(*Reprinted from Nature*, *Vol.* 179, *pp.* 678–679, *March* 30, 1957)

Protein Synthesis in *Sphinx ligustri* Pupæ

At the beginning of the pupal stage of *Sphinx ligustri*, a lysis of larval tissues occurs. In winter, during diapause, the pupa is a bag full of blood containing a suspension of partially broken down larval tissues. In spring, diapause is broken, formation of adult tissues is speeded up and the moth becomes rapidly ready to emerge. Most of the amino-acids used for adult protein synthesis come from larval tissues; the question is whether the larval proteins are hydrolysed to free amino-acids before being used for adult protein synthesis, or whether adult proteins are built up from larger units that might be carried by phagocytes which are known to destroy the larval tissues. In order to approach an answer to this question, we decided to compare the mean specific rates of protein synthesis (rate of synthesis/amount of proteins) from one free amino-acid, namely glycine, in *Sphinx ligustri* pupæ, either in diapause or at the moment of the development of the adult organs.

112 μgm. of glycine-1-^{14}C (433,000 c./min.) was injected into the body cavity of each of five *Sphinx ligustri* pupæ. After a time, specified later, the animals were cut longitudinally into two, the blood washed away, the remaining tissues dissected from the cuticle and put into 20 per cent trichloracetic acid to stop enzymic actions. The tissues were washed with water, cooked in boiling water and ground. They were put in a bag with some ordinary glycine and submitted to dialysis to eliminate the free amino-acids and especially the radioactive free glycine: the process of dialysis was repeated seven times. The non-dialysable fraction was submitted to hydrolysis with 6 *N* hydrochloric acid for 24 hr. and a neutral amino-acid fraction isolated from the hydrolysate with the use of ion-exchangers ('Dowex 2' and 'Dowex 50').

A portion of the neutral amino-acid fraction was used for the determination of the amount of the non-blood protein glycine following the ion-exchange method of Moore and Stein[1] (see Table 1) (the non-dialysable glycine is mostly protein glycine; we shall call it protein glycine).

The greater part of the neutral amino-acid fraction was used for the determination of the non-blood

Table 1

		Sphinx ligustri				
		I	II	III	IV	V
Non-blood protein glycine	Total amount (mgm.)	0·35	6·75	4·15	0·49	0·59
	Nitrogen determination on isolated sample (theor. 18·66 per cent)	18·5	18·7	18·6	18·4	18·7
	Total activity (c./min.)	31,200	14,700	30,800	1,600	1,090
	Specific activity (c./min./m.mole)	250,000	103,000	720,000	254,000	139,000
Pupal stage		Transforming into adult			Diapausing	
Duration of experiment		2 hr.		24 hr.		
Amount of glycine injected		112 μgm.; 433,000 c./min.				

protein glycine radioactivity. A carefully weighed amount of ordinary glycine was added as a carrier and the glycine was isolated as the α-nitronaphthalene-sulphonate, which was recrystallized several times in water. The sulphonate in aqueous solution was poured on a column of 'Amberlite *IR*-120(H)' and the glycine was eluted with 3 *N* ammonia; the regenerated glycine was recrystallized in water/ethanol/diethyl ether and submitted to Kjeldahl nitrogen analyses. The glycine crystals were used for the radioactivity determinations; this radioactivity was found not to change on recrystallization.

From these results, the specific activities of the non-blood protein glycine of the *Sphinx ligustri* could be calculated.

The experiment was carried out in June. *Sphinx* I, II and III had been maintained continuously in an unheated room in a garden; when injected, the formation of the adult was well on the way, antennæ and wings being recognizable. *Sphinx* IV had been maintained at 2° C. during winter and transferred to the unheated room in April; at the time of the experiment the animal was still in diapause. *Sphinx* V had been kept continuously in a room at 25° C.; at the time of the experiment, it was also in diapause. From what is known for other Lepidoptera, specimen

IV was likely to produce the adult within a few weeks, whereas specimen V would never become adult.

After the radioactive glycine injections, the five animals were put in the room at 25° C. so that the rates of incorporation of glycine into proteins could be compared.

Sphinx I and II were killed 2 hr., the other three 24 hr., after the injections.

Specific rates of protein synthesis or turnover-rates can be directly compared on the basis of the specific radioactivity of the protein glycine after injection of radioactive glycine, only if the specific radioactivity of the glycine used for protein synthesis is the same in all animals compared at any time after the injection. (The term 'turnover' is used only when there is a 'steady state', that is, no net protein synthesis, as is probably the case during diapause.)

When the same amount of radioactive glycine is injected into all the animals, the specific activity of the free glycine in the free amino-acid pool at the time 0 after the injection depends only on the quantity of free glycine in the pool (hæmolymph + intracellular). In the hæmolymph, the quantity of free glycine is slightly higher in the diapausing *Sphinx* (600–750 µgm.) than when the transformation into adult has started (400–700 µgm.) (Duchâteau and Florkin, unpublished results).

The decrease of the free glycine specific radioactivity after the injection depends on the rate of utilization of free glycine. Is this rate large or not? If it were not, the specific radioactivity of the free glycine could be considered constant during the experimental period, and the discussion would be simplified. However, the comparison of *Sphinx* I and II on one hand, and *Sphinx* III on the other, does not support this view: if the specific activity of the free glycine had not changed during the 24 hr. following the injection, one would expect to find the activity of the non-blood protein glycine in *Sphinx* III to be about twelve times as high as that in *Sphinx* I and II; the ratio is actually much less (whether calculated from specific or absolute activities).

It seems likely that the turnover of free glycine is higher in pupæ that are in the process of forming the adult than in pupæ that are still in diapause. We tentatively draw the conclusion that, on the average during the 24-hr. experimental period, the specific radioactivity of the free glycine in the free amino-acid pool was rather lower in *Sphinx* III than in *Sphinx* IV and V, and consequently the mean specific rate of non-blood protein synthesis from free glycine is much higher in the transforming pupa

than in the diapausing animal. This seems to support the view of adult protein synthesis directly from the free amino-acid pool.

We wish to thank Dr. J. Leclercq for rearing the insects and for helpful discussion of the results.

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Jan. 17.

[1] Moore, S., and Stein, W. H., *J. Biol. Chem.*, **192**, 663 (1951).

Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.