Components isolated from the brushes of male noctuid moths

	Component	mol. wt.	%	Component	mol. wt.	%
Leucania impura (Hueb.)	Benzaldehyde	106	80	Iso-butyric acid	88	20
Leucania conigera (Schiff.)	Benzaldehyde	106	80	Iso-butyric acid	88	20
Leucania pallens (L.)	Benzaldehyde	106	80	Iso-butyric acid	88	20
Polia nebulosa (Huf.)	Benzaldehyde	106	8	Benzyl alcohol	108	10
	2-Phenyl ethanol	126	70			
Mamestra persicariae (L.)	Benzaldehyde	106	10	Benzyl alcohol	108	2
	2-Phenyl ethanol	126	85			
Mamestra brassicae (L.)	?	166	85+			
Phlogophora meticulosa (L.)	6-Methyl-hept-5-en-2-one	126	63			
	6-Methyl-hept-5-en-2-ol	128	28			
	2-Methyl butanoic acid	128	9			
Apamea monoglypha (Huf.)	Pinocarvone	150	95			

6-methyl-hept-5-en-2-one and the corresponding alcohol. The equivalent of 1 µg per brush of 6-methyl-hept-5-en-2-one was extracted from a sample of 300 brushes of *P. meticulosa*. The total quantity produced by one moth will certainly be much greater since moths trapped in the field would probably have mated at least once before capture, everting the brushes and losing a high proportion of scent. Losses also occur through chemical change (e.g. benzaldehyde to benzoic acid).

The male scents have been identified without reference to their biological function. The apparently identical nature of the scent of 3 *Leucania* species would suggest that at least between these species the male scents are not acting to promote species isolation. We do not know whether females can or do distinguish between the slight differences in the male scents of *M. persicariae* and *Polia nebulosa*. Both are very closely related. In contrast, the divergence between the scents of the undoubtedly congeneric *Mamestra* species suggests that in this genus the male scent may have been instrumental in speciation. Pinocarvone, found in *A. monoglypha*, has not previously been isolated from insects, though pinenes, derived from their host trees are used by bark beetles (Coleoptera) as sex pheromones and in aggregation[5].

It seems likely that the scents will have the same function in all Noctuidae since the brush-organs are morphologically virtually identical in the family. Chemical composition of the scents agrees with sub-family divisions. Both species of Amphipyrinae (*P. meticulosa* and *A. monoglypha*) secrete terpenes, the 5 species of Hadeninae secrete aromatic compounds. This correlation is being extended as material becomes available[6].

Zusammenfassung. Die flüchtigen Anteile aus den männlichen Duftorganen von 7 Gattungen der Noctuiden wurden gaschromatographisch identifiziert.

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[6] This work was supported by the Science Research Council.

Effect of a Growth-Promoting Factor on Protein Synthesis and Amino Acid Transport in vitro

In a recent paper we reported on a growth-promoting factor obtained from an extract of calf muscle. Gel filtration of the extract on a Sephadex G-25 column indicated that the active substances in the extract were polypeptides[1]. Previously, KOSTYO[2] had suggested that the action in vitro of growth hormone on protein synthesis and on amino acid transport into the diaphragms of hypophysectomized rats is mediated by a protein or polypeptide; this protein or polypeptide, first synthesized under the influence of growth hormone, would be responsible for the later stimulation of the membrane-transport mechanism. Considering our finding and the possibility suggested by KOSTYO, we have examined the effect in vitro of the growth-promoting polypeptides obtained from calf muscle on amino acid transport into the diaphragms of hypophysectomized rats, and on protein synthesis. There we used as an indicator of protein synthesis the incorporation of ^{14}C-leucine into proteins, and as an indicator of transport the intracellular accumulation of ^{14}C-labelled α-amino-isobutyric acid (AIB).

In our experiments, 32 female hypophysectomized rats, body weight 70–80 g, purchased from Hormone Assay Laboratories (Chicago) were used. The rats were sacrificed by decapitation and hemidiaphragms were prepared by the procedure of KOSTYO and KNOBIL[3]. Each hemidiaphragm was incubated in 10 ml Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2 mg glucose per ml.

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The medium was gassed for about 5 min before incubation with a mixture of 95% O_2 and 5% CO_2. The flasks were sealed with tightly fitted glass stoppers and kept in a shaking bottle for 2 h.

Extracts of calf muscle were prepared as described earlier[1]. After gel filtration on Sephadex G-25 in the present experiments 2 fractions (34 and 40) were used which were biologically active in stimulating ^{35}S-incorporation into chick embryo cartilage. In some flasks, 25 µg bovine growth hormone (BGH) obtained from the National Institute for Medical Research, Mill Hill, London, was added.

