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Kambysellis, M.P., P.Hatzopoulos, and E.M. Craddock. New York University (New York) and State University of New York (Purchase) New York USNA. Rapid in vivo incorporation of radioactive amino acids into vitellogenin proteins of Drosophila grimshawi.

In our studies on the timing of vitellogenin protein synthesis in D.grimshawi (Kambysellis, Hatzopoulos & Craddock 1984), we have found that by feeding the flies for a brief time (1-5 minutes) with radioactive amino acids, sufficient label is introduced into the flies to permit in vivo incoporation into proteins for several days. In representative experiments outlined here, groups of five mature 18 day old females were starved for 15 minutes, and then introduced into a feeding chamber which consisted of a plastic shell vial (10 cm long, 3.5 cm in diameter, Connecticut Valley Biological Supply Co., Inc.) into which was inserted a piece of Kimwipe paper (2x1.5cm) four layers thick. This paper was saturated with 100 µl of a 20% sucrose solution containing a mixture of radioactive amino acids, namely 25 µCi each of ^{3}H-aspartic acid (s.a. 10.0 Ci/mmole), ^{3}H-serine (s.a. 16.8 Ci/mmole), ^{3}H-glycine (s.a. 15.0 Ci/mmole), ^{3}H-lysine (s.a. 68.0 Ci/mmole) and ^{3}H-leucine (115.2 Ci/mmole), and 100 µCi ^{35}S-methionine (999.8 Ci/mmole). The paper was then placed in the middle of the vial which was lain on its side as is usual for culturing the large Hawaiian Drosophila. Once introduced into the vial, the flies are attracted immediately to the sucrose-amino acid mixture and feed continuously for about one to one and a half minutes. The same feeding chamber can be used to feed up to ten groups of flies in each experiment. Following feeding (pulse), the flies were placed either on regular Hawaiian Drosophila medium (Wheeler & Clayton 1965) or in empty vials for the chase period. At the end of variable periods of chase, the groups of five flies were anaesthetized, their hemolymph collected (Kambysellis 1984), and the fat bodies and ovaries dissected, homogenized in 50 µl of 50 mM Tris-HCl pH 8.2, 0.25M NaCl buffer, and centrifuged for one minute in an Eppendorf centrifuge. The aqueous phase

