

course, might elicit eating in the early postoperative periods, but thus far we have had very little success with a number of foods tried: Purina Laboratory Pellets, a mash of 50 percent powdered laboratory food and water by volume, the same mash sweetened with dextrose, straight dextrose, and fresh ground beef. Yet some of our animals would eat bitter baker's chocolate, although in smaller quantities than milk chocolate.

Once rats recover the ability to eat postoperatively, they maintain the same intake of laboratory food and water as comparable normal animals and can regain and hold their preoperative weights. Nevertheless, it seemed worth while to determine more adequately whether or not recovery was complete. Some preliminary comparisons between six normal rats and six recovered rats were made. (i) When fed laboratory powder diluted with nonnutritive cellulose (25 percent by weight), both normal and recovered rats increase their gram intake by about 25 percent, thus maintaining their caloric intake. The recovered animals, however, reduce their intake for the first 3 to 4 days of cellulose-dilution, whereas normal animals make the adjustment within the first day. (ii) Both normal and recovered animals strongly prefer chocolate and evaporated milk when these are offered in addition to laboratory powder and water and reduce their intake of laboratory powder and water by 50 and 75 percent, respectively. (iii) The recovered animals prefer pure corn oil much more than do normals when it is offered in addition to laboratory powder and water. Controls take less and less oil on successive days and the recovered animals take more and more, until, by the end of 3 days, the controls are consuming less than one-half as much oil as the recovered animals.

These findings suggest a number of interpretations. (i) It seems that the lateral hypothalamic lesions impair an excitatory mechanism important in eating. Supporting this idea is the finding of Delgado and Anand (5) that stimulating this region through implanted electrodes can greatly increase eating behavior. (ii) The loss of eating behavior is only temporary. This fact itself is not surprising, for the same recovery of function has also shown up following changes in sleep (6), temperature regulation (6), and emotion (7) induced by lesions of the hypothalamus and related subcortex. The real questions are: What is the nature of the recovery (and therefore the loss)? What is the neural mechanism involved in the recovery? (iii) Some insight into the nature of the loss of function has been gained by plotting the course of its recovery. From our findings with evaporated milk, chocolate, and corn oil, we propose the hypothesis that fat may elicit eating behavior in operated animals sooner and more readily than other foods. Rephrased in more general terms, lesions of the lateral hypothalamus may change the rat's reactions to certain stimulus-aspects of the diet. At first rats will respond to no food stimulus postoperatively. After some recovery, certain food stimuli (provided by fats?) will elicit eating but others still will not. Finally, the recovered rats seem

responsive to enough of the stimuli provided by laboratory food to eat it as normals do, but they still seem to have a heightened fat-appetite.

#### References and Notes

- \* Present address: Department of Psychology, Harvard University.
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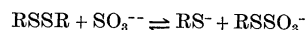
8 July 1954.

## Amperometric Determination of Disulfides in Intact Proteins

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In studies on disulfide bonds of proteins involved in blood coagulation (1, 2), it was found that the amperometric technic for —SH determinations (3) could be adapted, with modifications, to the measurement of —S—S— also. The basic principle, outlined by Kolthoff and Lingane (4), is the sulfitolysis of the protein —S—S— according to the following reaction:



Thus, 1 mole —S—S— yields 1 mole —SH after reaction with sulfite.

One milliliter of an aqueous protein solution, treated with a minute amount of antifoam emulsion (5), is added to 28 ml of 90 percent ethanol containing sufficient  $\text{NH}_4\text{OH}$ ,  $\text{NH}_4\text{NO}_3$ , and ethylenediamine tetraacetate to make the concentrations 0.25, 0.05, and  $3 \times 10^{-5}$  molar, respectively, in a final volume of 31 ml of reaction mixture; 2 ml of cold saturated  $\text{Na}_2\text{SO}_3$  then is stirred into the titration mixture, and the titration with  $10^{-3}M$   $\text{AgNO}_3$  is started immediately.

