Synthesis and Breakdown of Proteins and Ribonucleic Acid in *Tribolium confusum*, Duval

Since very few studies concerning the synthesis of nucleic acids and proteins in insects have been so far undertaken[1] it is desirable to investigate the ability of *Tribolium confusum*[2], Duval to synthesize RNA and protein at the various stages of its growth and development. The previous work of DEVI et al.[3] indicated a close relationship between RNA contents and growth of the insect at different stages of its life cycle. The present investigation shows how far RNA and protein synthesis are related to growth of this insect. The results of our investigation on the incorporation of C^{14} leucine into proteins and C^{14} uridine into RNA of *Tribolium confusum*, Duval at the various stages of insect's life are presented in this communication.

For this study a definite number of larvae 18–20 h old was allowed to grow in a synthetic diet containing 74.5% dextrose, 20.0% casein, 4.0% salt mixture, 1.0% cholesterol, 0.5% yeast powder plus sufficient quantity of vitamin B complex mixture and either C^{14} leucine or C^{14} uridine. The insects used in this work were taken from a pure stock continually reared for the last ten years on whole wheat flour supplemented with 5.0% dried brewer's yeast and maintained at $28 \pm 1°C$ and at a constant humidity of $70 \pm 5\%$. On every third day a certain number of the insects (having the same total weight) was taken out, washed three times with 0.85% cold saline, homogenized in 2 ml of the same medium at 0°C and then deproteinized by adding an equal volume of 10% cold TCA. The precipitate was washed, plated and counted in a window-less gas flow counter (Nuclear Chicago) according to the procedures described by DEVI et al.[4] and DEVI and SARKAR[5], except that where C^{14} uridine incorporation into cellular RNA was studied the acid-insoluble material was not heated in 5% TCA at 90°C for 15 min.

Figure 1 (a) indicates that the rate of incorporation of C^{14} into proteins increases slowly but regularly in the initial stages of growth of the insect but during the latter part of the larval period (6 to 12 days) the rate of incorporation of C^{14} increases sharply; during 13 to 16 days of its life the specific activity of the labeled protein does not significantly change, whereas at the pupal stage (17–20 days), the specific activity is dropped considerably but increases very slowly thereafter. The experiments were discontinued at the beginning of the reproductive cycle of the female insects.

A fairly identical picture has also been noted as shown in Figure 1 (b) in the case of incorporation of C^{14} uridine into cellular RNA. The rate of incorporation steadily increases over the entire period of the larval period although maximum incorporation occurs between 4–10 days; a few days earlier than the maximum incorporation of C^{14} leucine into protein is observed. During the pupa stage a steady drop in C^{14} uridine uptake occurs, and the radioactivity slowly increases thereafter.

Since the specific activity of the labeled protein or RNA does not significantly change between 12 to 17 days in the former case and 10 to 15 days in the latter case it may be concluded that the rate of incorporation of C^{14} into proteins or RNA and its release from the labeled protein or RNA balance each other during this time. The drops in the amino acid incorporation during 17–20 days and in the uridine incorporation during 15–18 days of insect's life might possibly be due to the absence of any active transport of C^{14} from outside (diet) during this period. It should be remembered that the insects do not take any food during this period. It may also be possible that the rate of protein or RNA catabolism during this period exceeds the rates of their synthesis.

Since the increased rate of amino acid incorporation into proteins (between 6–12 days) corresponds with the active growth period of the insect, the protein synthesis may be considered as an index of growth of the insect. Figure 2 represents such a growth curve of *Tribolium confusum*, Duval. Our present results suggest that RNA synthesis precedes the protein synthesis as should be because RNA is known to be directly involved in protein synthesis[6]. Our results also indicate that in larva the protein synthesis exceeds the protein catabolism while in pupa the reverse is true.

