938, **122**, 333.