Quantitative results are obtained only when the reagents are added in the order given, when 90 percent ethyl alcohol is used, and when an excess of  $\text{Na}_2\text{SO}_3$  is present. It is essential that the  $\text{Na}_2\text{SO}_3$  precipitate remain in the titration mixture, since some of the protein is adsorbed on it. This precipitate does not interfere with the titration, provided that the platinum electrode is rotated above the level of the precipitate.

Table 1. Disulfide values in oxidized glutathione, insulin, and prothrombin.

Biological substance	Quantity of biol. substance in titration mixture	10 <sup>-3</sup> M AgNO <sub>3</sub> used in titration (ml)	N per milliliter or milligram of biol. substance (mg)	—S—S— per mole of biol. substance (mole)	
				Our data	Data from literature
Oxidized glutathione*	0.5 mg	0.82		1.00	
	.25 mg	.42		1.03	
	.10 mg	.16		0.98	
	.05 mg	.08		.98	
Insulin†	1.0 ml	.85			2.91‡
	.05 ml	.42	0.268	2.81	2.98§
	1.0 ml	.21			
	0.5 ml	.11	.066	2.83	
Prothrombin	5.0 mg	.30	.156	3.86	
	2.0 mg	.12	.157	3.86	4.10¶
Prothrombin**	0.1 ml	.07	.167	4.06	

\* Nutritional Biochemicals Corp.; calculated on basis of 13.7 percent N.

† Lilly Research Laboratories, T-2842; calculated on basis of 15.55 percent N and mol. wt. 5700.

‡ Harfenist (7); beef insulin, component A.

§ Harfenist (7); beef insulin, component B.

|| Purified bovine prothrombin (Seegers); reconstituted from frozen-dried state; N corrected for 2.42 percent ash.

¶ Laki *et al.* (8); calculated from 3.14 g amino acid per 100 g protein expressed as cystine/2 and from mol. wt. 62,700.

\*\* Purified bovine prothrombin (Seegers); thawed from liquid-frozen state; calculated on basis of 15.5 percent N, mol. wt. 62,700.

It is necessary that the Na<sub>2</sub>SO<sub>3</sub> be freshly prepared, cold, and fully saturated, and that it be used within 45 min after preparation. Under the foregoing conditions, —S—S— does not react with Ag<sup>+</sup>. It was observed that higher protein concentrations could be employed in the presence of the antifoam emulsion. Passage of nitrogen through the titration mixture was unnecessary (6). Typical results obtained with the method are shown in Table 1.

Multiple determinations, using variable concentrations, have been made on oxidized glutathione, insulin, prothrombin, thrombin, fibrinogen, and thromboplastin. Recovery of oxidized glutathione was virtually complete when it was added to the proteins used in this work. The results obtained by this method are in

good agreement with those obtained by others on *hydrolyzates* of insulin (7) and of prothrombin (8).

#### References and Notes

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9 July 1954.

## Communications

### Few Students Want Culture

Harry J. Fuller's communication [*Science* **120**, 546 (1 Oct. 1954)] reminds me of a day at a public examination for a Ph.D. degree in surgery when the young candidate had reported satisfactorily on much research work on surgery of the stomach. Then a bombshell fell when one of the professors with a historical turn of mind asked who was Dr. W. Beaumont, and the candidate did not know! The professor then asked if he, the young physician, had ever heard of Alexis St. Martin, and he had not. This so upset the committee that the degree was not granted.

After 40 years of teaching medicine to graduates and undergraduates I have the unhappy feeling that most of the men whom I have watched as undergraduates, interns, residents, and graduate students, preparing for a specialty, were not interested in becoming learned and widely experienced and wise. They were interested in getting a certificate of 3 years' attendance that would enable them to take an examination, which, if passed, would give them a listing as a specialist. The less effort used in getting this certificate, the better.

The saddest moment in my teaching life came one day when, going into a library that had been used for half a century by hundreds of graduate medical stu-