ences in the same direction, and the parameters were found to represent the left-hand coordinate system. The absolute configuration of Achromycin thus obtained (Ib) was consistent with that determined by DOBRYNIN et al.[6]. A projection of the structure down the *c* axis is given in Figure 1.

At this stage we re-examined the absolute configuration of pillaronone monobromoacetate by using the same instrument and the same computer program. 39 Bijvoet pairs out of 40 supported the previous result. The opposite configuration at 4, 4a and 12a of pillaronone to tetracyclines was thus confirmed. Though it might require further investigations to apply the absolute configuration obtained with pillaronone directly to pillaromycin A, it seems to be improbable that the configurations of the 3 asymmetric carbon atoms were simultaneously reversed in the chemical processes from the latter to the former. In this way the production by bacteria of the same genus of structurally related antibiotics with opposite chirality at A ring was demonstrated.

The close similarity among hydrochlorides of Achromycin, Aureomycin and Terramycin was recognized in their conformations. The 4 statements made by DONOHUE et al.[3] for Aureomycin hydrochloride, verified by CID-DRESDNER[5] for Terramycin hydrochloride, have proved to be true also for Achromycin hydrochloride. For instance, an abnormal amide group, with the C-N bond length shorter than the C-O bond was also found in the present analysis (Figure 2). Bond distances in Figure 2 suggest that double conjugation is maintained with 2 carbonyl groups and the amide group.

Similarities were also found in their hydrogen bonding. The molecule is held together through a three-dimensional net of hydrogen bonds to the chlorine ions, each chlorine being attached to 4 different molecules. It was clarified that the hydroxyl group (proton donor) in Terramycin and the chlorine atom (proton accceptor) in Aureomycin have no significant influence on intermolecular interactions and, therefore, on crystal structures of their hydrochlorides.

*Zusammenfassung.* Durch Röntgen-Strukturanalyse wurde die absolute Konfiguration des Antibiotikums Achromycin ermittelt. Sie ist zu derjenigen des verwandten, aus Kulturen von *Streptomyces flavovirens* stammenden Pillaromycins A entgegengesetzt.

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*22 October 1970.*

## Incorporation of $C^{14}$-Leucine in vivo by the Meal Moth *Plodia interpunctella* During Development

Amino acid incorporation is used as a criterion of protein synthesis. No one would disagree with the statement that protein synthesis is a measure of nucleic acid metabolism. Thus, during the metamorphosis of several insect species, the ratio of RNA/DNA increases just prior to adult emergence or during the early phases of adult development[1,2]. This event is correlated with similar increases in amino acid activation[3] and transamination[4] both of which are related to protein synthesis. Several workers have used labelled amino acids in studies of protein synthesis in various insects. Most studied the biosynthetic capacity of different tissues at different developmental stages. SHIGEMATSU[5], working with the fat body of the last larval instar of *Bombyx mori*, showed that marked biosynthetic activity coincided with an increased haemolymph protein content. PRICE[6,7] showed that fat body from 4-day-old *Calliphora erythrocephala* larvae had the highest rate of incorporation, while a marked decline followed at later stages and by the sixth day very little incorporation was found. CHIPPENDALE and KILBY[8] examined the relationship between the proteins of the haemolymph and the fat body during metamorphosis of the large white butterfly, *Pieris brassicae*. They also noted[9] that the fat body and midgut, but not the haemolymph, in mid-fifth instar larvae of *Pieris* are active sites of protein synthesis. The purpose of this study is to investigate the rate of protein synthesis at different developmental stages of the insect *Plodia interpunctella*.

*Material and methods.* The insects were cultured in our laboratory in cylindrical glass tubes $10 \times 20$ cm. As rearing medium, a mixture of chicken mash, glycerine and honey in a 6:1:1 proportion was used. The room temperature was between 28–30 °C and the relative humidity 40–50%. Under the above conditions the total life cycle of the insect, from the day of hatching untill the day of adult emergence was at least 28 days. The ages used here were: Larvae 15-day-old ($L_{15}$); 18-day-old ($L_{18}$); Spinning Larvae [(SL) last instar, which developed a green hue]; Pre pupae [(PP) immobile spinning larvae]; Pupae 4-day-old ($P_4$); 6-day-old ($P_6$); and 8-day-old ($P_8$); and newly emerged adults males and females. $8 \times 10^{-3}$ μCi of radioactive solution were injected into each individual with an 'Agla' micrometer syringe. The animals were homogenized with a *Tris* buffer solution of pH 7.5 at 2 and 4 h after injection. The homogenate was boiled for 10 min and placed in a water-bath with mechanical shaking at 37 °C for 2 h, after adding an equal volume of 1 *N* NaOH. The mixture was then centrifuged in a 'Sorvall RC 2B' centrifuge for 20 min at 1510 *g*. The precipitate was discarded and the supernatant was used for further study. The proteins were precipitated by adding 3 *M* trichloroacetic acid. Each etherdried sample of precipitated proteins was taken in acetone and dried on the tared planchet before being introduced in the