The gradual loss of radioactivity might be due, as has been suggested earlier, to the breakdown of labeled protein by intracellular proteases or labeled RNA by nucleases. That such a process of protein and RNA catabolism

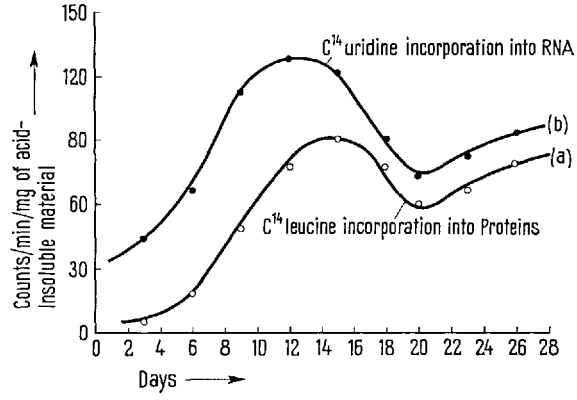

Fig. 1

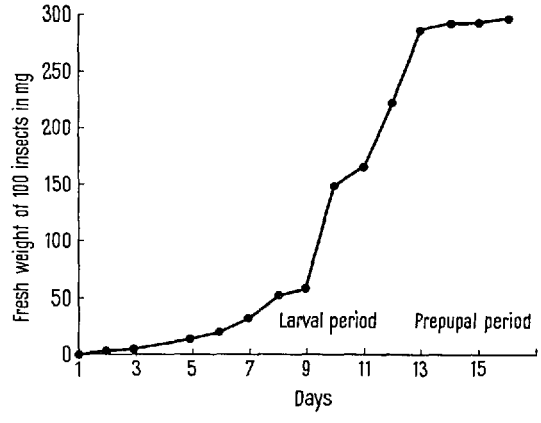

Fig. 2

[1] B. BHEEMESWAR, Proc. of the IX. Int. Congr. of Biochemistry, on Insect Biochemistry, vol. 11 (Pergamon Press, London 1959), p. 78.

[2] The life cycle of *Tribolium confusum*, Duval is divided into five well defined phases such as: (a) Embryonic stage (6 to 0 days), (b) Larval stage (0 to 13 days), (c) Prepupal stage (14 to 17 days), (d) Pupal stage (18 to 21 days), and (e) Adult stage.

[3] ANIMA DEVI, A. LEMONDE, UMA SRIVASTAVA, and N. K. SARKAR, Exp. Cell. Res. *29*, in press (1963).

[4] ANIMA DEVI, S. LERMAN, and N. K. SARKAR, Nature *190*, 1193 (1961).

[5] ANIMA DEVI and N. K. SARKAR, Nature *191*, 1094 (1961).

[6] J. BRACHET, in *Biological Role of RNA* (Elsevier Publishing Company, Amsterdam 1960).

is continuously going on in the insect cell is illustrated in the Table. In order to show this, insects previously labeled with C^{14} leucine or C^{14} uridine are transferred and allowed to grow in a nonradioactive diet. At regular intervals a definite number of them are withdrawn, washed, homogenized and deproteinized by adding 5% cold TCA. The precipitate was washed, plated and counted as described before. The results of the Table show a regular decline in the radioactivity in the acid insoluble precipitate until very little activity is retained by the insect. These results can only be interpreted on the basis of continuous breakdown of proteins by intracellular proteases and RNA by RNase in the cells of the insect.

The progressive loss of radioactivity in *Tribolium confusum* due to continuous degradation of proteins and RNA by intracellular proteases and RNase's respectively during the life cycle of the insect

	Counts per min per mg of TCA - insoluble material					
	8th day	11th day	14th day	17th day	20th day	24–26th day
Protein labeled with C^{14}-leucine	40–45	18–20	6–8	3–4	very little	nil
RNA, labeled with C^{14}-uridine	90–95	50–60	35–40	14–16	4–6	practically nil

The insects were fed radioactive diets containing either C^{14}-leucine or C^{14}-uridine for 7 days; then on 8th day the radioactivity in the acid insoluble material was determined; a definite number of the insects was removed to a jar containing non-radioactive diet. At regular interval the radioactivity retained by the insect was determined. For experimental details see the text.

In summary, from this study one can conclude that the insect *Tribolium confusum*, Duval, shows great variations in the activities of those enzymes involved in the synthesis and breakdown of proteins and nucleic acids during its entire life cycle. The results, obtained in the course of this investigation, further indicate that RNA synthesis precedes protein synthesis and the protein synthesis can be considered as an index of growth of the insect[7].

