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PIGMENT IN SILKWORM SYSTEMS AS STUDIED BY ELECTRON SPIN RESONANCE. I

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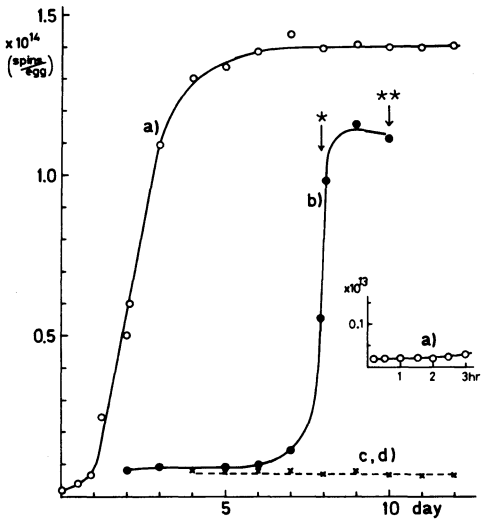
It has been shown that the formation of the pigments, i.e., ommochrome and melanine, as followed by ESR can be used to investigate hibernation, fertilization and the growth in each instar period in the silkworm system.

It has been shown in a previous paper [1] that silkworm hibernating eggs give ESR signals which increase in intensity and become constant, corresponding to about 1.4×10^{14} electron spins per egg (See Fig. 1-a). Since this result suggests high potency of the ESR technique to investigate biological activities in living systems nondestructively, we have continued to investigate the source of the ESR signals, and to examine the time dependence of these signals not only in the eggs but also in hatched worms and pupae. ESR was also measured in eggs of different genetic species, white, red, and normal. [2,3] On the bases of these experiments it has been suggested that the ESR signals are probable due to pigments in the eggs. In the present work, non-hibernating and non-fertilized eggs have been examined by means of ESR to make this point clearer. It will also be shown that ESR is useful to determine the time the real onset of fertilization and to follow the ecdysis of the hatched worms. Biological significance of these findings will also be discussed.

ESR measurement on eggs was carried out in the experimental conditions as described in the first paper [1], and those on the hatched worms by placing the freeze-dried worms in the sample tubes. The sample worms were taken at each measurement out of the same hatch. All measurements were carried out at 25°C.

The results of ESR measurements on eggs are shown in the Fig. 1, which refer to the effects of hibernation and fertilization. Experiment has been performed twice with respect to the concerns with hibernation. The results of both experiments agree well with each other.

Fig. 1 Spin Content of Silkworm Egg.



(a) hibernating } fertilized
(b) non-hibernating }
(c) hibernating } non-fertilized
(d) non-hibernating }

* body pigmentation
** hatch

In Fig. 1, the signal intensity of the hibernating eggs, J106 x Daizo F_2, increases rapidly for the first 4 days after oviposition until it reaches a plateau of about 1.4×10^{14} spins/egg, whereas that of the non-hibernating ones of the same strain does not increase immediately after the oviposition but keeps a low value of 0.9×10^{13} spins/egg, until the egg reaches the stage of head pigmentation of the embryo. The latter finding strongly suggests that the ESR signal is directly related with the formation of pigment. It must be noted that the time at which the maximum intensity appears in b of Fig. 1 corresponds to the stage of body pigmentation. According to the literature [4], the pigment of the hibernating egg is ommochrome, and that of the non-hibernating eggs at the stages of head and body pigmentation is malanine. Then, the two kinds of ESR signals can be assigned as follows: the signals of the hibernating and non-hibernating eggs are ommochrome and melanine, respectively. It should be noted that the present result of ESR reveals the fact as the problem to be solved, why the formation of ommochrome is gradual and that of melanine abruptive (See, Fig. 1). This will be studied in the future.

