Pyruvic Acid in the Juice of Onion (*Allium Cepa*)

SOME years ago, during a search for the presence of reducing substances in plants, I obtained a positive Simon and Piaux[1] (nitroprusside) test for pyruvic acid in onion juice. Owing to the intensity of the reaction it seemed worth while to estimate and identify pyruvic acid.

Several mature onions (*var.* Suttons *A*1) which had been pulled up and stored for one month were minced, and then squeezed through linen. 40 ml. of the juice were immediately made 5 per cent with respect to trichloroacetic acid. The precipitate was centrifuged off and well washed with 5 per cent trichloroacetic acid. The supernatant fluid and washings were then filtered. Pyruvic acid was estimated in the filtrate by weighing the 2 : 4-dinitrophenylhydrazine derivative, using the method of Simon and Neuberg[2]. The pyruvic 2 : 4-dinitrophenylhydrazone weighed 0·1260 gm., corresponding to 0·1034 gm. of pyruvic acid per 100 ml. of onion juice.

The pyruvic 2 : 4 dinitrophenylhydrazone melted at 209° uncorr., gave a deep red colour with 6 per cent alcoholic potash, and a lemon-yellow colour with 85 per cent sulphuric acid. Once recrystallized from ethyl acetate, it melted at 213° uncorr. (found (Weiler): C, 40·00; H, 2·89; N, 20·80 per cent. $C_9H_8O_6N_4$ requires C, 40·30; H, 3·00; N, 20·89 per cent).

Judging from the nitroprusside test, there is no pyruvic acid in the intact onion. It is very rapidly formed from a precursor, or precursors, when the onion tissue is wounded (that is, minced or ground up in a mortar). This was demonstrated by the following experiment, which was repeated on individual onions several times with the same result.

An onion was weighed and cut in half, one half was quickly placed in a mortar under twice its weight by volume of 10 per cent trichloroacetic acid, and the other half under the same volume of water. Both halves were then cut up under the acid and water respectively, ground up with a little sand, and centrifuged. The same volume of acid was then added to the supernatant fluid from the water extract, and both made up to the same volume with water. Both were then saturated with ammonium sulphate and filtered. The trichloroacetic acid extract of half the onion gave a negative test for pyruvic acid, while the water extract gave a very strong positive test.

Cutting and grinding the onions up under 0·5 *M* sodium fluoride does not prevent the formation of pyruvic acid.

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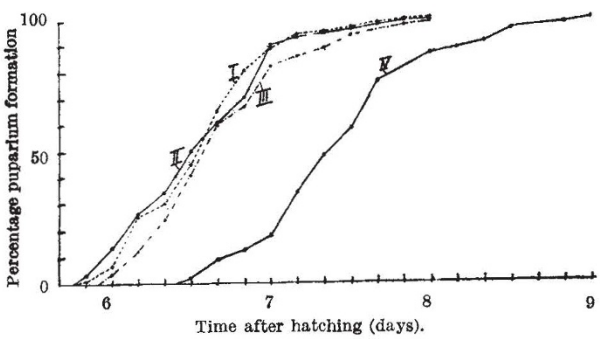
Inhibitory Effects of the Corpora Cardiaca and of the Corpus Allatum in *Drosophila*

THE function of the corpora cardiaca in Diptera is, at present, obscure. According to unpublished data of M. Thomson (cit. by E. Thomsen[1]), an extract of the corpus cardiacum of the blowfly (*Calliphora*), when injected into blinded shrimps (*Leander*) with maximally expanded chromatophores causes a strong contraction of the red and black pigment. Whether the corpus cardiacum acts similarly on the pigment cells in Diptera is unknown. Two other effects of the corpora cardiaca, obtained in transplantation experiments on *Drosophila hydei*, will be reported here.

One of the effects of implanted corpora cardiaca consists of an inhibition of the normal colouring of the puparium, the latter only attaining a brown colour instead of the normal red colour characteristic for *Drosophila hydei*.



PERCENTAGE DISTRIBUTION OF TIME OF PUPARIUM FORMATION.
I = control series. Mean = 6·62 ± 0·04 days (n = 119).
II = fat body series. Mean = 6·62 ± 0·06 days (n = 61).
III = corpora allata series. Mean = 6·75 ± 0·06 days (n = 58).
IV = corpora cardiaca series. Mean = 7·51 ± 0·07 days (n = 56).

