first instar; this is not surprising, since the death of Mw, Ml[2] and Mfla homozygotes and ClB males in the first instar,[2] as well as of almost all of the wild type larvae which die during the larval period, indicates that the first instar is a crucial period in development.

Delay in onset of pupation. In the y w/+ stock, it was observed that triplo-X larvae do not begin to form puparia until about 24 hours after the mean pupation time of XY and XXY sibs at 25°C. Some *XXX* larvae did not pupate until 7 days after oviposition, and 2 were still in the larval stage at 15 and 18 days respectively. This is the longest delay in onset of pupation reported for any genotype of *D. melanogaster* and is the first delay known to be attributable to a duplication. Dobzhansky[3] has reported a delay in total time of development of superfemales, but the lengthened development has not hitherto been recognized as due to a delay in puparium formation.

Conclusions. The lethal effect of the triplo-X condition is chiefly exerted upon the pupal stage. A few triplo-X zygotes die in the larval period, especially at the stage just before pupation. The onset of pupation of triplo-X larvae is delayed more than 24 hours at 25°C.

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The Estimation of Histidine.

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The estimation of arginine, histidine and lysine in small amounts of protein by the silver precipitation method[1] has been satisfactorily employed by Miller,[2] Mazur,[3] Plimmer,[4] and others. However, Abderhalden and Siebel[5] were only successful in the determination of

2 Brehme, K. S., *Genetics*, 1938, **23**, 142; *Am. Nat.*, 1937, **71**, 567.
3 Dobzhansky, Th., *Biol. Bull.*, 1930, **59**, 128.
1 Block, R. J., *J. Biol. Chem.*, 1934, **106**, 457.
2 Miller, E. J., *Biochem. J.*, 1935, **29**, 2344.
3 Mazur, A., *J. Biol. Chem.*, 1937, **118**, 631.
4 Plimmer, R. H. A., and Lowndes, J., *Biochem. J.*, 1937, **31**, 1751.
5 Abderhalden, E., and Siebel, H., *Z. physiol. Chem.*, 1935, **238**, 169.

arginine and lysine. The latter investigators reported large losses of histidine during the purification procedures in which $HgSO_4$ and $CuCO_3$ are employed. It has been recognized that histidine is the most difficult of the basic amino acids to determine accurately[6] and we take this occasion to present some details of the modifications of the 1934 procedure.

Histidine is precipitated from the protein hydrolysate at pH 7.4 with $AgNO_3$ and $Ba(OH)_2$.[1] The precipitate is centrifuged, washed and decomposed with H_2S after suspending in dilute H_2SO_4 (*cf.* [7] for apparatus). The Ag_2S is removed and the filtrate and washings are concentrated *in vacuo* (*cf.* [7]). The filtrate is adjusted to pH 3.5-4.5 with $Ba(OH)_2$, and after removal of the $BaSO_4$, the histidine solution is concentrated to approximately 20 cc. and decolorized with charcoal. The precipitate is filtered, thoroughly washed with hot H_2O and the filtrate is diluted to volume (100 cc.). Histidine may be determined directly as follows.

(a) *Colorimetric* (Kapeller-Adler). 2 cc. portions (containing 0.5-1.0 mg. of histidine per cc.) of the sparkling histidine sulfate solution are pipetted into 6 test tubes graduated at 10 cc. Into 6 other similar tubes, an equal quantity of histidine standard is placed. (For best results, standard and unknown should contain approximately the same amounts of histidine.) The 12 tubes are set up alternately and an excess of Br_2 in dilute acetic acid (*cf.* [7]) is added. The bromination is allowed to proceed for 10 minutes, care being observed that all the solutions are colored yellow at the end of this period. The excess Br_2 is removed by a drop of As_2O_3,[8] and 2 cc. of NH_4OH reagent (*cf.* [7]) per tube are added immediately. The contents of the tubes are mixed and they are placed in mildly boiling H_2O for 5 minutes. At the end of this period, the solutions are cooled in ice for 10 minutes and diluted to volume with NH_4OH reagent. The contents of each group of test tubes are mixed and the colors matched in the usual fashion.

(b) *Gravimetric* (nitranilic acid). In 1936, Town[9] claimed that nitranilic acid (2,5-dihydroxy-3,6-dinitroquinone) specifically precipitates glycine from an ethanolic solution of the products of protein hydrolysis. Stein[10] found that the amount of glycine in

[6] Block, R. J., *J. Biol. Chem.*, 1934, **105**, 455; 1937, **119**, 765.

[7] Block, R. J., *The determination of the Amino Acids*, Burgess Publishing Co., Minneapolis, Minn., 1938.

[8] Conrad, R. M., and Berg, C. P., *J. Biol. Chem.*, 1937, **117**, 351.

[9] Town, B. W., *Biochem. J.*, 1936, **30**, 1833.

[10] Stein, W. H., personal communication.

gelatin estimated by Town's method was greater than that determined by Bergmann and Fox[11] and that this discrepancy could be explained by the partial precipitation of the basic amino acids along with glycine. We have found that nitranilic acid can be used successfully for the isolation of histidine as follows:

The remainder of the histidine solution (*cf.* a) is concentrated *in vacuo* (pH 3.5-4.0) and an excess of nitranilic acid (freshly dissolved) in H_2O, $(CH_3)_2CO$, or CH_3OH is added. The finely crystalline, yellow precipitate is filtered off, washed and dried at 110°. The gravimetric and colorimetric estimations check very closely, on analysis of 15 brain proteins, if the factor 0.403 x the weight of the yellow precipitate is employed. Further work along this line is now in progress.

In certain instances, further purification of the histidine fraction is desirable before estimation of the amino acid. In such cases, the histidine sulfate solution is concentrated to approximately 120 cc. and an excess of Denige's reagent (*cf.* [7]) is added. The suspension is centrifuged for 20-30 minutes and the filtrate is discarded. The precipitate is suspended in H_2O and decomposed with H_2S. After removal of the HgS and H_2S, the histidine solution is adjusted to pH 5-6 with $Ba(OH)_2$. $BaSO_4$ is removed and washed. The filtrate is boiled and it is made alkaline to litmus by the addition of minimal quantities of $BaCO_3$. An excess of $CuSO_4$* and a further quantity of $BaCO_3$ to turn red litmus blue are added. The suspension is boiled 2-3 minutes longer, cooled in ice, centrifuged, washed, and after acidification of the filtrate with H_2SO_4, the Cu is removed by H_2S. The solution is concentrated *in vacuo* (pH 3.7-3.9) and the histidine is determined.

Results indicating the probable errors of the method, which are based on more than 10 experiments, each starting with 0.1355 gm. of histidine hydrochloride hydrate are given:

	Loss (%)
Histidine silver precipitate (decolorized)	1-2
" mercury "	4-5
" copper filtrate	2-3
" diflavianate precipitate	15-25
" nitranilate " †	less than 3

[11] Bergmann, M., and Fox, S. W., *J. Biol. Chem.*, 1935, **109**, 317.

* The use of $CuSO_4$ in place of $CuCO_3$ or $Cu(OH)_2$ was suggested by Dr. Lawrence Morris of Louisiana State University.

† Histidine nitranilate, prepared from histidine hydrochloride hydrate, may be recrystallized from water.