(the non-radioactive TMP peak) because this mutant cannot phosphorylate TMP to TDP. This process is, however, not essential for the uptake of TDP by this strain, hence the specific radioactivity of [^{32}P]TDP was not reduced during the membrane transport ([^{32}P]TDP peak of the figure, strain 70-23-107).

Discussion. Kawasaki and Yamada[15] attempted to demonstrate the uptake of free T by *E. coli* using a T kinase defective mutant. In their mutant, however, the active uptake of T occurred only during 2–3 min after the start of reaction. On the basis of their data they considered the negative charge on TDP to be important for the accumulation of intracellular T. Hence the participation of T kinase in T uptake by *E. coli* has not completely been excluded. But the data presented in this report, showing that TDP is transported into *E. coli* strain 70-23 without the participation of T kinase, exclude this uptake system from the scope of so called 'group translocation'. The accumulation of cold TMP in strain 70-23-107 could possibly be explained by the preliminary data that the activity of alkaline phosphatase is enhanced to some extent in this strain. As alkaline phosphatase is known to be localized in the periplasmic space TDP is possibly dephosphorylated by this enzyme outside the cell membrane. In addition to studies of uptake, a method for the synthesis of [^{32}P]TDP and [^{32}P]TMP with high specific radioactivity is described in this paper. The activities obtained were 10 and 5 mCi/mmole for [^{32}P]TDP and [^{32}P]TMP, respectively.

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Isolation of the two paragonial substances deposited into the spermatophores of *Acanthoscelides obtectus* (Coleoptera, Bruchidae)

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Summary. Aqueous extracts from spermatophores of *Acanthoscelides obtectus* (Coleoptera, Bruchidae) have been chromatographed on Sephadex G 25 and G 15 columns, the active fractions being further purified by preparative SiO_2 TLC. The isolation and properties of 2 biologically active compounds are reported. The paragonial substance A has a favourable action upon oogenesis, is a low molecular weight molecule (500–1000) and gives 1 single amino acid on hydrolysis, corresponding to glycin on TLC. The paragonial substance B is toxic, or an inhibitor of oogenesis at lower concentration, thus showing an antagonistic effect.

Previous research[2-5] has shown that male secretions produced by one of the accessory glands (tubular glands) of the Coleopterae (Bruchidae) *Acanthoscelides obtectus* influence female reproductive activity. These products, the so-called paragonial substances[3], are deposited into the spermatophores during mating and migrate into the hemolymph some h later. The isolation of the crude active compounds[3] was achieved from aqueous extracts of spermatophores, collected at the end of copulation. It has been possible to isolate 2 paragonial substances, substance A which stimulates oogenesis, and substance B which on the contrary inhibits oogenesis and is toxic at higher concentrations. In the present work, which involved different fractionations of the extracts from some 8000 spermatophores obtained through dissection, we report the final isolation of the 2 chromatographically homogeneous products, which should later on permit the determination of their chemical nature.

The experiments were carried out on spermatophores collected by dissection of the female abdomen, just after copulation. They were immediately extracted with water at 20–40 °C, followed by vacuum concentration. Sephadex G 25 coarse, G 25 superfine and Sephadex G 15 were successively used for chromatographic fractionation. Aliquots from each fraction were taken and applied to SiO_2 TLC for control. After developing with butanol-acetic acid-water 4:1:1, staining with ninhydrin at 100 °C (1% in acetone in spray) the active compounds were looked for in connection with previous results[3] before the activity was tested. Column chromatography was performed with help of an automatic Elugraph apparatus with double recording of the absorbance at 220 and 280 nm. For preparative TLC, SiO_2 plates of 0.25 mm thickness were self-made from Merck HR silica gel, following the usual method. For analytical TLC, commercial plates (Merck) were used directly. Final purification steps were accomplished through repetition on previously washed plates, using acetone and water for washing before development. Finger printing of purified samples was carried out by 2-dimensional TLC developing with butanol-acetic acid water 4:1:1 and then 70% propanol in water.

For the biological activity determination, the purified products were dried under vacuum and dissolved in physiological serum before injection in the abdomen of 4-day-old virgin females, each receiving 1 µl of solution. After injection, the females were reared in presence of bean seeds during 10 days at 27 ± 2 °C and 70% relative humidity. The

Biological activity of the different fractions

Compound	Concentration	Number of females	Ovarian production	Signification of the difference with control	Biological activity upon oogenesis	Toxicity (% of mortality)
Physiological serum (control)	0	45	60.6±2.0			0
1	$0.4 \cdot 10^{-3}$ µg/µl	34	59.6±3.5	t=4.8 (+)	−	0
2	$0.2 \cdot 10^{-3}$ µg/µl	40	71.7±3.2	t=5.7 (+)	−	0
	$0.5 \cdot 10^{-3}$ µg/µl	32	72.3±4.1	t=4.9 (+)	+	0
3	$0.2 \cdot 10^{-3}$ µg/µl	32	49.1±5.1	t=4.7 (+)	−	47%
	$0.5 \cdot 10^{-3}$ µg/µl	43	50.9±7.8	t=2.3 (+)	−	72%

presence of seeds of the host plant induces egg laying and prevents oocyte retention into the abdomen. This retention could of course mask the eventual effect of the injected fraction. At the end of the experiment, the fecundity and the ovarian production is checked by reference to control insects receiving an injection of 1 µl of physiological serum. The fecundity is expressed as the number of eggs layed, and the ovarian production is the sum of the eggs layed plus the number of mature oocytes retained in the abdomen of each female insect.

