amine, 0·2 mgm.; nicotinamide, 0·8 mgm.; uracil, 1·5 mgm.; riboflavin, 10 μgm.; biotin, 0·1 μgm.; vitamin B_{12}, 63·0 μgm.; and distilled water to 1,000 ml. (*p*H 7·6). Stock cultures of *Cl. histolyticum* were maintained in 'Pyrex' tubes (25 × 200 mm.) containing 40 ml. of this medium to which 1 gm. of dry, fat-free bovine heart was added. The stock cultures were transferred at monthly intervals and were refrigerated in the interim. The culture medium used in the production of collagenase consisted of the following constituents in 1 l. of medium: Solution 1, casein hydrolysate (enzymatic), 40 gm.; sodium succinate, 1·25 gm.; sodium thioglycollate, 1·0 gm.; $Na_2HPO_4.12H_2O$, 5·8 gm.; KH_2PO_4, 0·48 gm.; gelatin, 2·0 gm. Solution 2 (stock vitamin preparation), calcium pantothenate, 1·0 mgm.; pyridoxamine, 2·0 mgm.; riboflavin, 0·1 mgm.; nicotinamide, 8·0 mgm.; uracil, 15·0 mgm.; biotin, 10·0 μgm.; vitamin B_{12}, 63·0 μgm. Solution 3, dextrose (50 per cent), 5·0 ml. After preparation of solution 1, the incomplete medium was adjusted to *p*H 7·6 with 5 *N* sodium hydroxide. Solution 2 was then added and the combined medium was then placed in an autoclave for 15 min. at 15 lb./sq. in. After cooling, Seitz-filtered glucose (solution 3) was added aseptically. 9-l. 'Pyrex' carboys containing 6·5 l. of medium were used. The inoculum was prepared in 500 ml. of broth of the same composition as that being used to produce collagenase. The maximum yield of enzyme was obtained after 48 hr. incubation at 24–25° C. The bacterial cells were removed by filtration through 'Hyflo Super Cel' and the *p*H of the filtered broth adjusted to 7·5–7·6 with 5 *N* sodium hydroxide. Collagenase activity was measured by hydrolysis of undenatured Achilles tendon collagen as described in ref. 3. One collagenase unit was defined as the amount of activity catalysing the solubilization of 1 mgm. of 40–60 mesh collagen under standard conditions. It was found that the foregoing relatively simple procedure provided broths with collagenase activity that averaged 107 units of enzyme a ml. The average yield of purified preparations of collagenase expressed as collagenase *A* was 140 mgm./l. of fermentation beer.

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Crystallization of Deuterated Ribonuclease

We have prepared deuterated bovine pancreatic ribonuclease (Armour, lot 381–059) by the procedures of Hermans and Scheraga[1]. Both $RNaseH_{20}$ (all but 20 exchangeable H's exchanged) and RNase all-deuterated crystallized from 55 per cent (v/v) aqueous 2-methyl-2,4-pentanediol (deuterated solvent) in form II[2]. The intensities of the X-ray reflexions along the principal axes were identical within experimental precision to those of non-deuterated crystals out to $2\theta = 60°$ (1·5 Å. resolution); at higher angles RNase gives poor reflexions. The lattice constants were identical to those of undeuterated crystals within the normal variation (± 0·05 Å. and ± 0·03°). The small differences between deuterium bonds and hydrogen bonds do not change the structure of RNase within the limits observable by X-ray diffraction. Detectable changes might be produced in the case of a crystallizable protein with a transition temperature near room temperature instead of the high transition temperature (61° C.) of RNase[3].

The deuterated crystals were prepared by Miss Sylvia Scapa and Mrs. H. R. Bello; diffraction patterns were taken by Miss E. De Jarnette.

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Properties of Xanthine Dehydrogenase in the Silkworm, *Bombyx mori* L.

The enzyme which catalyses the oxidation of purine derivatives xanthine and hypoxanthine to uric acid has been demonstrated in several insects[1–5]. In the silkworm, *Bombyx mori*, it functions as a dehydrogenase. This inference is based on the fact that the formation of uric acid and oxygen consumption of the fat body homogenates show marked increases only when methylene blue is present as a hydrogen carrier[6,7]. The inference seems to be justifiable from the work of Remy *et al.*[8] using chicken liver. To gain further knowledge of the nature of xanthine dehydrogenase in the silkworm, it will be desirable to use the highly purified enzyme as in milk[9,10] and liver[11] oxidase preparations.

The present report deals with preliminary work on the properties of the xanthine dehydrogenase in the silkworm. The enzyme was prepared from the fat body according to the methods of Kielley with a few modifications (Hayashi, unpublished). The enzyme activity was determined from oxygen consumption and uric acid formation by a method similar to that described in the previous paper using crude tissue homogenates of silkworm larvæ, with methylene blue as a hydrogen carrier between xanthine dehydrogenase and molecular oxygen[7].

Purified preparations of the silkworm enzyme are yellowish-brown in colour. The enzyme has two absorption bands with maxima at 360 and 405–410 mμ, the latter disappearing when reduced by hydrosulphite. The absorption spectrum of the silkworm enzyme is similar to that of the chicken liver enzyme[8] and aldehyde oxidase[12]. The difference spectrum was not conspicuously characteristic of flavin and is probably a compound of flavin plus an unknown group.

In recent work it has been reported that xanthine oxidative enzyme from some animal tissues contains iron, molybdenum and flavin as the prosthetic groups. The mammalian liver xanthine oxidase of Kielley[11] has an Fe : Mo : flavin ratio of 4 : 1 : 1 and the chicken liver xanthine dehydrogenase of Remy *et al.*[8] has a ratio of 8 : 1 : 1. From the purified preparation of xanthine dehydrogenase from silkworm fat body, it was determined that the ratio is 9 : 1 : 1, thereby showing closer resemblance to the latter rather than the former prosthetic group.