Table III

Antituberculosis Activity in Guinea Pigs of Vinactane Sulfate at Varying Dose Levels. *Treatment Started:* 28th day, post-infection. *Duration of Treatment:* 60 days.

Dose Vinactane Administered	Number of Animals	Average Gross Involvement				Average Total Involvement
		Spleen	Lungs	Liver	Site of Inoculation	
10·0 mg	11	10·0	6·4	7·7	3·6	27·7
5·0 mg	15	6·3	10·0	6·7	4·7	27·7
2·0 mg	12	18·8	10·8	18·8	8·3	56·7
Pretreatment Controls*	5	26·0	10·0	16·0	10·0	62·0
Untreated Controls (88th day)	13	19·2	20·8	23·5	10·0	73·5

* The pre-treatment controls were sacrificed at the commencement of therapy (28th day, post-infection) in order to establish the extent of tuberculous involvement.

tained for 21 consecutive days. In the 38th day, post-infection, all surviving mice were sacrificed. According to the results obtained, therapy with 1·0 mg Vinactane can be delayed for nine days after infection and yet exert a strong antituberculosis activity. At the 0·5 mg dose level, treatment with the antibiotic can be delayed for two days beyond the time of infection and still yield excellent activity.

Chemotherapeutic Activity in Tuberculous Guinea Pigs. Female guinea pigs, approximately 500 g in weight, were infected subcutaneously in the groin with 1·0 ml amounts of a 1:50 dilution of a 7-day old culture of H37Rv. Twentyseven days after receiving the infecting dose, the animals were tuberculin tested (intracutaneous injection of 0·1 ml of 1% "O.T.") and all responded with a strong positive reaction. Groups of 12 animals each were, on the 28th day following infection, treated with 10·0, 5·0 and 2·0 mg Vinactane sulfate, administered intramuscularly once daily for 60 days. The normal animal diet was supplemented with greens and ascorbic acid, the latter added to the drinking water (0·5 mg/ml) on alternate days. On the last day of therapy (88th day, post-infection) all animals were sacrificed. The gross organ involvement was determined according to the method described by FELDMAN and KARLSON[1]. Maximal values of 40, 70, and 100 respectively were assigned to animals with slight, moderate, and extensive degrees of total organ involvement. The results of this experiment are shown in Table 3 and indicate that Vinactane is capable of exerting appreciable anti-tuberculosis activity in guinea pigs at doses of 2·0 mg per day.

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Zusammenfassung

Vinactan, eine antibiotische Substanz, isoliert von *Streptomyces vinaceus*, besitzt bedeutende antituberkulöse Aktivität in infizierten Mäusen und Meerschweinchen. Erheblicher Schutzeffekt in tuberkulösen Mäusen wurde beobachtet, wenn die Therapie nicht sofort, sondern erst 9 Tage nach der Infektion einsetzte und die Behandlung mit 1 mg täglich während 21 Tagen durchgeführt wurde. In tuberkulösen Meerschweinchen, die mit 5 mg Vinactan täglich subkutan während 60 Tagen behandelt wurden, war die Entwicklung tuberkulöser Infektion in Milz, Lunge und Leber erheblich vermindert.

[1] A. G. KARLSON and W. H. FELDMAN, Ann. N. Y. Acad. Sci. *52*, 637 (1949). – W. H. FELDMAN, Amer. Rev. Tuberc. *48*, 248 (1943).

Occurrence of an Enzyme Acting on Xanthopterin-B in *Bombyx mori*

A pterin like pigment, xanthopterin-B (the suffix "B" coming from *Bombyx*)[1] is found in the integument of larvae of the mutant, "lemon" (*lem*)[2] and "yellow lethal" (*lem*^{l})[3], of the silkworm, characterized by the yellow colour of their skin. It is also found in the wings of the yellow butterflies, *Eurema* and *Colias*. Both in chemical properties and biological activity, xanthopterin-B has a similarity to xanthopterin and to folic acid[4]. However, xanthopterin-B has not been isolated in a pure form for the determination of its structural formula. It shows a strong yellow fluorescence and like folic acid is very unstable to light, especially in acid solution. The relation between xanthopterin-B, uric acid and melanin in the skin of several mutants of the silkworm has been studied from the stand-point of biochemical genetics[5].

