A substance isolated by ion-exchange chromatography could perhaps be contaminated with a substance which is unretarded in the chromatographic system, since a complex between the two substances might be formed. The ease of separation of most of the MSH activity from the corticotropin and the constant amount of residual activity[9] provided some evidence that this residual activity was intrinsic. The fact that MSH activity was lost on treatment with peroxide and regained on heating with a thiol, together with the ACTH activity[10], and together with a physico-chemical change (the retention volume on chromatography)[8,11], provided further evidence that the activity was a property of corticotropin itself, but this argument was weakened by the similar behaviour of the activity of purified melanophore-stimulating peptide[4].

The chromatographic separation of two substances from partially regenerated corticotropin A_1, one possessing both ACTH and MSH activities and one inactive in both tests, provides strong evidence that both activities are intrinsic to one molecule. Both peaks would probably be active if the activities were due to a contaminant which formed a complex stable under the conditions of chromatography. The simplest explanation of the results is that whenever a molecule of the faster-running material is converted into the slower-running it regains both activities, although more complex explanations can be proposed. Thus corticotropin appears to possess intrinsic MSH activity although it has less than 1 % of the potency of the melanophore-stimulating peptide.

I am most grateful to Professor F. W. LANDGREBE for the gift of the purified melanophore-stimulating peptide, and to him and to Drs. M. P. STACK-DUNNE, E. B. ASTWOOD, M. S. RABEN and I. N. ROSENBERG for permission to mention assays and unpublished results. I am also grateful to Professor F. G. YOUNG, F.R.S., for advice and encouragement, and to Mr. B. KETTERER for advice and discussion. I am indebted to Miss M. B. THOMAS for skilled technical assistance.

Department of Biochemistry, The University, Cambridge (England) H. B. F. DIXON

[1] M. S. RABEN, I. N. ROSENBERG AND E. B. ASTWOOD, *Federation Proc.*, 11 (1952) 126.
[2] B. J. BENFEY AND J. L. PURVIS, *J. Am. Chem. Soc.*, 77 (1955) 5167.
[3] A. B. LERNER AND T. H. LEE, *J. Am. Chem. Soc.*, 77 (1955) 1066.
[4] J. PORATH, P. ROOS, F. W. LANDGREBE AND G. M. MITCHELL, *Biochim. Biophys. Acta*, 17 (1955) 598.
[5] M. A. SAYERS, G. SAYERS AND L. A. WOODBURY, *Endocrinology*, 42 (1948) 379.
[6] R. W. PAYNE, M. S. RABEN AND E. B. ASTWOOD, *J. Biol. Chem.*, 187 (1950) 719.
[7] E. B. ASTWOOD, M. S. RABEN, R. W. PAYNE AND A. B. GRADY, *J. Am. Chem. Soc.*, 73 (1951) 2969.
[8] H. B. F. DIXON AND M. P. STACK-DUNNE, *Biochem. J.*, 61 (1955) 483.
[9] P. H. BELL, *J. Am. Chem. Soc.*, 76 (1954) 5565.
[10] M. L. DEDMAN, T. H. FARMER AND C. J. O. R. MORRIS, *Biochem. J.*, 59 (1955) xii.
[11] H. B. F. DIXON, *Biochim. Biophys. Acta*, 18 (1955) 599.
[12] F. W. LANDGREBE AND H. WARING, in *Hormone Assay*, edited by C. W. EMMENS, Academic Press, New York, 1950, p. 141.
[13] L. HOGBEN AND D. SLOME, *Proc. Roy. Soc. B.*, 108 (1931) 10.

Received November 19th, 1955

Bombixin, a sex attractant discharged by female moth, *Bombix mori*

Immediately after emergence both the male and the female of *Bombix mori* copulate. The female moth takes up a characteristic calling position as if she would discharge some kind of scent from her hip in order to attract the male insect.