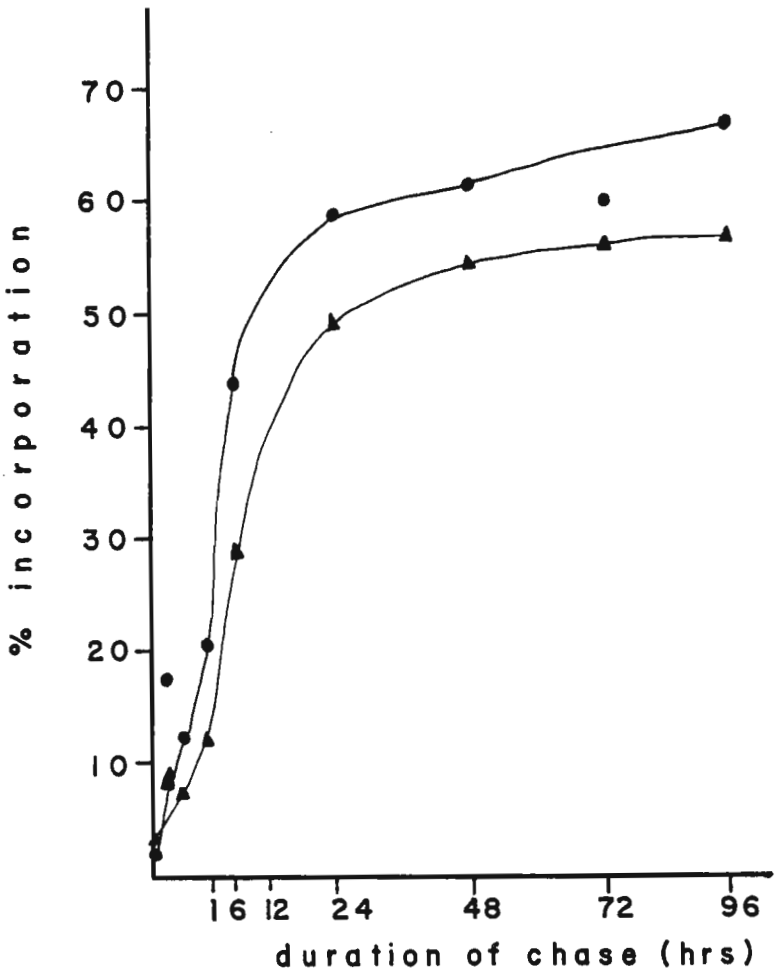

Fig. 1. Incorporation of radioactive amino acids into TCA insoluble proteins. Percentage of incorporation was determined by dividing the number of TCA precipitable counts in a particular sample by the total cpm in that same sample. Notice that the scale on the horizontal axis has been expanded for the first hour of the chase to disclose the four observations during that period.

Table 1. In vivo incorporation of ^{3}H- and ^{35}S-amino acids into proteins in adult female D.grimshawi. TCA precipitable counts in aliquots of tissue homogenates from groups of five 18-day old female flies fed radioactive amino acids for 1 min, followed by variable periods of chase, with the flies (a) maintained on food, or (b) starved for the chase period.

	(a) flies on food			(b) flies starved		
Chase period	hemolymph (1µl≅1 fly)	fat body (one)	ovaries (one pr)	hemolymph (1µl≅1 fly)	fat body (one)	ovaries (one pr)
0'	2,760	826	1,104	333	376	428
15'	1,816	521	2,143	2,047	754	1,639
30'	1,224	1,058	3,289	1,520	890	2,123
60'	1,740	1,117	4,802	3,963	2,575	6.090
6 hr	64,307	26,831	108,023	34,119	20,142	102,489
24 hr	75,602	48,344	248,557	-	27,106	127,111
48 hr	47,323	64,132	245,044	45,164	53,514	334,700
72 hr	-	45,885	251,302	-	-	-
96 hr	-	31,375	227,406	-	-	-

between the lipid and precipitated material was removed for analysis. Aliquots from each sample were assayed for: (a) total cpm; (b) incorporation of radioactive amino acids into TCA insoluble proteins; and (c) the presence of specific proteins on 7-12% T gradient SDS polyacrylamide gels.

As shown in Table 1, sufficient radioactivity was incorporated into proteins in all three tissue samples almost immediately after feeding. The low initial incorporation, which represents about 2% of the total radioactivity found in the tissues at that time (Fig. 1) steadily increased to reach 50% incorporation by 12 hr chase in the ovaries and 24 hr chase in the fat body (fig. 1). Maximum incorporation into TCA insoluble material was reached by 24 hr in hemolymph (Table 1) and 48 hr in the fat body of flies maintained on food for the chase. Incorporation into the ovary showed a different pattern, due to the uptake and storage of yolk proteins. The slow incorporation during the first hour of chase was followed by a sharp increase in the next 6 hr, reaching a maximum 24 hr and maintaining a plateau until 72 hr, after which a decrease was observed. In the flies maintained for the chase period in dry vials, where the precursor radioactive amino acids were not diluted by feeding on non-radioactive medium, incorporation continued to increase in all three tissues until the death of the flies between 48 and 72 hr of chase.

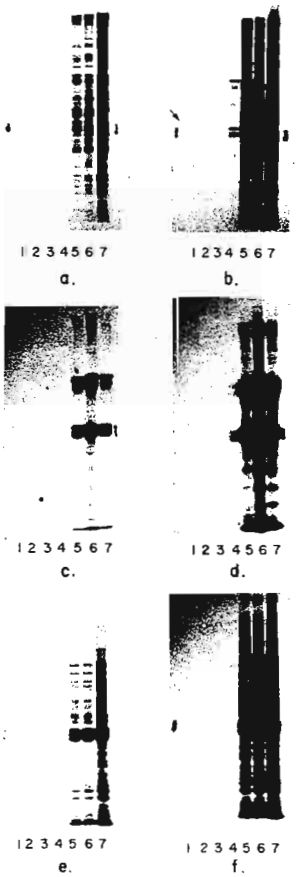

Fig. 2. Fluorographs of SDS-PAGE showing labeled proteins in fat body (a,b), hemolymph (c,d), and ovary homogenates (e,f). Samples were taken at various intervals after feeding the flies for one min with radioactive amino acids. The durations of the chase period are as follows: no chase (lane 1), 15' (lane 2), 30' (lane 3), 60' (lane 4), 6 hr (lane 5), 24 hr (lane 6) and 48 hr (lane 7). (a,c,e) represent 4 days exposure and (b,d,f) 13 days exposure of the X-ray films. The arrow points to the vitellogenin proteins. Black dots indicate the positions of the three yolk proteins present in control egg homogenates.