Résumé. Dans notre étude, l'incorporation de la leucine-C^{14} dans les protéines et de l'uridine-C^{14} dans le RNA chez le *Tribolium confusum* s'est révélée maximale durant la phase la plus active de croissance, et minimale durant la phase de pupe, pendant laquelle l'animal ne mange pas. La synthèse du RNA précède celle des protéines, tel que prévu. Nos résultats montrent aussi une continuelle dégradation des protéines par des protéases intra-cellulaires et du RNA par des RNases.

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Department of Biochemistry, Faculty of Medicine, Laval University, Quebec (Canada), October 22, 1962.

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Occurrence of 'Partially Acid-Fast' Cells in Cultures of Genus *Staphylococcus* and Genus *Micrococcus*[1]

The occurrence of 'partially acid-fast' (PAF) cells in cultures of *Escherichia coli* was noted previously[2]. The cells were demonstrated by a simple staining method[3], which resembles the MACHIAVELLO[4–7], CASTAÑEDA[8], and KÖSTER[9,10] stains.

These experiments were extended to Gram positive cocci, namely, genus *Staphylococcus* and genus *Micrococcus* growing on nutritive agar (Difco), free from any agents such as antibiotics.

Smears were prepared from cultures incubated at 37°C for 17 and 41 h, and cultures incubated at room temperature for four weeks. The 'partially acid-fast' stain revealed PAF positive (red stained) and PAF negative (blue stained) cocci in all cultures of both genera (Figure). The positive forms were always outnumbered by the negative forms.

It has been stated that the carbol fuchsin penetrates the dead cells of *Staphylococci* more easily than living cells[11]; however, division of PAF positively stained cells was clearly observed in these experiments (see illustration). This division sometimes leads to development of small microcolonies consisting of PAF positive cocci.

A total of 66 strains of *Staphylococci* and 62 strains of *Micrococci* were studied. In the *Staphylococci*, 87% were PAF positive and in the Micrococci, 38%. Thus, the *Staphylococci* are more apt to produce PAF positive forms.

Most of the cultures contained Gram negative cocci as well as Gram positive forms, and it was shown that the PAF character coincides with the Gram positive cocci. This observation was made by comparison of color photomicrographs taken from the same area of smears stained first by the PAF method and later by the Gram technique. Thus, the Gram positive cocci can now be subdivided on the basis of staining into PAF positive and PAF negative forms.

[1] These studies were done with the aid of the Medical Research Council of Canada. Grant MA-729, 1962.

[2] G. NOGRADY, XIIIth Meeting of the Canadian Public Health Association, Seigniory Club, Montebello (Québec, Canada), December 6th (1962).

[3] *PAF staining method:* Stain with alcalinized 0.2% basic fuchsin solution (w/v) for 5 min at room temperature. Rinse with distilled water. Dip in 5% acetic acid for 1 sec and rinse again. Counterstain with 5% aqueous methylene blue for 1 min (saturated solution in 95% ethyl alcohol). Blot and dry without rinsing. Basic fuchsin should be prepared daily. Alcalinize 50 ml basic fuchsin solution (23°C) with 3 drops of n/1 Sodium hydroxyde (which must be stored in polyethylene bottle to avoid silicate contamination).

[4] A. MACHIAVELLO, Zentralbl. Bakt. Abt. I. Orig. *139*, 291 (1941).

[5] A. W. STABLEFORTH and I. A. GALLOWAY, *Infectious Diseases of Animals* (Butterworth, 1959), vol. 1, p. 63.

[6] H. ZINSSER, F. FITZPATRICK, and H. WEI, J. exp. Med. *69*, 179 (1939).

[7] P. F. ZDODOVSKII and H. M. GOLINEVICH, *The Rickettsial Diseases* (Pergamon Press 1960), p. 170.

[8] M. R. CASTAÑEDA and S. J. ZIA, J. exp. Med. *58*, 55 (1933).

[9] K. HANSEN and H. KÖSTER, Dtsch. tierärztl. Wschr. *44*, 739 (1936).

[10] H. KÖSTER in K. HANSEN and H. KÖSTER, Dtsch. tierärztl. Wschr. *44*, 739 (1936).

[11] S. D. ELEK, *Staphylococcus pyogenes and its Relation to Diseases* (Livingstone 1959), p. 50.