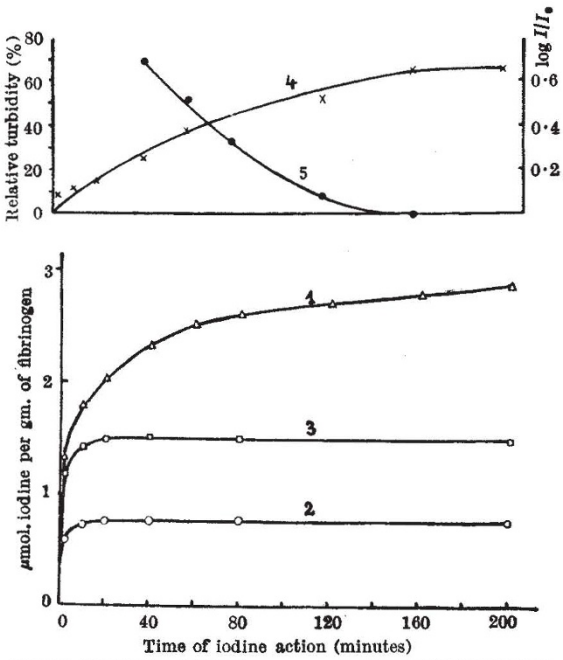
Action of Thrombin on Iodinated Fibrinogen

FIBRINOGEN dissolved in 30 per cent urea solution readily consumes iodine even at 0° C. Analysis has shown that the action of iodine is twofold: part of the consumed iodine is substituted in the tyrosine rings in the fibrinogen molecule, and part of the iodine oxidizes certain of its groups. Thus as a result of the action of iodine, fibrinogens, iodinated and oxidized to various extents, can be prepared. To obtain such iodinated fibrinogens, a known amount of $N/100$ iodine solution was allowed to act on samples of fibrinogen dissolved in slightly alkaline 30 per cent urea solution. After various incubation times the action of the iodine was stopped by removing the remaining free iodine with thiosulphate. The urea was then dialysed off and the fibrinogen precipitated isoelectrically several times and finally dissolved in slightly alkaline water. The iodine



Curve 1, total amount of iodine consumed. Curve 2, iodine bound to fibrinogen. Curve 3, calculated quantity of iodine used up for the substitution of tyrosine (taking two iodine atoms per tyrosine molecule, and two for the oxidation of two hydrogen atoms during substitution). Difference between curves 1 and 2 gives the quantity of iodine used for oxidation. Curve 4, optical density of the yellow fibrinogens. Curve 5, relative turbidity of iodinated fibrinogens due to the action of thrombin

bound to fibrinogen was then estimated. It was found that the iodine bound to the fibrinogen reached its maximum value in the first few minutes and then remained constant. This maximum value corresponds to two iodine atoms per tyrosine residue of fibrinogen. Simultaneously, the Millon reaction for phenolic groups disappeared.

The constant increase of consumption of iodine shows that the iodine not only enters the fibrinogen molecules but also oxidizes certain groups. As a result of this oxidation, yellow fibrinogen preparations were obtained, and the intensity of colour was found to increase proportionally with the iodine consumed.

Fibrinogens recovered from urea solution clot normally, but none of the fibrinogen exposed to the action of iodine forms a clot on the addition of thrombin. Thrombin, however, still has a certain effect on these fibrinogens, since after its addition there is a shift in the isoelectric point of the fibrinogen towards the alkaline side. There is also a decrease in the solubility of the fibrinogen molecules, which causes turbidity in the otherwise clear solutions. The turbidity was found to decrease as the yellow colour increased. The results are summed up in the accompanying graph.

In some earlier experiments[1], it was found that the formation of the fibrin takes place in two stages; and the role of the thrombin is to modify the fibrinogen molecules, which then polymerize to fibrin.

The experiments with iodinated fibrinogens showed that thrombin was still able to modify these iodinated molecules which, however, lost the property of polymerizing to fibrin. The iodinated fibrinogens become insensitive towards thrombin only when the oxidation by iodine, and correspondingly the development of yellow colour, has exceeded a certain value. This suggests that thrombin requires for its action those groups of the fibrinogen molecules which are oxidizable by iodine to a yellow colour, or which are oxidizable with the same ease as those which give rise to yellow colour.

Since tyrosine iodinated in the same way gives a similarly coloured product, it is not unlikely that the tyrosine residues in the fibrinogen molecule are responsible for the yellow colour.

Yellow iodinated fibrinogens on which thrombin has no action still form mixed clots with untreated fibrinogen, provided that the yellow fibrinogen is not added in excess over normal fibrinogen. The excess of the yellow iodinated fibrinogen inhibits the clot formation of fibrinogen.

A detailed account of this work will appear elsewhere.

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[1] Laki, K., and Mommaerts, W. F. H. M., *Nature*, **156**, 664 (1945).

Nucleic Acids in *Drosophila* Eggs, and *Y*-Chromosome Effects

CALLAN'S recent extension[1] of our study of the effect of the *Y*-chromosome on the egg cytoplasm of *Drosophila*[2,3] has given results apparently at variance with those we obtained. A brief comment at the present time may be of some use, in clarifying the issues involved for further work.

The data presented by Callan pose two problems. One is the difference of technique: we used the ultra-violet absorption; he used a test for the carbohydrate component of the nucleic acids. Both methods have their weaknesses; but it is fair to say that the results to date have been in good agreement when tests have been made on the same tissues. As Callan points out—and this brings us to the second point—it is not easy to make a direct comparison of the two methods for the stages of oogenesis used in the ultra-violet study. He therefore used eggs laid over a one-hour period, hoping in this way to obtain large enough numbers of eggs at a uniform stage for analysis by the method he used. Unfortunately, however, in *Drosophila* the first twelve cleavage