Fluorographs of the proteins resolved by gradient SDS-PAGE show that in the fat body (Fig. 2a,b), amino acids were incorporated into vitellogenin proteins almost immediately after the pulse, and by one hour of chase (lane 4), the three newly synthesized vitellogenin proteins and another unrelated protein of about 75,000 daltons were the predominant labeled proteins. By 6 hr, scores of other proteins had also been synthesized, some of which subsequently became even more abundant in this tissue than the vitellogenin proteins. Incorporation of labeled amino acids into proteins continued for four days with only a slight decrease in intensity.

In hemolymph (Fig. 2c,d), the first proteins to be detected in trace quantities at the 15 min chase were the three vitellogenins, followed by two larger proteins in the range of 75,000 daltons. These serum proteins together with the vitellogenins rapidly increased in quantity after one hour of chase, and reached a maximum by 24 hr. They remained as the predominant hemolymph proteins throughout the chase.

In the ovaries (Fig. 2e,f), the appearance of the vitellogenin proteins was similar to that in the hemolymph. They were detected following 15 min chase and by 6 hr represented the predominant class of egg proteins. Several other less abundant proteins showing a pattern of electrophoretic mobility similar to that of proteins in the fat body were also found after the 6 hr chase. Whether these are identical proteins in the two tissues is not known.

It is of interest to notice the quantitative differences in the three vitellogenins between tissues. In the fat body, the V_2 protein is synthesized in lower quantitites than the V_1 and V_3 proteins, while in the hemolymph and ovary all three proteins are present in roughly equimolar amounts. This observation supports the suggestion that the vitellogenins synthesized in the D.grimshawi ovary (Kambysellis, Hatzopoulos & Craddock 1983) are secreted into the hemolymph prior to their sequestration by the oocyte. Experiments to document this assumption are now in progress.

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References: Kambysellis, M.P., P.Hatzopoulos & E.M.Craddock 1983, Genetics 104:s39; Kambysellis, M.P. 1984, DIS 60: ; Kambysellis, M.P., P.Hatzopoulos & E.M.Craddock 1984, W.Roux's Archiv. submitted; Wheeler, M.R. & F.E.Clayton 1965, DIS 40:98.

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Studies of Drosophilidae (Diptera) in Yugoslavia. V.Collections from Mljet.

At the end of August and beginning of September 1982, we collected Drosophilidae ssp. from two localities on the Adriatic island of Mljet: Pomena and St.Mary.

Pomena is characterized by an abundant vegetation of aleppo pine (Pinus halepensis), holm oak (Quercus ilex) and other kinds of plants characteristic of those forests. At this place we also studied the dispersal rate of D.subobscura, see Taylor et al. 1984. St.Mary is a small island about 300 m in diameter, in a bay of Large Lake on Mljet; it is an island about 300 m in diameter, in a bay of Large Lake on Mljet; it is an island within an island. Here a Benedictine Monastery, St.Mary, was built in the twelfth century and has since been renovated to become a small hotel. Although this location is much drier than Pomena, several species of pine (Pinus pinea, Pinus halepensis), cypress (Cupressus sempervirens), bay (Laurus nobilis) and olive (Olea europea) are found here, as are many cactic (especially Opuntia ficus indica). The ground is covered with characteristic grasses and rocks. In both localities the flies were caught by sweeping nets over fermenting mixed fruit (watermelon, grape and apple) baits exposed on open plates. Flies were preserved in alcohol and brought to the laboratory for identification. In Pomena they were collected only in the evening; at St.Mary they were collected in the mornings as well.

The results are shown in Table 1. It can be seen that in the relatively "wilder" habitat, Pomena, subobscura flies are six times as numerous as melanogaster/simulans flies. This ratio is reversed at St.Mary, where many more melanogaster/simulans flies were collected, the ratio being 1:2.5.

The ratio between sibling species, melanogaster:simulans, is also different in these two localities: at Pomena it is 1:4.3, while at St.Mary it is 1.2:1.