

	Percentage				
	A	B	C	D	E
Boys (214, or 45.2 per cent.)	2.3	30.4	55.1	6.6	5.6
Girls (259, or 54.8 per cent.)	2.3	13.1	57.5	15.9	11.2
Entire group (473)	2.3	20.9	56.5	11.6	8.7

showing. This is also brought out by taking a general average for boys and for girls. On the basis of A=4, B=3, C=2, D=1 and E=0, the boys show an average of 2.17 or C+, and the girls 1.80 or C-. Even when the 45 girls with the lowest records are eliminated, so that an equal number of boys and of girls are compared, the average for these 214 girls is but 2.09, a distinctly lower average than for the entire group of boys.

These statistics show clearly that, for the group in question, the study of geology is markedly easier for boys than for girls. From contact with the individual students, I believe that an important element in this difference is that the boys adjust themselves more readily to the changes in methods of instruction, in passing from high school to college, especially to the demand for a greater degree of initiative on the part of the student and to the greater emphasis placed on cause and effect relations as contrasted with mere memorizing of facts from a prescribed assignment in a text-book. However, the exceptional girl can and does do work equal to that of the exceptional boy.

Geology, though more difficult for the average girl than for the average boy, appears to furnish her a more attractive way of fulfilling the science requirements of a college course than do biology, physics or chemistry. During the last few years, freshman classes have been composed of about 55 per cent. boys and 45 per cent. girls, while the geology group under discussion consists of 45 per cent. boys and 55 per cent. girls. Contrasted with this distribution, boys generally outnumber girls at least two to one in the elementary courses in biology, physics and chemistry. This would seem to indicate that older girls, in common with the ninth-graders studied by Smith, find biology, physics and chemistry more difficult or, at least, less attractive, than do boys. In contrast, geology is more difficult for the older girl than for the older boy, but not for those of ninth-grade age.

These data and conclusions are, of course, for a group of students from one city institution and receiving instruction from one individual and that a man. It would be interesting to learn whether or not statistics from institutions with different settings and in other localities confirm these conclusions.

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### THE ACTIVATION OF UREASE

THE recent studies by Waldschmidt-Leitz and co-workers<sup>1</sup> on the significance of sulhydryl compounds as activators in a large number of enzymic processes led to a reinvestigation of the factors affecting the activity of urease.

It has long been known that heavy metals "poison" urease, whereas cyanides, H<sub>2</sub>S and certain amino acids "protect" the enzyme or activate it after it had been "poisoned." The action of these protective substances has been ascribed largely to the effect upon the poisoning or inhibiting influence of the heavy metals (Cu, Hg, Ag) by the formation of complex ions, as with cyanides and amines, or of insoluble compounds, as with sulfides. But since the heavy metals catalyze the oxidation of the thiol (SH) group in cysteine, glutathione and similar compounds, while cyanides and sulfides reduce dithiol, -S-S-, groups to thiol, -SH, groups, it is plausible to suspect that some thiol compounds may play a part in the activity of urease. This suspicion was strengthened by the application of the nitroprusside test for -SH to various jack bean meal extracts and to crystalline urease preparations of widely varying range of activity. It was found at once that the more active preparations gave a stronger nitroprusside test than the weaker ones. Furthermore, the most active jack bean meal in our possession (164 units per gram of meal) was found to contain 70 mg of reduced glutathione per 100 grams, using the combined methods of Vivario and Lecloux<sup>2</sup> and of Kühnau.<sup>3</sup> That the -SH compound was glutathione rather than cysteine was surmised from the fact that the highly specific Sullivan reaction was negative before and positive after hydrolysis. On partial oxidation by means of aeration of highly active crystalline urease prepared with the use of solvents carefully freed of metals,<sup>4</sup> it was observed that the -SH reaction became weaker with the decrease in activity. The activity could be restored to the original titer by cyanides, cysteine and reduced glutathione, but only partially restored by alanine. The loss of activity could be prevented if the air current used for oxidation was saturated with aqueous cyanide vapor or by the addition of a sufficient excess of cysteine before aeration was begun.

That the -SH groups are not solely responsible for urease activity is suggested by our observation that urease solutions oxidized by aeration or by H<sub>2</sub>O<sub>2</sub> to the complete loss of activity can be reactivated but to a very slight extent by means of -SH compounds, sulfides or cyanides.

Experiments now in progress indicate that it may

<sup>1</sup> Summarized in *Naturwissenschaften*, xix, 964, 1931.

<sup>2</sup> *Arch. intern. de Physiol.*, xxxii, 1, 1930.

<sup>3</sup> *Biochem. Zeit.*, ccxxx, 353, 1931.