Results. Isolation of the products. The figure shows the technique followed, together with the corresponding quantitative aspect. Chromatography of aqueous extracts on Sephadex columns[3] provides a fraction (F) which is biologically active. Final purification of this fraction is achieved by preparative TLC on silica gel as shown.

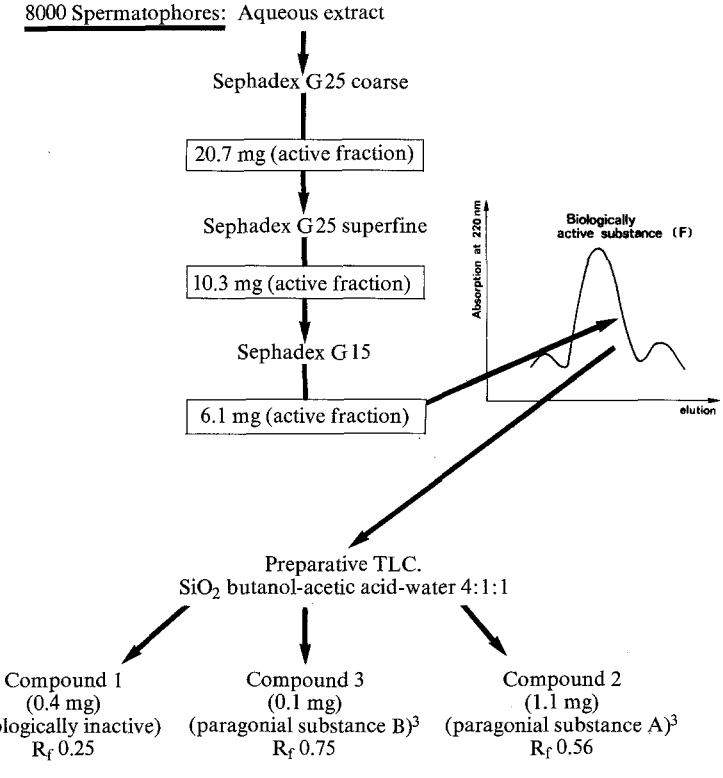
Determination of the biological activity of the fractions: The table shows the biological activities of different compounds, comparing the ovarian production (test of activity upon oogenesis) and the toxicity.

- Compound 2, injected into the abdomen of virgin females, stimulates oogenesis at the concentrations of 0.2–$0.5 \cdot 10^{-3}$ µg/µl and is not toxic. In all cases, the ovarian production is found to be higher than with the control insects and the differences are significant.
- Compound 3 injected at a concentration of $0.5 \cdot 10^{-3}$ µg/µl provoked considerable mortality (72%) and the surviving females have a reduced ovarian production. At lower concentrations, the toxicity of paragonial substance 3 is reduced, but the ovarian production still remains lower than in the control experiment.

These 2 purified compounds correspond to the paragonial substance A (compound 2) and paragonial substance B (compound 3) analyzed in a previous study[3].

Assay of characterization of paragonial substance A: The UV end absorption noticed for paragonial substance A in water in the region 190–210 nm may indicate the presence of peptide linkages in the product. The results of the different Sephadex column chromatographies permit us to fix the limits of the mol.wt between 500 and 1000. Paragonial substance A was hydrolyzed in 6 N HCl in a sealed tube at 110 °C for 24 h, the solvent was evaporated under vacuum, and the residue dissolved in a drop of water was applied to SiO_2 TLC developped with butanol-acetic acid-water 4:1:1, when it gave a single spot with ninhydrin corresponding to a glycine standard (R_f 0.40).

Discussion. A relatively simple procedure is thus reported for a final step purification of paragonial substances A and B from the insect named in the title. Due to the very small amounts of the products isolated so far, it has not yet been



Scheme of isolation of the 2 paragonial substances A and B from the Coleoptera *Acanthoscelides obtectus* and quantitative report from 8000 spermatophores obtained through dissection. Fingerprints of paragonial substances A and B on 2 dimensional SiO_2 TLC (butanol-acetic acid-water 4:1:1 and propanol-water 70%) show homogeneity.