As seen in Fig. 1-c and 1-d, ESR active material is not formed in the non-fertilized samples beside it presents the signal of spins of about $0.7 \sim 1.0 \times 10^{13}$ spins/egg which is originally contained in the sample. The variation of the latter value refers to the range of the variation of the experimental values. It is seen in Fig. 1-a that some induction period of about 120 minutes exists before the ESR active material is formed in the fertilized hibernating egg. We assume that this induction period refers to the necessary time for the sperm to penetrate and to

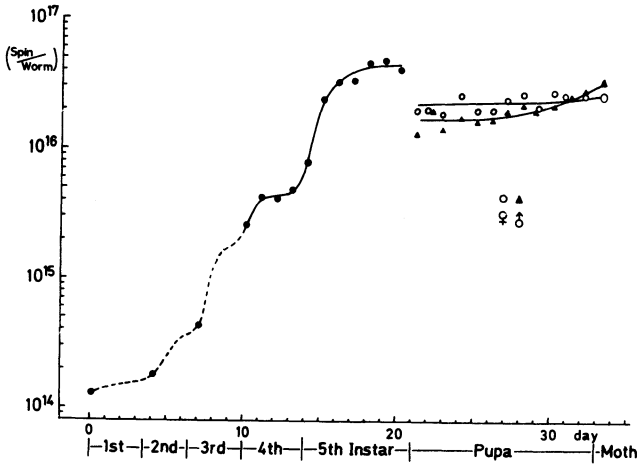
meet the nucleus of the egg. Namely, if the fertilization is defined as the meet of the sperm with the egg-nucleus, it does not occur at the time of connection, whereas it does by the meet of the sperm with the egg-nucleus. In Fig. 1-a, the time t = 0 refers to that of the connection which is actually the same with that of oviposition. Microscopic observation of eggs agrees with the present finding that the real fertilization, meet of the sperm with the egg-nucleus, occurs in about 2 hours after oviposition.

Besides the facts observed in the time dependence of the ESR signal intensity in the eggs, more direct evidence with respect to the pigmental origin of the ESR signal of hibernating egg is obtained by the quantitative determination of the spin content in serosa following. Namely, 20 dormant normal hibernating eggs of HIKO have been smashed and treated by ethyl alcohol, then serosa and embryo picked out each separately. Serosa freeze-dried, which weights 0.095 mg for 20 eggs, presents 10×10^{13} spins/egg and embryo no spin. As the original egg contains about the same amount of spins, this result refers to the fact that the spins are dominantly located in serosa and not in embryo. According to the finding of entomology, ommochrome is present in serosa. It is also easily assumed that pigmental material works as the source of ESR signal. Accordingly we assign the ommochrome in serosa to the source of ESR signal. It has also been known that this pigment is produced after fertilization, then it gathers into serosa with the formation of the latter, and finally, as the hatch time comes close, it is swallowed into embryo following the disintegration of serosa. These facts suggest that the ESR signal simply refers to the formation of pigment and does not directly related with cell devision. The latter remark has been referred to already by us [3], and Egami and Higashinakagawa have also confirmed it more recently [5].

Time dependence of the ESR signals due to melanine in the hatched worms has been measured as shown in Fig. 2. As stated above, worms were placed in the sample tube of ESR, and the intensities of the ESR signals were measured in the usual way. The characteristics of the time dependence in the 4th and 5th instars resemble to each other, as well as to that of egg that the intensity rises rapidly in the early stage of each instar period, then gradually levels off to a plateau, at the end of which the worm casts the skin and enters the next instar period. The dotted lines drawn for the earlier instar periods have been drawn simply with the expectation that the same trend in the 4th and the 5th instars holds in those

instars. It must be noticed that a similar pattern of the time dependence is observed in the eggs where the pigment is formed without any feeding from outside. Hence there must be some motives in the eggs after fertilization to produce the pigment, and also some mechanism which causes the cease of the formation of pigment. More detailed analysis of pigment is being carried out. The results will be reported in the near future.

Fig. 2 Total Spin Content in Silkworm vs Time.



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