Using 5% sodium citrate as a solvent, xanthopterin-B in the tissue extract of the *lem* larvae separated into two components on a paper chromatogram. We designated the two components as xanthopterin-B_1 (*Rf* value, 0·27) and xanthopterin-B_2 (*Rf* value, 0·32)[4]. The two spots, however, overlapped when a common solvent such as butanol-acetic acid-water (4:1:1) was used (*Rf* value, 0·45). Aside from sodium citrate the following solvents were also useful for differentiating the two components: sodium acetate, sodium chloride, ammonium chloride and urea (all 5% solutions). Both xanthopterin-B_1 and -B_2 show yellow fluorescence, but the former fluorescents much more strongly and is also more unstable toward some chemical treatments. In the integument of the *lem* larva four pterins or pterin like pigments, leucopterin, leucopterin-B ("isofluorescyanine [fluorescyanine B]"[6], or probably identical with isoxanthopteria[7])

[1] Y. HIRATA, K. NAKANISHI, and H. KIKKWA, Bull. Chem. Soc. Japan *23*, 76 (1950).

[2] T. HAMA, Y. MAKI, and H. ARUGA, Zool. Mag. *58*, 219 (1949). – K. NAKANISHI, Y. HIRATA, and H. KIKKWA, Jap. J. Genetics *25*, 78 (1950).

[3] Y. UMEYA and M. TSUJITA, Bull. Sericult. Exptl. Station *13*, 329 (1951).

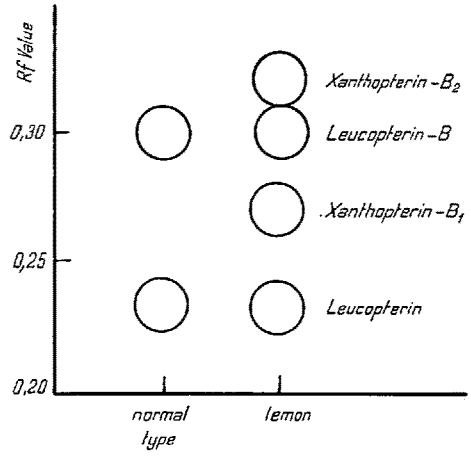
[4] H. ARUGA and N. YOSHITAKE, unpublished.

[5] H. ARUGA, N. YOSHITAKE, and R. ISHIHARA, J. Sericult. Sci. Japan *22*, 1 (1953).

[6] M. POLONOVSKI, C. ALCANTARA, and R. G. BUSNEL, C. r. Acad. Sci. *235*, 1703 (1952).

[7] Y. HIRATA and S. NAWA, C. r. Soc. Biol. *145*, 661 (1951).

xanthopterin-B_1 and -B_2 can be differentiated[1]. In the normal (wild type) larva, there exist only two pterins, leucopterin and leucopterin-B (Figure). The content of leucopterin-B in the *lem* mutant is less than in the wild type. These facts led us to carry out some experiments on the metabolism of pterin like pigments in the mutants of the silkworm. During the course of these experiments, we observed the disappearance of the fluorescence of xanthopterin-B_1 when the latter was incubated with tissue extracts from wild type larvae. As shown below an enzyme (or enzymes) appears to be responsible for this change.



Paper chromatogram of the extract of the integument of the silkworm. solvent; 5 % sodium citrate. *Rf* value and colour of fluorescence: xanthopterin-B_2; 0·32 (yellow); leucopterin-B, 0·30 (purple); xanthopterin-B_1 0·27 (yellow); leucopterin, 0·23 (pale blue).