possible to ascertain their structures, in spite of the fact that paragonial substance A is a relatively small molecule with only 1 amino-acid. This compound is indeed rapidly transferred from the spermatophore into the hemolymph and the stimulation of oogenesis appears[4] 16–24 h later. Baumann[6,7] has described a paragonial substance PS_2 from *Drosophila funebris*, also active upon oogenesis, and also a low mol.wt glycine containing product. However, in the absence of structure elucidation and of direct comparison, it is not possible to conclude that the 2 products are identical. The biological activity of paragonial substance B is quite original but difficult to analyse. However, the combined action of paragonial substances A and of the antagonistic (versus toxic) paragonial substance B may create a balance ensuring the regulation of ovarian production and oocyte maturation. As has been shown in a number of cases, particularly in insects, the control of reproduction could result from the interaction of 2 antagonistic hormonal factors[8,9]. It is necessary to add that the possibility that the paragonial substance B is a toxic artefact produced by the degradation of paragonial substance A during the manipulations cannot be excluded for the moment. But even, in this case, the fact that an antagonistic property is obtained is also of great interest.

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Effect of tryptophan on tyrosinase in relation to vitiligo[1]

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Summary. Tryptophan can inhibit DOPA (3,4-dihydroxy phenylalanine) conversion to melanin both by the enzymatic and the nonenzymatic route. Its role has been studied in relation to vitiligo.

Tryptophan is recorded to be an accelerator of tryptophan pyrrolase[3]. Tryptophan pyrrolase has been found to have an antagonistic relationship with tyrosinase during induced depigmentation and repigmentation in *Bufo melanostictus*[4]. It was, therefore, of interest to examine the role of excess tryptophan on tyrosinase in *Bufo melanostictus* which has been used as an experimental animal in the studies of pigment metabolism in relation to vitiligo[5], and also in mammalian system using black mice. In the present communication we report the effect of tryptophan on different aspects of melanin formation.

Materials and methods. L-DOPA and L-tryptophan were purchased from Sigma Chemical Co., USA. Other reagents were of the purest variety possible.

Studies on DOPA auto-oxidation. DOPA auto-oxidation in the formation of melanin was studied in the presence of different concentration of tryptophan.

Studies on enzyme level. Tyrosinase and tryptophan pyrrolase activity of ventral skin and liver of *Bufo melanostictus* (b. wt 40–50 g) and black mice (b. wt 20–25 g) were estimated after 7 days treatment with 1 mg tryptophan per day per animal.

Tryptophan pyrrolase activity was measured according to Knox[6] as slightly modified by Spiegel[7]. The liver and the ventral skin were dissected out. The homogenates of the tissues (12.5%) were prepared with 0.14 M KCl containing 0.0025 M NaOH, pH 7.0–7.5 and used as an enzyme source. The enzyme activity was measured after incubating with 0.03 M L-tryptophan in 0.2 M phosphate buffer (pH 7.0) solution for 1 h at 37 C. The enzyme activity was expressed in terms of μM of kynurenine/mg of protein.

Tyrosinase activity of the liver and the ventral skin were estimated according to Pomerantz[8] by measuring the rate of formation of dopachrome from L-DOPA at 37 °C under the following conditions: L-DOPA (1 μM), sodium phosphate buffer, pH 7.4 (35 μM), enzyme (0.2–0.3 units), total 1 ml. The enzyme activity was expressed as μM of dopachrome formed/min/mg protein.

Results and discussion. It is evident from the experiment that addition of tryptophan can inhibit DOPA auto-oxidation and this inhibition is proportional to the graded amounts of tryptophan (figure). In addition to this observation, tyrosinase activity has been found to be inhibited both in the skin and liver (table 1) of *Bufo melanostictus* along

Table 1. In vivo effect of tryptophan on tyrosinase and tryptophan pyrrolase of ventral skin and liver of *Bufo melanostictus* (n = 30)

Treatment	Tissue	Tyrosinase* (mean ± SD)	Tryptophan pyrrolase** (mean ± SD)
Control	Ventral skin	14.3 ± 2.61	3.02 ± 1.20
	Liver	9.2 ± 1.72	3.1 ± 0.59
Tryptophan (1 mg/day/toad for 7 days)	Ventral skin	7.8 ± 1.15	5.4 ± 2.01
	Liver	5.7 ± 0.86	11.2 ± 1.70

* μM of dopachrome/min/mg of protein ($p < 0.001$); ** μM of kynurenine $\times 10^{-2}$/mg of protein ($p < 0.001$).

Table 2. In vivo effect of tryptophan on tyrosinase and tryptophan pyrrolase of ventral skin and liver of black mice (n = 20)

Treatment	Tissue	Tyrosinase* (mean ± SD)	Tryptophan pyrrolase** (mean ± SD)
Control	Ventral skin	20.6 ± 2.24	4.5 ± 1.11
	Liver	7.1 ± 0.88	2.9 ± 0.58
Tryptophan (1 mg/day/mice for 7 days)	Ventral skin	15.7 ± 1.18	6.9 ± 2.71
	Liver	1.62 ± 0.70	7.3 ± 1.12

* μM of dopachrome/min/mg of protein ($p < 0.001$); ** μM of kynurenine $\times 10^{-3}$/mg of protein ($p < 0.001$).