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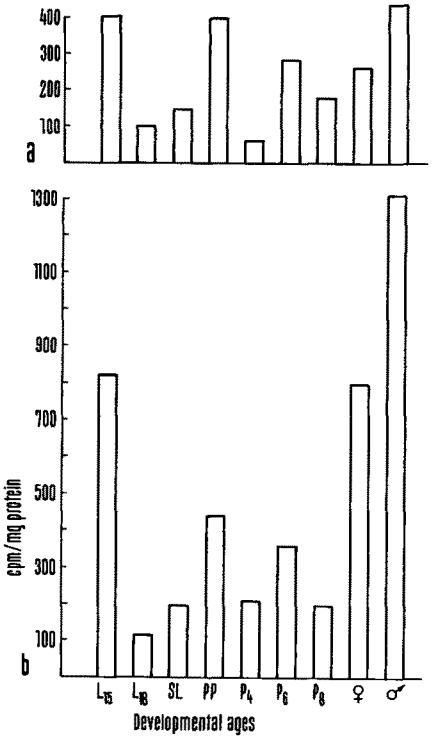
counter. The radioactivity was measured with an automatic Nuclear Chicago gas flow counter. Proteins were determined by the method of LOWRY[10].

*Results and discussion.* From the Figure it is seen that there are significant differences in the incorporation of radioactive leucine at several of the developmental stages of the insect. Thus, in the early larval stage $L_{15}$, the uptake by total animal is greater than in the late larval stage $L_{18}$. This finding parallels results of PRICE[6,7,11], who reported that in blowfly larvae cultured at 25 °C the high rate of protein synthesis in the fat body decreases rapidly in the late larvae (aged from 4 to 6 days). Further, CHIPPENDALE and KILBY[9] found that the relative in vivo protein synthesis in mid-fifth instar *Pieris brassicae* larvae is high in the fat body and midgut, which may be considered active sites of protein synthesis. In the pupal stage we found 2 other smaller peaks of increasing rate of protein synthesis. These appeared in the stages of pre-pupa and 6-day-old pupa. This observation can be correlated with the changes occurring in the fat body. BUTTERWORTH et al.[12] observed changes in the fat body in the late pre-pupal stage when proteinaceous granules, which were stained with fast green, began to appear. On the other hand, WALKER[13] reported that during the pharate pupal stage, each fat body cell becomes packed with many granules and fat vacuoles. CHIPPENDALE and KILBY[8] suggested that fat body stores haemolymph proteins during the pharate pupal stage.




Incorporation of $C^{14}$-leucine into protein of different developmental stages of *Plodia interpunctella*. $C^{14}$-leucine was injected (a) 2 h and (b) 4 h before the animals were sacrificed.

A significant increase of protein synthesis was observed in the adults. This finding supports the suggestion of CHIPPENDALE and KILBY[9] that some other tissues may also participate in haemolymph protein synthesis. Thus, except for the fat body, other cells or differentiated tissues like midgut become possible active sites for haemolymph protein synthesis. It is of interest that the males showed a rate of protein synthesis twice as high than the females. No ready explanation is available for this finding.

*Zusammenfassung.* Die Einbaurate für Leucin-$^{14}$C in die Körperproteine ist in 15tägigen Larven und Adultinsekten von *Plodia interpunctella* bedeutend höher als in den Zwischenstadien. Bei letzteren zeichnen sich Vorpuppen und 6tägige Puppen durch eine höhere Einbaurate aus. Männliche Falter inkorporieren mehr Leucin als weibliche.

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## Dissociation of Avidin-Biotin Complex in vivo

Avidin, a toxic glycoprotein, which renders biotin unavailable for animal and microbial growth combines firmly with biotin to yield an avidin-biotin complex with a dissociation constant of $10^{-15}M$[1]. The avidin-biotin complex was found to be extremely stable. It is stable over a wide pH range[2,3] and towards heat, even steaming at 100 °C for a short period[4,5]. Furthermore, biotin, when combined with avidin, cannot be liberated from the avidin-biotin complex by ordinary proteolytic enzymes[6].

In contrast to the effectiveness of avidin to cause egg white injury upon oral administration, GYÖRGY and ROSE[7] found that its injection was shown to have a curative effect on the same deficiency disease. This was explained by FRAENKEL-CONRAT and FRAENKEL-CONRAT[8] that biotin in the avidin-biotin complex presumably is released under physiological conditions and then fellows a path similar to that of free biotin. In the present investigation tracer techniques have been used to further confirm this problem.

Pure avidin used in this study was prepared by the method of MELAMED and GREEN[9] with an activity of 13.4 U/mg.

Avidin-radiobiotin complex (A–B*) was prepared from appropriate amount of avidin saturated with excess amount of D-biotin-carbonyl-$^{14}$C. The excess free radiobiotin was removed from the solution by dialysis against $0.2M$ ammonium carbonate. The undialyzed material, A–B*, was lyophilized and dissolved with appropriate amount of normal saline. 4 male rats (Sprague-Dawley