In the first experiment, 1 µC of ^{14}C-leucine was added to each flask. After incubation the tissue was homogenized in 10% trichloracetic acid (TCA), the precipitate was spun down and washed once in 3 ml 10% TCA. Excess of non-labelled leucine was added. The precipitate was then heated for 15 min at 90 °C in 30 ml 10% TCA. The insoluble material was extracted with 3.0 ml 0.4 *N* NaOH and the insoluble residue rejected. The dissolved protein was reprecipitated by addition of 5 ml 10% TCA. The precipitate was then washed 3 times with a mixture of ether-ethanol-chloroform (2-2-1), once with ether and finally dried in a vacuum dessicator. The dried protein was weighed and dissolved in 2 ml hyamine and 10 ml permablend was added (Packard Instrument Co.). The samples were counted in the scintillation spectrometer without and with addition of a known internal standard. The results are expressed in disintegrations per minute (DPM) per mg of dry weight protein.

In the second experiment the uptake of ^{14}C-AIB was measured. The amount of the amino acid in the extracellular compartment of the muscle is calculated as the product of the volume of the extracellular compartment and the concentration of the amino acid in the incubation medium, assuming that, after 2 h incubation, equilibrium exists between the amino acids in the extracellular space and in the incubation medium. To each 10 ml of incubation medium were added 1.66 µC of C^{14}-AIB. After incubation the tissue was weighed and homogenized with 3.0 ml 10% TCA. An aliquot (1.0 ml) of the supernatant was taken and diluted with 10 ml of the scintillation fluid. The same procedure was applied to 1 ml of the incubation medium. The radioactivity was measured with and without an internal standard. The results are expressed as the distribution ratio between the radioactivity in the intracellular fluid and the radioactivity in the medium (DPM per µl intracellular fluid: DPM per µl incubation medium). The results obtained with ^{14}C-leucine in the first experiment are shown in Table I.

Table I. Effect of BGH, fraction 34 and 40, on the incorporation of ^{14}C-leucine into proteins of diaphragm of hypophysectomized rats.

Added to incubation fluid	No. of samples	DPM/mg of protein ± S.E.
No addition	8	180 ± 11
BGH	8	367 ± 16[a]
Fraction 34	8	250 ± 14[a]
Fraction 40	8	277 ± 16[a]

[a] Statistically significant difference ($p < 0.01$) as compared to the control.

Table II. Effect of BGH, fraction 34 and 40, on the distribution ratio of ^{14}C-AIB in the diaphragm of hypophysectomized rats

Added to incubation fluid	No. of samples	DPM/µl intracellular fluid: DPM/µl incubation medium
No addition	8	1.62 ± 0.11
BGH	8	3.48 ± 0.26[a]
Fraction 34	8	3.65 ± 0.09[a]
Fraction 40	8	8.92 ± 2.23[a]

[a] Statistically significant difference ($p < 0.01$) as compared to the control.

Both fractions obtained from calf muscle (34 and 40) increased significantly the incorporation of ^{14}C-leucine into the diaphragm proteins, although the stimulation was less obvious than in the group where BGH was added.

The results obtained in the second experiment with ^{14}C-AIB are shown in Table II. From the distribution ratios in this experiment it is seen that fractions 34 and 40 both significantly increased the concentration of the ^{14}C-AIB in the cells of the diaphragms, compared to its concentration in the incubation medium.

The present results, obtained in vitro, support the earlier demonstration in vivo of the growth-promoting action of factors from the extract of calf muscles. From the results with ^{14}C-AIB, the primary effect of these factors seems to be stimulation of the membrane-transport. Our experiments cannot decide whether the increased protein synthesis caused by fractions 34 and 40 is solely a consequence of a higher intracellular amino acid concentration or whether these factors act also on the process of protein synthesis. However, the effect on transport may be nonspecific because in earlier studies it was found that the factors from muscle increase the incorporation of ^{35}S-labelled sulphate into calf costal and chick embryo cartilage[4, 5]. It was also reported that in the presence of growth hormone muscle tissue releases more sulphation factor activity into the incubation fluid than the control[5], suggesting that sulphation activity in muscle is growth-hormone dependent[6].

If the growth-promoting factors from muscles are indeed dependent on growth hormone this would agree with KOSTYO's hypothesis that the growth hormone effect on protein synthesis and on amino acid transport is mediated by some factor which could be a polypeptide.

Zusammenfassung. Es wird nachgewiesen, dass sich in der Muskulatur ein Faktor befindet, der eine Anreicherung der α-Amino-isobuttersäure in den Zellen hervorruft und der auch in vitro den Einbau von Leucin und damit die Proteinsynthese fördern kann. Ob dieser Faktor als Übermittler für Wachstumshormon wirkt, ist noch nicht abgeklärt.

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