Since not enough pure xanthopterin-B_1 was available for investigating the enzymatic reaction, in most cases we used the heated extract of the skin of the *lem* larva as a substrate. 200 mg of aceton dried, defatted and hot alcohol denatured powder of the skin of the *lem* larvae (5th instar) was ground with 5 ml water and the mixture was heated for 10 min at 80–90°C. The cooled extract was centrifuged and the yellow supernatant was used as a substrate. To avoid the effect of light all procedures were carried out in the dark room under a red lamp. The enzyme solution was prepared as follows: the skin of a normal larva (5th instar) freed from internal organs and adipose tissues was washed with water and weighed after removing excess water with filter paper. The minced skin was homogenized with ten volumes of M/15-phosphate buffer (pH 7·2). The mixture was left standing for 15 min and then centrifuged. The supernatant was used as an enzyme preparation. The skin extract of the *lem* larva was also prepared in the same way. In order to compare the enzymatic activity of the normal and *lem* larvae, the following mixtures with a few drops of toluol were incubated in the dark for 3 h at 30°C: (1) 0·5 ml normal extract + 0·5 ml substrate solution + 0·5 ml water, (2) 0·5 ml heated normal extract (80°C 5 min) + 0·5 ml substrate solution + 0·5 ml water, (3) 0·5 ml normal extract + 0·5 ml substrate solution + 0·5 ml *lem* extract, (4) 0·5 ml heated normal extract + 0·5 ml substrate solution + 0·5 ml *lem* extract. For control, mixtures of 0·5 ml phosphate buffer, 0·5 ml substrate solution and 0·5 ml *lem* extract or water were also incubated. At the end of the incubation each mixture was analyzed directly by paper chromatography (5 % sodium citrate or acetate as a solvent). Under the above conditions, it was observed that after incubation the yellow fluorescent spot of xanthopterin-B_1 had disappeared in cases (1) and (3). The spots of xanthopterin-B_2 and other pterins were intact in all cases. The same results were obtained using the reextracted solution of xanthopterin-B_1. Although the yellow colour of the mixtures (1) and (3) decreased with the disappearance of fluorescence of xanthopterin-B_1, it could not be decided whether the yellow colour of the crude mixtures was exactly proportional to the concentration of xanthopterin-B_1 present.

[1] One more fluorescent substance is found in the integument of the *lem* larva by using two dimensional paper chromatography. With the one dimensional method the spot for this substance probably overlaps with that of leucopterin-B [H. ARUGA and N. YOSHITAKE, unpublished).

Table I
Distribution of the enzyme in the several tissues of the silkworm

		Normal (+lem)	Lemon (lem)
5th instar	Integument: cuticle	−	−
	Integument: epidermis	+	±
	Adipose tissue	+	±
	Body fluid	−	−
	Ovary	±	−
	Testis	±	−
pupa	Adipose tissue	+	±
	Body fluid	+	±
	Ovarian Tube	+	±
	Testis	±	±
Egg (diapause)		+	±

The pH optimum of the enzyme lies in the range of 6–8 (phosphate buffer) and the activity is mostly destroyed by heating at 60°C for 5 min. It is inhibited by potassium cyanide and monoiodoacetate (final concentration, *M*/100) but not by sodium fluoride or hydroxylamine. A carefully prepared aceton powder of the skin retains its original activity.

Table II
Distribution of the enzyme and xanthopterin-B in the integument of several mutants of the silkworm.

Strain	Remark	Accumulation of xanthopterin B	Occurrence of the enzyme
p*	White part	−	+
	Black pigmented (melanin) part	−	+
plem	Yellow part	+	±
	Black pigmented (melanin) part	±	±
od	Translucent	−	+
odlem	Translucent yellow	±	±
lem	Yellow skin colour	+	±

* *p* (Striped) is characterized by the black melanin bands in each segment.

