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NEW DATA ABOUT DL-DICARNITINE AS A GROWTH FACTOR FOR TENEBRIO MOLITOR LARVAE

BY

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Mealworm larvae *(Tenebrio molitor* L.) were known to require a hydrosoluble factor (1) which has been identified as carnitine (2). Using two different strains of *Tenebrio molitor* (3), reared on a diet similar to that described by *Fraenkel* (4), I was able to confirm the basic facts regarding carnitine deficiency and to prove that carnitine can be replaced by synthetic DL-dicarnitine (5, 6).

In an earlier paper (7), it was stated that a few larvae (at least one in ten in some experiments) had been reared successfully and produced a pupa on a diet where carnitine could only be available as traces in rice starch. *Fraenkel* (8) failed however to find any carnitine activity in starch. Although samples of starch could easily differ each from the other in that respect, it was interesting to find out whether some genetic types of mealworms did not differ from the others in having no (or very little) need for dietary carnitine.

I. Experiments with higher biotine doses (5 μg per g dried food)

Methods

The synthetic media were prepared and used exactly in the same conditions as those previously described (6). Only larvae of the strain F were tested. But instead of using a number of larvae obtained on the same day from eggs laid by a single colony, the offsprings of several groups of females were selected, i.e.:

Overdruk uit „Voeding", Jaargang 16, No. 8 (1955), 785—790

a) 20 larvae hatched on September 5th, 1953 (control, no carnitine)
b) 20 „ „ „ October 23rd, 1953 (receiving 3.2 µg dicarnitine)
c) 21 „ „ „ December 20th, 1953 „ „ „
d) 42 „ „ „ „ 20th, 1953 (control)
e) 9 „ „ „ „ 23rd, 1953 „
f) 15 „ „ „ January 11th, 1954 „

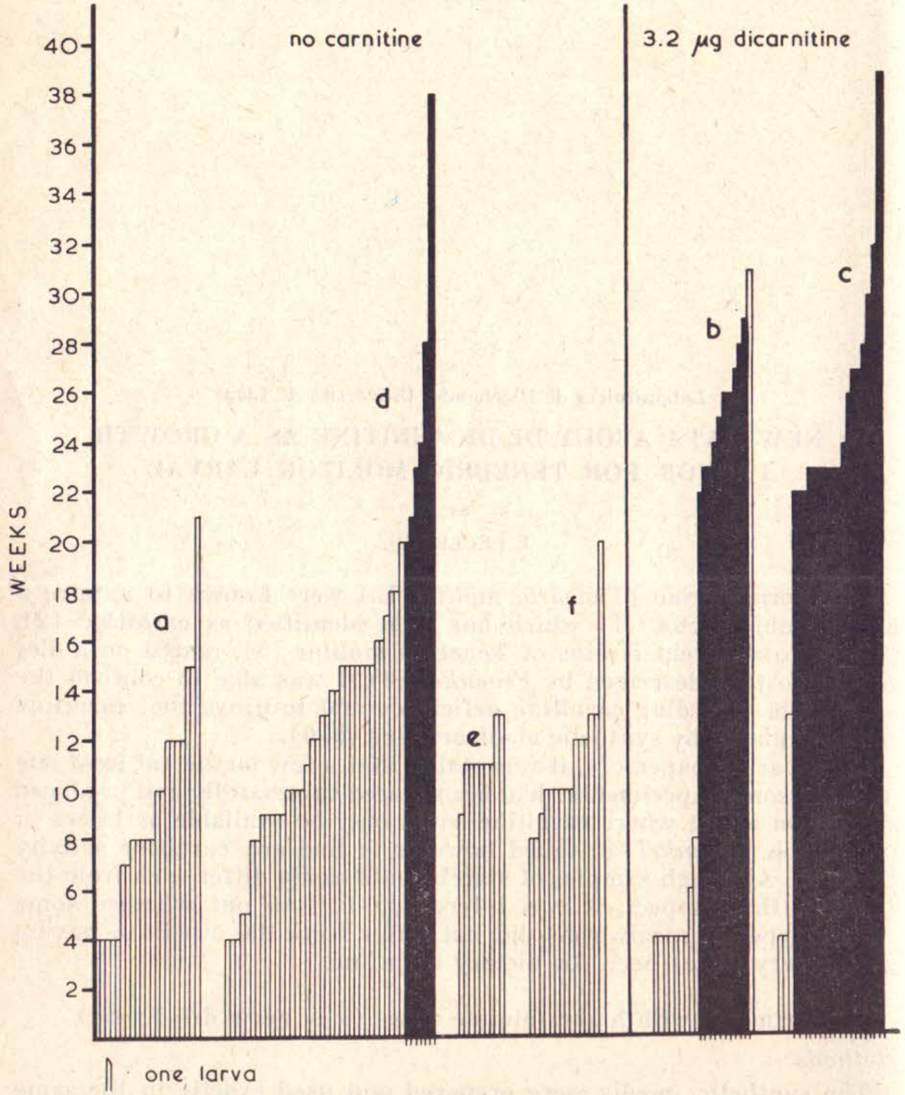

Fig. 1. Larvae of *Tenebrio molitor* (*strain F*) grown individually, ab ovo, on a synthetic diet without or with 3.2 µg DL.-dicarnitine.
Each rectangle represents one single larva, the rectangle is blackened when the larva succeeded in producing a pupa, otherwise death occurred during the week corresponding on the scale.

