the writer has encountered in the literature no other instance of the involvement of *P. lunatus* in any interspecific hybrid combination, it was deemed advisable to publish now this preliminary report of progress, even though the subsequent breeding behavior of the F_1 hybrids is yet to be established.

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Niacin and Niacinamide Biosynthesis in Insects[1]

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It has recently been shown that niacin can be formed from tryptophan in mammals (*1*), but cannot be formed in the larval stage of insects, such as *Drosophila melanogaster* (*2*), *Tenebrio molitor,* and *Tenebrium confusum* (*3*), which require both tryptophan and niacin in their larval growth.

This paper reports an increase of the amount of niacin and niacinamide during the metamorphosis of the pupal stage in the silkworm *Bombyx mori.* We determined these two substances in various stages of the pupa, which live for a considerable period (7–12 days) without food. Female pupae of the Japanese and Chinese hybrid (J 115 × C 108) were used. The samples were kept at a constant temperature of 28° C.

The determination of niacinamide was made by Kato and Shimizu's method (*4*), and that of total niacin by the microbiologic assay method, using *Lactobacillus arabinosus.*

The results of the experiments are given in Tables 1 and 2 and in Fig. 1. It was found that in the period from the second to the eighth day both the total niacin and the niacinamide reached a maximum on the eighth day, and that the amounts of these substances were twice as great as on the second day. They suddenly decreased on the ninth day. The formation of total niacin was found to be greater than that of niacinamide at each period.

From the above results, we conclude that niacin and niacinamide are formed biosynthetically in silk-

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TABLE 1

TOTAL NIACIN CONTENT OF SILKWORM PUPAE, DETERMINED BY THE MICROBIOLOGICAL ASSAY METHOD WITH *L. arabinosus*

Material and age in days	Wt of 5 fresh pupae (g)	Titration value (ml)	Calibration curve (γ)	Dilution factor	Content of total niacin (γ)	Total niacin content (γ/g)	Total niacin content (γ/individual)
Female pupae							
Second day	7.50	2.55	0.08	3150	252	33.6	50.4
" "	8.30	2.75	.09	3150	284	34.2	56.8
Fifth "	7.84	2.85	.10	3325	333	42.4	66.6
Sixth "	7.27	3.10	.12	3325	399	54.8	79.8
Eighth "	7.19	3.70	.18	3325	599	83.3	119.8
Ninth "	6.70	3.25	0.14	3588	502	74.9	100.4

TABLE 2

NIACINAMIDE CONTENT OF SILKWORM PUPAE, DETERMINED BY KATO AND SHIMIZU'S METHOD

Material and age in days	Wt of 5 fresh pupae (g)	Titration value (ml)		Niacinamide added (γ)	Dilution factor to total individuals	Niacinamide (γ) in total individuals	Estimated value (γ), titrated with 2γ/ml of niacinamide standard solution		Dilution factor	Recovery (%)	Niacinamide content (γ/g)	Niacinamide content (γ/individual)
		a	b	c	$f=$	$\frac{acf}{b-a}$	a'	b'	f'			
Female pupae												
Second day	7.50	0.080	0.120	10	6.0	120	0.160	0.240	120	96	16.0	24.0
" "	8.30	.085	.125	10	6.0	128	.170	.250	120	96	15.4	25.6
Fifth "	7.84	.105	.140	10	6.3	189	.210	.280	120	84	24.1	37.8
Eighth "	7.19	.125	.160	10	6.3	225	.250	.320	120	84	31.2	45.0
Ninth "	6.70	0.120	0.162	10	6.3	180	0.240	0.324	120	101	26.8	36.0

a, a', no niacinamide added.
b, b', niacinamide added.