Tables I and II show the distribution of the enzyme in the several tissues and mutants of the silkworm. The activity was clearly observed in the epidermis and adipose tissues of normal larvae and also in the normal eggs and pupae, but could not be found in the body fluid of normal larvae. Little or no activity could be found in several tissues of the yellow mutant examined. It should

be noticed in Table 2 that as long as the *lem* gene is present in homozygous, the enzyme activity is very low. However, the lack of xanthopterin-B does not necessarily indicate the presence of the enzyme, since, for instance, in the mutants "Striped" marking (p^s) and "od-translucent" (*od*), these genes have also an effect of suppressing the accumulation of xanthopterin-B[1].

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Résumé

Nous avons démontré qualitativement chez le *Bombyx mori*, l'existence d'un ferment qui attaque la xanthoptérine-B, une substance fluorescente du type ptérinique qui se trouve dans les intéguments des larves de race jaune «lemon», et se transforme en une substance non-fluorescente. Le ferment se trouve dans les épidermes, tissus adipeux des larves et dans les œufs et cocons des *Bombyx* de type normal. Chez les mutants de la race jaune, l'activité du ferment est faible ou nulle dans bien des tissus. Cependant on ne peut pas nécessairement dire que la présence de ce ferment conduit à l'absence de la xanthoptérine-B, parce que l'accumulation de xanthoptérine-B est supprimée par l'action d'autres gènes chez les mutants «Striped marking» (p^s) ou «od-translucent» (*od*). Dans ces derniers l'activité du ferment est en effet très faible.

[1] H. ARUGA, N. YOSHITAKE, and S. ISHIKAWA, J. Sericult. Sci. Japan *20*, 399 (1951).

Concerning the Biogenesis of Lysergic Acid

Recently VAN TAMELEN[1] has proposed a biogenetic route to lysergic acid (VI, $R = CH_3$) having its origin in the coupling of a dihydronicotinic acid (or derivative) with a quinone imine derived from 5-hydroxytryptophan.

[1] E. E. VAN TAMELEN, Experi. *9*, 457 (1953).

The present writer would like to suggest the relatively simple and direct biogenetic pathway to lysergic acid involving the condensation of tryptophan (I) with citric acid (III).

The transformation of IV → V by dehydration with accompanying loss of carbon dioxide is based on extensive precedent, e.g., the conversion of citric to itaconic acid[1] and the formation of desmethylaxerophthene in the synthesis of vitamin A acid[2].

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Zusammenfassung

Ein biogenetischer Weg zur Lysergsäure, bestehend in der Kondensation von Tryptophan mit Zitronensäure, wird vorgeschlagen.

[1] R. ANSCHÜTZ, Ber. dtsch. chem. Ges. *13*, 1542 (1880).
[2] N. L. WENDLER, H. L. SLATES, N. R. TRENNER and M. TISHLER, J. Amer. chem. Soc. *73*, 719 (1951).

Oestrus Activity in Fat-Tailed Sheep During the Longest Days

The importance of day-length as a controlling environmental factor has been shown in poultry[1], old-world birds[2], and in a variety of mammals. Farm animals could be classified in this respect into three classes, those which are short-day breeders, such as sheep[3] and goats[4]; those which are long-day breeders, such as poultry, including pheasants[5]; and those which are not particularly responsive to daylight environment, such as rabbits. The conventional view of photoreception is that light received by the eye starts an unknown

[1] E. O. Whetham, J. Agric. Sci. *23*, 383 (1933).
[2] J. R. BAKER, Tabul. Biol. Berl. *15*, 333 (1938).
[3] E. S. E. HAFEZ, J. Agric. Sci. *42*, 189 (1952).
[4] T. H. BISONNETTE, Physiol. Zool. *14*, 379 (1941).
[5] T. H. BISSONNETTE and A. G. CZECH, J. Wildlife Management *5*, 383 (1941).