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Distribution of Sulfur in Crystalline Insulin.

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The distribution of the sulfur in insulin has long been a subject of interest. Crystalline insulin contains 3.06 to 3.38% of sulfur.[1] Only part of the total sulfur could be accounted for by cystine, the Folin-Marenzi method yielding 9-10% and the Sullivan method 8-9% of cystine.[2] Methionine has been claimed to be absent.[2, 3]

A reliable technique for the estimation of methionine in proteins has been established by Baernstein[4] and further developed to include the iodometric determination[4] of cysteine and homocysteine. The Folin method for the determination of cystine has been adapted to the Pulfrich Photometer,[5] resulting in greater specificity. It has been found that the Sullivan method gives more constant and reliable results if the time interval between additions of color reagent and sulfite is regulated[6] (10 seconds measured with a stopwatch).

It was, therefore, thought desirable to determine the distribution of the sulfur in crystalline insulin with the aid of these improved techniques. A sample of such material (crystalline insulin, Lilly T-800) was kindly put at our disposal by Eli Lilly and Company. The insulin was repeatedly extracted with petroleum ether and dried *in vacuo* at 100°C. Its sulfur content (Pregl) was found to be 3.11 and 3.14%. The ash content was 1.5%. Certain observations[7] may indicate that 1-3% of the total sulfur of this preparation is in the form of sulfate. The presence or absence of such traces of sulfate could not be ascertained directly on account of the large amounts of crystalline insulin necessary.

The experimental data are presented in Table I. The determinations by Baernstein's methods were carried out as described,[4] ex-

1 Scott, D. A., *J. Biol. Chem.*, 1931, **92**, 281.

2 *cf.* Jensen, H., Evans, E. A., Pennington, W. D., and Schock, E. D., *J. Biol. Chem.*, 1936, **114**, 199.

3 Freudenberg, K., Dirscherl, W., and Eyer, H., *Z. physiol. Chem.*, 1930, **187**, 89.

4 Baernstein, H. D., *J. Biol. Chem.*, 1934, **106**, 451; *J. Biol. Chem.*, 1936, **115**, 25, 33.

5 Kassell, B., *J. Biol. Chem.*, 1935, **109**, xlix.

6 Rossouw, S. D., and Wilken-Jorden, T. J., *Onderstepoort J. Vet. Sci.*, 1934, **2**, 361.

7 Kassell, B., and Brand, E., unpublished experiments.

TABLE I.
Cystine and Methionine Analysis of Crystalline Insulin.
(Lilly T-800, Total S—3.13%, Ash—1.5%.)

Exp. No.	Insulin mg.	Hydrolysis‡ Acid used 5 cc.	Time hr.	Cystine Sullivan %	Folin (Photo.) %	Baernstein %	Methionine (vol. iodide) %
1	51.8	6N-HCl	6	10.5	10.8		
2	32.6	6N-HCl	8	10.8	11.0		
3	28.7	6N-HCl	17	11.5	11.1		
4	45.4	57% HI	6			11.2*	0.8†
5	157.6	57% HI	6			11.2*	0.6†
Average				10.9	11.0	11.2	0.7
Calculated as S					2.94		0.15
Percent of Total S					94		5

*Corrected for decomposition (H_2S ≃ 0.8% Cystine).[7]
†Corrected for methyl mercaptan formation[7] ≃ 0.05% Methionine.
‡Bath temperature for HCl—130°C., for HI—150°C.

cept for some minor modifications and corrections.[7] The small amounts of insulin used in the estimations precluded the accurate determination of homocysteine, but the presence of its lactone in the HI digests was established. There were no sulfhydryl compounds in the HCl hydrolysates, and the modified Folin photometric determination[5] indicated the probable absence of disulfides other than cystine.

The average cystine content of the hydrolysates was 11.0%. The Sullivan method, the Folin photometric method, and the Baernstein method gave practically identical results. The figures seem to indicate that hydrolysis for 6-8 hours liberates all of the cystine.

The methionine content of the preparation was 0.7%. This amount is so small that, to exclude the possible presence of methionine-containing impurities in our sample, further experiments on different and specially recrystallized samples of crystalline insulin may be necessary.

In these experiments 99% of the total sulfur of a preparation of crystalline insulin was accounted for, 94% by cystine and 5% by methionine. It is interesting to note that the sulfur distribution of insulin resembles that of wool.[8]

[8] Barritt, J., *Biochem. J.*, 1934, **28**, 1.

Note: Drs. duVigneaud and Miller informed us that their unpublished experiments indicate a cystine content of crystalline insulin higher than heretofore obtained.