$$\text{Recovery} = \frac{f'(b'-a')}{c} \times 100.$$

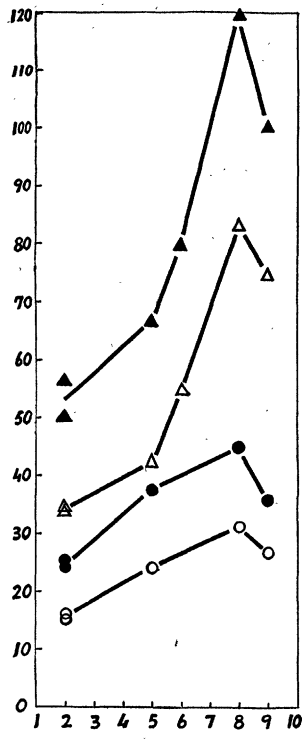

FIG. 1. Variation of the total niacin and niacinamide content in the pupal stage of *Bombyx mori*. Ordinate: content of total niacin and niacinamide (γ). Abscissa: age of pupae in days. ▲, Total niacin per individual; ●, total niacin per g; △, niacinamide per individual; ○, niacinamide per g.

worm pupae, although we cannot yet identify tryptophan as the precursor.

It is natural that *Tenebrio* and *Drosophila* have no mechanism of niacin biosynthesis, because they receive niacin in their food in the larval stage, whereas according to Kikkawa (*5*) 3-hydroxykynurenine is absent or very scanty in the larval stage of the silkworm, but becomes suddenly increased biosynthetically at the beginning of the prepupal stage and is maintained through almost the whole pupal stage except the last short period. We can therefore readily suppose that the niacin biosynthesis is carried on in the pupal stage.

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An Oxidative Metabolite of Desoxycorticosterone

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The general procedure of perfusing an isolated organ with a circulating medium enriched by the addition of a selected steroid precursor has found utility both as a means of determining the metabolic path of the precursor under conditions resembling those present in the intact animal (*1*) and as a method of biosynthesis (*2*). We have employed this procedure in a study of the metabolism of desoxycorticosterone (DOC) in a mammalian liver.

The liver is known to play an important role in the inactivation of DOC, as judged by the failure of this hormone to elicit a full physiological response when it is administered orally or introduced into a site drained by the portal circulation (*3, 4*). Studies based on urinary excretion products (*5–9*) in various species have shown that the administration of DOC (or its acetate) is followed by an increased elimination of pregnanediol, generally isolated and characterized as sodium pregnanediol glucuronide. The amount of administered DOC which can be accounted for as pregnanediol usually varies from 1% to 15%. In Addisonian patients a similar conversion of 11-dehydrocorticosterone to 11-keto-pregnanediol has been reported (*10*). Assays of the blood sera of monkeys have indicated a partial transformation of DOC acetate into progesterone (*11*). The administration of progesterone is also known to give rise to pregnanediol (*6*) and to pregnane-3α-ol-20-one (*12*).

More recently Schneider and Horstmann (*13*) have incubated DOC with rat liver tissue and have observed reduction of the conjugated unsaturated system in Ring A, shown by ultraviolet spectroscopy and by the isolation of allopregnane-3β, 21-diol-20-one. These investigators have also demonstrated that extensive attack takes place on the side chain, involving cleavage or reduction beyond the stage of the α-glycol, as they observe a loss of formaldehydogenic steroids during incubation. This result is explainable by the formation of pregnanediol or, as Schneider and Horstmann suggest, by the possibility of cleavage to a 19-carbon atom steroid. There is, however, some evidence (*14*) that administered DOC does not give rise to 17-ketosteroids, and no metabolite of DOC having other than 21 carbon atoms has previously been reported.

In our procedure rat livers were perfused via the superior vena cava with oxygenated, citrated beef blood containing about 500 mg of added DOC per liter. Adsorbable constituents were removed from the hemolyzed perfusate by activated carbon, and these materials were then removed from the carbon by extraction with chloroform and benzene in a Soxhlet apparatus.

The Soxhlet extract was subjected to extensive chromatography on silica columns, terminating in a final column on which the steroidal portion was partitioned, on 65 times its weight of silica, into 40 fractions by means of increasingly polar mixtures of ethyl acetate and benzene. Elution in the vicinity of 1:2 ethyl acetate-benzene resulted in crystalline fractions containing unaltered DOC and a 20-carbon atom transformation product, difficult to separate by direct chromatography because of its close resemblance to DOC in its behavior on the column.

Identification of this material as 3-keto-4-etiochol-