Summing up, 86 larvae were tried in control diets without carnitine and 41 were offered a diet containing 3.2 µg of DL-dicarnitine *) per g of dried synthetic diet. Smaller doses of DL-dicarnitine were also tested, their data will be discussed in a further paper.

Results

The results obtained are presented in the graphs of the fig. 1. These graphs have been drawn with the same method and the same scales as those illustrating a previous paper (6).

Conclusion

It is clear that the susceptibility of *Tenebrio molitor* larvae to carnitine deficiency varies to a great extent from one stock to the other. There is also a considerable individual variability. There are even larvae which can grow successfully without any dietary carnitine at all.

Should we consider the possibility that certain chemicals (such as "vitamin-free casein"?) used in the diets still carry traces of carnitine, or another possibility that some microbiological processes are involved when a few larvae develop without carnitine, it would still be certain that carnitine is a growth factor generally required by *Tenebrio molitor* larvae. When DL-dicarnitine is added to the diet of a weakly susceptible stock (cf. graphs c and d on fig. 1, obtained with larvae of the strictly same origin), survival and ability of larvae to produce a pupa are always considerably increased.

II. Experiments with lower doses of biotine, with or without choline

In the diets used so far, the amount of biotine was much higher than the dose used by *Fraenkel* (4): 5 µg instead of 0.25 µg per g of dried synthetic food. The question has been raised that certain differences between my results (6) and those of *Fraenkel* (4) were due to a certain toxicity of biotine. Furthermore, the amount of nicotinic acid in the media was only 25 µg/g. According to *Fraenkel, Blewett* and *Coles* (1), the necessity of choline chloride in the diet of *Tenebrio molitor* was still questionable and the fact is, that this vitamin was omitted in the diets first used (7) which permitted a limited number of complete developments. It was thus necessary to carry on experiments with a lower dose of biotine (0.25 µg/g), with a higher dose of nicotinic acid (50 µg/g) and with or without choline chloride. Results obtained in such conditions would be more strictly comparable to those reported by *Fraenkel* (4) although differences could still be expected owing to the fact that *Fraenkel* rears his *Tenebrio* at 30° C (instead of 27°) **), that *p*-aminobenzoic acid and inositol are omitted in my diets, and possibly too because our mealworms' strains would be genetically different.

*) "Bicarnésine Labaz" kindly supplied by the "Laboratoires Labaz S.A.", Brussels.
**) Experiments now in progress indicate that such a difference in temperature conditions does not affect the general trend of the results.

The larvae tried in the following conditions all belonged to the strain F and hatched between December 23rd, 1953 and January 11th, 1954. They were thus of the same stocks as those which served the controlled experiments labelled e) and f) above.