<sup>4</sup> J. B. Sumner, *Ergebn d. Enzymforsch.*, i, 295, 1932.

be possible to separate the -SH containing activator component from urease in a manner analogous to that employed for aginase and cathepsin by Waldschmidt-Leitz.<sup>5</sup>

It is of interest to note that while the activity of deamidizing enzymes, arginase and urease, is enhanced or conditioned by the presence of certain reducing groups, Salaskin and Krivskii<sup>6</sup> have observed that synthesis of urea in the perfused liver from ammonium carbonate required the presence of an abundant supply of oxygen in the form of oxyhemoglobin. It is thus evident that the direction as well as the relative activity of an enzymic process may well depend upon the oxidation reduction potentials of the system.

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#### FERROUS IODIDE AND LINOLEIC ACID IN VITAMIN A DEFICIENCY. A REPLY TO CERTAIN CRITICISMS

To those students of vitamins who have not read our brief reports it may appear that Reed, Mendel and Vickery, in *SCIENCE* for September 30, 1932, have actually repeated our experimental procedure with ferrous iodide and supplemental linoleic acid and that their negative findings cast some doubt on the studies reported by us. This is not the case, for neither the authors cited nor the other workers who have similarly failed to repeat our work have duplicated our experiments.

We have been too busy in evaluating the rôle of Vitamin B in our diets and in studying the influence of iodine added to various oils and fatty acids, and even to oxygenated cod-liver oil, to report our findings in detail. Then, too, we have had a certain reticence, born of experience about attempting to furnish advice to those so long in the field as some of the critics of our work. It would seem, however, that perhaps a few very elementary facts regarding dosage of depleted animals might have been gratuitously offered in our summaries.

(1) We administered our ferrous iodide and supplemental linoleic acid, *indirectly*, in small amounts of food. All the persons who have reported negative results with ferrous iodide gave the medicine directly, syringing it into the mouths of highly depleted rats, without added food. No medical adviser, such as our critics consulted regarding xerophthalmia, would countenance such drastic treatment. It is to be noted that in the experiment listed (*loc. cit.*) cod-liver oil was not thus offered.

(2) But, even if ferrous iodide and linoleic acid

<sup>5</sup> *Zeits. physiol. Chem.*, clxxxviii, pp. 17 and 31, 1929.

<sup>6</sup> *Zeits. physiol. Chem.*, cxcvi, 121, 1931.

are given in food, one can not expect recoveries when only 5 per cent. of yeast is used, as in the diet of Reed, Mendel and Vickery. We used 10 per cent. yeast (Vitavose), of a brand recommended highly by those who had tested several kinds. This Vitavose contains .0125 per cent. of iron and is quite rich in yeast fats. Reports on the yeast fats have appeared from time to time in the *Biochemical Journal*, and we have had a considerable discussion of the significance of different sources of Vitamin B in the *Journal of Biological Chemistry*. For example, Norris and Church (*Jour. Biol. Chem.*, 89, 589, 1930) showed that several failures to recover rats, depleted of Vitamin A, with cod-liver oils were due to the amount of Vitamin B used. When 15 per cent. to 18 per cent. of the yeast vitamin was added, recoveries ensued. This is also in line with the report of Honeywell, Dutcher and Ely (*Jour. of Nutrition* 3, 491, 1931), who evaluated yeasts in their Vitamin A studies and who concluded that Vitamin A probably consists of two factors. Personal communications from a friend at Harvard University and from a worker in one of the Yale laboratories, regarding the value of yeast as an appetizer in Vitamin A recoveries, caused our group to test added Vitavose with our medicinal treatments. We extracted the Vitavose with ether for safety, and found that the animals attacked our mixture with greater avidity. But the crucial test came when, last year (1931), we split 16 litters of rats into four requisite groups and learned that with low Vitamin B, our linoleic acid and ferrous iodide combinations were of very slight benefit, and that the recoveries of our cod-liver oil controls were not as numerous as before. Perhaps low Vitamin B may account in part for the difference in results reported. But the fact that we ran our animals down on the deficiency diet, then added ergosterol, is also an important difference. Of course, ergosterol accentuates the mineralization of the animals, and with no added fats, and relatively little yeast fats, the digestive functions are much impaired. It has long been known by physiologists that fats and oils will facilitate the optimum absorption of minerals. Evidently the vitamin students have been getting their peculiar effects, largely by a mineral imbalance and by fat-starvation.

When we reduced the Vitamin B content of our diet, we found a high incidence of gallstones and urinary calculi. The presence of stones has been reported by several workers, using standard diets and without reference to the Vitamin B content. Since cod-liver oil contains fatty acids and some *iodin*, it may be significant in this connection that Seel and Creuzberg (*Archiv. exp. Pathol. p. Pharmak.*, 1931, 161, 674) have shown that iodine will improve the