The second effect is a delay in puparium formation, as shown in the accompanying figure. Curves I–IV represent the percentage distribution of puparium formation in four different experimental series. Curve I, serving as control, shows the time of puparium formation after injection of Ringer solution, while curve IV was obtained after implantation of 3–5 pairs of corpora cardiaca taken from 4-days old adult females. (For all experiments, larvæ aged 2 days 21 hours were used as hosts. As the corpora cardiaca are fused with the ganglion hypocerebrale, both tissues were transplanted together.) A comparison of the two curves shows that about 40 per cent of the controls had formed puparia before the first larva with implanted corpora cardiaca started pupation, and that 90 per cent of the controls had pupated at a time when only 20 per cent of the corpora cardiaca series had done so. The delay in pupation caused by the implantation of corpora cardiaca is also obvious from the means, the mean for the control series being 6·62 ± 0·04 days, that for the corpora cardiaca series 7·51 ± 0·05 days. The possibility that the delay in puparium formation was a non-specific effect of any adult tissue was excluded by testing the action of two other organs from adult females. Pupation occurred at the normal time when either the fat body (curve II) or the corpus allatum (curve III) were implanted into the hosts.

The actions of the corpora cardiaca on the colouring process of the puparium and on the time of puparium formation are independent from each other. This is shown by the fact that the corpora cardiaca of adult females, when implanted into older larvæ, exert no delaying action on puparium formation, while their inhibition of the colouring process remains unchanged.

The fact that no action whatsoever on the time of pupation was observed after implantation of corpora allata (curve III) is interesting from two more aspects. First, the absence of any accelerating effect on puparium formation agrees with the view expressed by Scharrer and Hadorn[2] and Vogt[3], according to which the pupation hormone originates only from large cells which form the lateral part of the larval ring-gland and which do not belong to the corpus allatum. Secondly, the absence of any delay of puparium formation in *Drosophila* differs from the inhibition of metamorphosis produced by the corpora allata in other insects. In these, either supernumerary larval moults were observed or moults intermediate between a larval and a pupal (or imaginal) moult. In certain instances, however, the inhibitory action was restricted to parts of the skin in the immediate neighbourhood of the implanted corpora allata[4]. In the present experiments on *Drosophila*, defects in the imaginal skin of the abdomen (the injections were made into the larval body cavity at the level of the abdominal segments) were observed in 52 per cent (n = 54) of the hatched flies of the corpora allata series. Similar defects, usually of smaller size, were obtained in 5 per cent (n = 119) of the controls, in 2 per cent (n = 60) of the fat-body series and in 0 per cent (n = 56) of the corpora cardiaca series. The defects were characterized by the absence of pigment, hairs and a typical cuticle, and extended occasionally over several segments. The statistically significant difference between the corpora allata experiment and the other three series establishes an inhibitory effect of implanted corpora allata on the development of the imaginal characters in the abdominal skin at the site of implantation. (In other insects, the inhibited areas show larval characters instead of imaginal ones. Imaginal characters might be expected when dealing with inhibition of the pupal moults in Diptera but not in the present instance of an inhibition of the imaginal moult, since the imaginal skin of *Diptera* consists of special 'imaginal' cells which never form larval structures during development.) If we may consider these actions to be analogous to the restrictive effect of implanted corpora allata occasionally observed in other insects, the inhibitory effect of the corpus allatum in Diptera would not differ in principle from that in other insects. Another indication of an inhibitory action of the corpus allatum in *Drosophila* was previously obtained in a study of the imaginal differentiation of the eye disks[5].

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Lack of Optomotor Reactions in a White-eyed Mutant of *Culex molestus*

THE white-eyed mutant of *Culex molestus*[1], recently detected in the Department of Entomology in the London School of Hygiene and Tropical Medicine, made it possible to repeat experiments performed on normal and white-eyed individuals of *Drosophila melanogaster*, *D. pseudoobscura*[2] and (unpublished) *D. subobscura*; in these experiments it was shown that the pigments normally separating the individual ommatidia in the compound eye are necessary for the perception of moving contours and that white-eyed individuals, regardless of genetical constitution, do not show optomotor reactions.

Wild-type *Culex molestus* shows optomotor reactions similar to those described for *Aedes ægypti*[3], and the methods developed for this species can be applied to test the former's behaviour. However, the simpler cylinder arrangement described for *Drosophila*[2] can be equally used for the mosquito, the only modification being a different glass container, namely, a flat-bottom vial 1½ in. wide and 4 in. long in which the mosquitoes can fly. When the striped cylinder is set in motion, the mosquitoes become agitated, and after a few seconds typical optomotor reactions are observed. The best reactions, namely, a turning of the flying mosquito following the rotation of the cylinder, were observed at a speed of about two rotations per second using a pattern of five black stripes interspersed with five equally wide white stripes (each 36° wide). Postures similar to those described for sitting flies[4] could also be observed in wild-type mosquitoes, when sitting on the bottom, ceiling or the walls of the vial. None of these reactions was ever observed in the white-eyed mutants. The insects did not become agitated nor did they circle or bend, when the striped pattern was set in motion; in fact they did not show any sign of movement perception. It should perhaps be mentioned that they did show phototaxis, as the normal mosquitoes do, but in contrast to the white-eyed *Drosophila*, they mostly flew away from strong light.