Utilization of the Carboxyl Group of Glycine for the Synthesis of the Amino-Acids of Silk by *Bombyx mori*

WHEN fasting and ready to spin their cocoons, five silkworms were each injected with 112 μgm. (2·1 μc.) of glycine-1-^{14}C.

Fibroin was isolated from the cocoons by the method described by Dunn *et al.*[1]. The four amino-acids—tyrosine, glycine, alanine and serine—were then isolated from the hydrolysed fibroin, using a modification of Stein and Moore's procedure[2]: the hydrolysed fibroin was freed of excess hydrochloric acid by repeated evaporation and then by percolating through a bed of 'Amberlite' *IR* 120 (H+). The amino-acids adsorbed on the resin were eluted with 3 *N* ammonia, which was then evaporated *in vacuo*. At this stage, the tyrosine was removed by filtration and purified by crystallization and finally by chromatography on a starch column, with butanol/water as solvent[3]. Tyrosine was completely radio-inactive. Glycine was precipitated as glycine nitronaphthalene sulphonate, alanine as azobenzene-*p*-sulphonate and serine as *p*-hydroxyazobenzene-*p*-sulphonate. The amino-acids were obtained by passing the sulphonates through an 'Amberlite' *IR* 120 (H+) column and then eluting with 3 *N* ammonia. They were crystallized several times in water–ethanol–diethylether.

The specific activities of the sulphonates and of the derived amino-acids remained constant throughout the crystallizations. The chemical purity of each amino-acid was checked by Kjeldahl nitrogen analysis and by paper chromatography in butanol/hydrochloric acid and in phenol/water. They were then oxidized and converted to barium carbonate. The radioactivity measurements were performed with a thin mica-window Geiger counter on barium carbonate deposits. Each amino-acid was decarboxylated with ninhydrin[4]. The carbon dioxide evolved was trapped in sodium hydroxide solution and converted to barium carbonate. The radioactivities of the isolated amino-acids and of their carboxyl groups, corrected for background and self-absorption[5], are summarized in Table 1.

Table 1

	Specific activities		Total activity per cocoon (counts/min.)
	Amino-acid (counts/min./mmole)	Carboxyl group (counts/min./m.equiv.)	
Injected glycine	$2·7 \times 10^9$	$2·7 \times 10^8$	
Isolated glycine	196,000	196,000	81,500
Isolated serine	65,700	63,600	7,500
Isolated alanine	18,200	18,600	5,400
Isolated tyrosine	0	0	0
			94,400

Total activity injected per worm, 400,000 counts/min.

From these results, one can conclude that: (1) One quarter of the total activity injected appeared in silk fibroin. (2) The injected glycine was found diluted about one thousand-fold in the silk, which was most probably not uniformly labelled. (3) Direct conversion of glycine to serine keeping the label in the C-1 position occurs in the silkworm. It is probably the result of the addition of a one-carbon unit as shown in mammals, birds and micro-organisms. (4) There is also a conversion of glycine to alanine which might proceed through serine and pyruvic acid. Whatever the steps of this transformation are, the reactions involved must introduce the C-1 of glycine exclusively into the carboxyl group of alanine. (5) Tyrosine has not incorporated any radioactivity from glycine-^{14}C, whereas in a previous communication we have shown a direct conversion of phenylalanine to tyrosine[6]. This seems to indicate that the phenylalanine–tyrosine pair is essential to the silkworm.

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Utilization of Formate for the Biosynthesis of Glycine Carbon-1 and -2 in *Bombyx mori*

JUST prior to spinning their cocoons, each of twenty silkworms was injected with 1·7 mgm. (25 μc.) of formate labelled with carbon-14. The fibroin, isolated by the method of Dunn *et* al.[1], was hydrolysed and the glycine isolated as nitronaphthalene sulphonate. This salt was recrystallized several times, then dissociated on a 'Dowex' 50 column; free glycine was eluted with 3 *N* ammonia and crystallized in water/ethanol/diethylether. Its chemical purity was checked by paper chromatography in two different solvents (phenol/*iso*propyl alcohol/water and butanol/acetic acid/water). The specific radioactivities of the nitronaphthalene sulphonate and of the free glycine remained constant throughout the recrystallizations.

The isolated glycine was decarboxylated[2]; C-1 was isolated as barium carbonate and C-2 as formaldimedone. The specific activities of the isolated glycine and of its two carbons are shown in Table 1; all determinations were performed with a thin mica-window Geiger counter on barium carbonate deposits (glycine and formaldimedone were oxidized and converted to barium carbonate before the assays) and the results corrected for background and self-absorption.

The degradation procedure was checked on synthetic glycine-1-^{14}C and glycine-2-^{14}C (Table 1).

It can be concluded that formate carbon is utilized for the synthesis of glycine C-1 and C-2 in *Bombyx mori*. 1·5 per cent of the injected radioactivity appeared in the fibroin glycine, while 2 per cent was found in fibroin serine which had a specific activity about six times greater than glycine.

Several biochemical pathways, known in other organisms, can be suggested to explain these results. Further work, namely the analysis of the distribution

Table 1. SPECIFIC ACTIVITIES
(counts/min./mmole or m.equiv.)

	Isolated	1-^{14}C	2-^{14}C
Glycine	68,400	33,600	280,000
C-1	20,300	33,800	158
C-2	43,500	42	277,000