6567

Sulfhydryl Compounds and Crystalline Urease.

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Perlzweig[1] states that both crystalline urease and extracts of jack bean meal give a positive sulfhydryl test with nitroprusside and that the more active preparations give stronger tests than the weaker ones. He estimates one of his samples of jack bean meal to contain the equivalent of 70 mg. of glutathione per 100 gm. and believes the substance in the meal to be glutathione, rather than cysteine, since the material is negative to the Sullivan reaction until after hydrolysis. He suggests that urease activity is due in part to the sulfhydryl compound and that it may be possible to separate a sulfhydryl activator from the urease proper.

We have independently observed that urease, prepared from jack bean meal by the Sumner procedure and twice recrystallized from 30% alcohol, produces a moderate amount of red color when treated with nitroprusside, ammonium sulfate and ammonia, and had ascribed this reaction to the presence of sulfhydryl groups attached to the urease molecule. It was recently shown that no co-enzyme for urease is present in the soy bean, or the jack bean.[2] However, in view of the statements of Perlzweig we have undertaken experiments to ascertain the connection between crystalline urease and the sulfhydryl groups.

Perlzweig refers to studies of Waldschmidt-Leitz and his students upon the significance of sulfhydryl compounds in enzymatic reactions.[3] Attention should be called to the fact that the claim made by Waldschmidt-Leitz that liver arginase is wholly inactive in the absence of sulfhydryl compounds, while it is active in the presence of hydrogen sulfide, cysteine, or reduced glutathione has been vigorously denied by Edlbacher, Kraus, and Walter,[4] as well as by Klein and Ziese,[5] who claim that arginase is never activated by cysteine or by glutathione, but instead is inhibited.

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[1] Perlzweig, W. A., *Science*, 1932, **76**, 435.

[2] Sumner, J. B., and Kirk, J. S., *Biochem. J.*, 1932, **26**, 551.

[3] Waldschmidt-Leitz, E., and collaborators, *Naturwis.*, 1929, **17**, 85; 1930, **18**, 644; 1931, **19**, 964; *Z. physiol. Chem.*, 1930, **188**, 17; 1931, **198**, 260.

[4] Edlbacher, S., Kraus, J., and Walter, G., *Z. physiol. Chem.*, 1932, **206**, 65.

[5] Klein, G., and Ziese, W., *Z. physiol. Chem.*, 1932, **211**, 23.

It is impossible to employ crystalline urease prepared by the Sumner procedure[6] directly for the nitroprusside test, since the preparation contains acetone, which itself gives an intense coloration with nitroprusside. Therefore we have prepared urease by the usual procedure, and have then recrystallized it from 30% alcohol. One sample of urease, after 2 recrystallizations, gave a nitroprusside test equivalent to about 0.02 mg. of glutathione per mg. of urease. On the other hand, the original jack bean meal, compared with pure glutathione, was found to contain per 100 gm., roughly the equivalent of 50 mg. of glutathione. Since this meal had 195 units of urease per gram, and since the urease had 135 units per mg., it may be calculated that of the total color-producing substance in 100 gm. of jack bean meal only about 3% could be credited to the urease. Furthermore, while the substance in jack bean meal which produces most of the color, is readily dialyzable through a collodion membrane, the substance in crystalline urease, responsible for the nitroprusside test, will not dialyse. We have dialysed from 1 to 3 cc. of urease, containing from 1600 to 4800 units, against 5 cc. of water for from 2 to 44 hours and have never been able to detect the least trace of sulfhydryl compound in the outer liquid, while we have found the dialysed urease to produce approximately the same amount of red color with nitroprusside as at the start.

Hence, it is certain that while jack bean meal contains a considerable amount of material which reacts similarly to glutathione, crystalline urease does not contain this substance, but does give a reaction with nitroprusside, either because the urease molecule contains sulfhydryl groups, or because the preparation contained some colloidal impurity which contains sulfhydryl groups.

Titration of urease with iodine, by a procedure similar to that used by Kühnau[7] for the determination of glutathione, shows that in dilute sulfuric acid 1 mg. of urease reacts with 0.1 cc. of N/500 iodine. Twice recrystallized and dialysed ovalbumin uses up only approximately one-half of this amount of iodine. The filtrate from urease, precipitated with trichloracetic acid, takes up no measurable amount of iodine. Urease was found to give a much weaker nitroprusside test after having been boiled than before, while, ovalbumin, as is well known, gives no nitroprusside test until after having been coagulated by boiling or by other means.

Having access to crystallization, it seemed rather absurd to attempt to separate the sulfhydryl compound from crystalline urease

[6] Sumner, J. B., *J. Biol. Chem.*, 1926, **49**, 435.

[7] Kühnau, J., *Biochem. Z.*, 1931, **230**, 353.

by adsorption on kaolin or any other adsorbent. Consequently we have recrystallized urease from 30% alcohol repeatedly in order to note whether it is possible to remove the sulfhydryl compound. After 3 recrystallizations the amount of color given by the nitroprusside test remains approximately the same as after one recrystallization. We believe, therefore, that the urease molecule itself contains sulfhydryl groups, or groups which give the nitroprusside test. These groups, if sulfhydryl, may account for the readiness with which urease is inactivated by silver, mercury and copper ions, and by quinone and other oxidizing agents, and why urease is protected by sulfhydryl compounds.

Finally, the dialysable substance in jack bean meal is not concerned with urease activity since urease which is freed from this material has high activity, and since addition of concentrated dialysate from jack bean meal exerts no favorable effect upon urease action.

We wish to thank Dr. D. B. Hand for his kindness in supplying us with pure glutathione.

6568

Transmission of Neurohumors in Animals by Other Means Than Blood and Lymph.

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It has been assumed that the contraction or expansion of dermal melanophores in fishes whereby these animals take on a light or a dark tint is dependent upon substances carried in the animal's blood and lymph (Parker[1]). Such an assumption appears to be justified by the fact that if a small amount of dissolved adrenalin is injected into the muscles of a dark chub, *Fundulus heteroclitus,* this fish within a quarter of an hour or less will become very light-colored. That the normal changes in tint shown by such a fish are brought about by other means than by dissolved substances carried in blood and lymph seems very probable from the following observations.

If a short transverse incision is made in the tail of a light-colored Fundulus, a dark stripe extending from the cut to the posterior edge

[1] Parker, G. H., Humoral agents in nervous activity with special reference to chromatophores. Cambridge, England, 1932, 79 pp.