Results

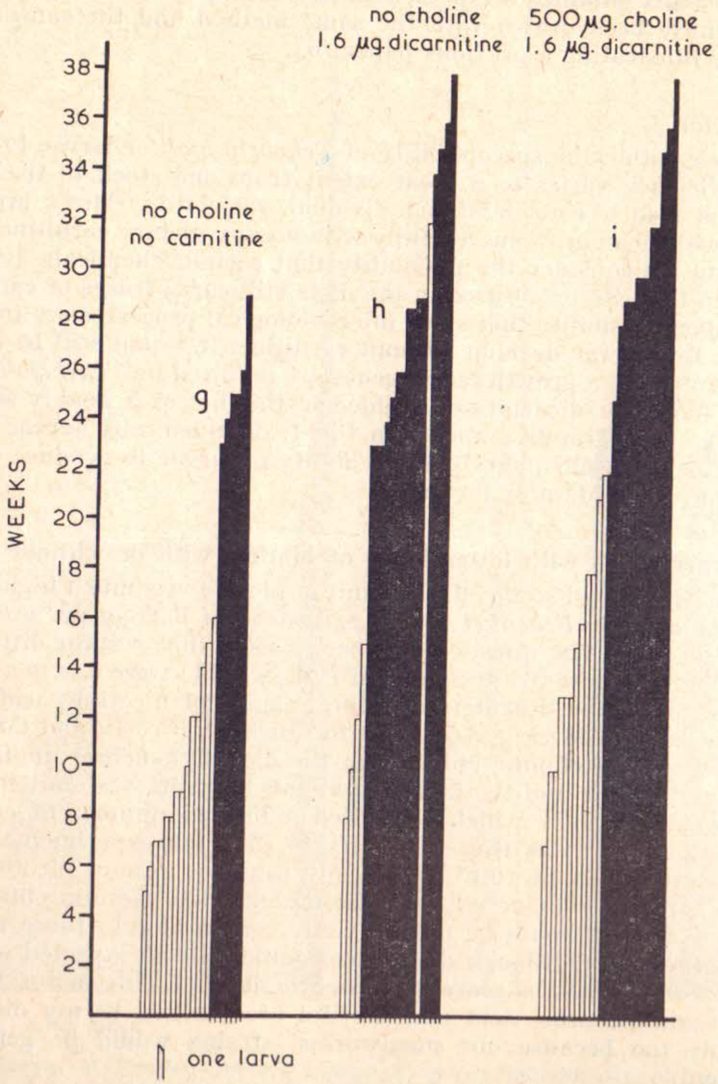

Fig. 2. Larvae of *Tenebrio molitor (strain F)* grown individually, ab ovo, on synthetic diets containing 0.25 µg/g nicotinic acid, with or without choline chloride and DL-dicarnitine.

Conclusion

Comparison of graph g (fig. 2) with graphs e and f (fig. 1) suggests that a high percentage of larvae (30 %) can survive and produce a pupa when biotine and nicotinic acid are brought to their optimal doses, choline chloride and carnitine being omitted. This result was quite unexpected for it shows that, at least in some genetic lines of the strain F:

1.— choline chloride is not at all necessary and may eventually be toxic;

2.— carnitine becomes unessential in the diet of some larvae which were likely (cf. e and f, fig. 1) to require it to survive and reach the pupal stage.

Comparison of graph h (fig. 2) with graph g (fig. 2) shows however that addition of DL-dicarnitine (at levels of 1.6 µg/g) is clearly beneficial. This confirms entirely the conclusions drawn previously (6) and above. As a matter of fact, survival is again compromised when choline chloride is added to the diet (cf. graph i, fig. 2).

It should be pointed out that the majority of the larvae which were able to develop completely on diets devoid of dicarnitine reached the pupal stage within a fairly normal number of weeks. Most of the late pupae obtained so far in all the experiments, occurred in diets containing dicarnitine. It is certain that there are fast-growing and slow-growing individuals in all the populations of *Tenebrio molitor* (even from the same stock, in the same strain and even in optimal natural food: unpublished data). It would seem that fast-growing individuals are the only ones which can, in some cases, grow without carnitine, while the slow-growing individuals succeed in surviving and developing into a pupa only when the growth promoting factor is available.

Summary

1.— A total of 192 larvae of *Tenebrio molitor* belonging to the same genetic strain (F) were put ab ovo (27° C, ± 75 % R.H.) on various diets, in order to check their susceptibility to carnitine deficiency.

2.— Susceptibility to carnitine deficiency varied to a great extent according to the genetic stock. There are colonies which produce offsprings including a few individuals able to grow successfully and to reach normally the pupal stage, without any dietary carnitine. These aberrant individuals seem to belong generally if not always to the group of "fast-growing individuals", very slow developments occurring only when dicarnitine is present in the food.

3.— Nevertheless, DL-dicarnitine acted as a growth promoting factor in all the cases where it was added. Levels of 1.6 and 3.2 µg (per g of dried synthetic food) permitted survival and normal development of a great majority of the larvae tested.

4.— At least with the genetic stock under consideration, choline chloride appears to be not only unnecessary but somewhat toxic.

References

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2. **Carter, H. E., P. K. Bhattacharyya, K. R. Weidman and G. Fraenkel:** Arch. Biochem. Biophys. 38 (1952), 405.
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5. **Dechamps, G., N. P. Buu-Hoi, H. Le Bihan et F. Binon:** C. R. Acad. Sci. 238 (1954), 826. (See also preceding paper by **F. Binon.**)
6. **Leclercq, J.:** Arch. int. Physiol. 62 (1954), 101.
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8. **Fraenkel, G.:** Arch. Biochem. Biophys. 34 (1951), 457.

Discussions

Q. : Dr *E. C. Owen* (Great Britain): Would Dr *Leclercq* please say whether the insects' symbionts complicated his experiments? Did he raise any insects from sterilized eggs?

A. : Dr *J. Leclercq* (Belgium): No attempt has been made so far to rear *Tenebrio molitor* in sterile synthetic foods, nor to try larvae hatched from sterilized eggs.

It is believed that, methodologically, it was best to work out:

a) nutritional requirements of the natural complex "*Tenebrio molitor + its symbionts*",

b) the variability of the responses of that complex,

c) the requirements of single genetic strains of the insect species alone, free from symbionts.

The latter point is thus considered as a further, though necessary, step which should be taken in account when all the questions raised in the previous paper are solved.