The male, when coming near the female, seems to feel excitement, flapping the wings exquisitely and lifting her abdominal hip. From a distance of 10 cm the male gradually approaches the female vibrating his wings violently and from a distance of 5 cm he goes more or less straight to her. Throughout this approach the female lifts her hip, putting out a tongue-like body from the end of the abdomen and seems to discharge some gaseous substance. The male takes a copulating attitude so long as the female scent substance is in the air, even in the absence of the female moth. This sexual scent seems very specific and showed no relation to ordinary odiferous substances. We call this sex attractant "bombixin".

In order to obtain bombixin as pure as possible, we treated hips of sixty thousand female moths after copulation (we think it is better to use virgin moths) with alcohol in a mortar by the addition of a small amount of quartz sand. After standing overnight the mixture was filtered. The filtrate which amounted to 15 l was evaporated under the reduced pressure to 2.2 l and saponificated by the addition of 600 grams of solid caustic soda at a temperature not exceeding

40° C. The whole was evaporated in vacuum and the alcohol was removed as far as possible at the lower temperature. The residue, after dilution with water, was extracted with ether six times and the ether extract, after being dried with sodium sulphate, was evaporated to a small volume. Benzene was added to it, and a white precipitate settled out. The whole mixture was passed through a column of alumina (BL-6) in order to remove impurities and pigments. The effluent was evaporated at a temperature below 60° C to remove the solvent, giving 4.5 grams of an orange wax, which contained plenty of bombicesterol. 3 grams of the extract was dissolved in a small amount of methanol by warming slightly. Bombicesterol separated on standing in a refrigerator, was filtered and the mother liquor evaporated gradually in a desiccator in the dark, when a crop of bombicesterol crystals again separated out. After filtration, the mother liquor, protected from oxidation in air by the addition of a drop of D,L-α-tocopherol, was evaporated gradually in order to remove methanol. The crystals of bombicesterol from the second and further crystallization contained the adhering sex attractant and attracted the male moth strongly. These crystals were treated with methanol and the methanolic extracts were united to the mother liquor. This procedure was repeated in order to remove bombicesterol as much as possible, and when the quantity of mother liquor reached about 1 ml the whole was evaporated strongly under reduced pressure to remove as much methanol as possible. The resulting brown syrup was subjected to the molecular distillation. The portion which distilled between 100° C and 110° C under 0.06 mm Hg pressure was taken separately. It is a thick faintly yellowish oil and 0.0005 γ of it has clearly the ability to excite the male moth and cause him to dance in a circle; 0.00025 γ of it is further able to make him vibrate his wings. On a paper chromatograph developed with butanol/acetic acid/H_2O (ascending method), the R_F of bombixin was about 1.0, with 85% phenol, 0.98, and with abs. benzene/abs. methanol (6:4), 0.82. In this case it was very interesting to use a male moth as the detector, in the following way. After the paper strip was developed with abs. benzene/abs. methanol and dried, it was passed slowly in front of the male moth. When the spot of the sex attractant approached the male, the insect became excited and went round the spot shaking his wings violently (Fig. 1).

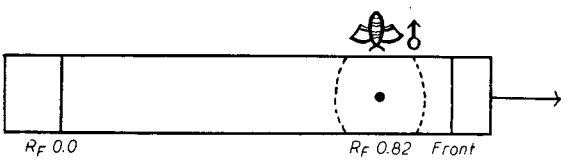

Fig. 1.

The elementary analysis showed that our bombixin had the following composition; C, 86.41%; H, 12.05%. In the spectral analysis this substance showed no remarkable specific absorption in the ultraviolet region. In the infrared spectral analysis there was some indication of the existence of an primary hydroxyl group. A portion of the sample was purified by chromatography in the absence of air and light and in a favourable case a sample active in $2 \sim 4 \cdot 10^{-5}$ γ was obtained. But the yield was very minute.

When our study had proceeded so far, we were informed by a friend that there had been a report on the same subject by A. BUTENANDT, who isolated in 1938 a male attracting substance from the female of *Bombix mori* by a method different from ours (*Angew. Chem.*, 54 (1941) 89), which was said to be physiologically active in 0.01 γ. But, as described above, our bombixin is far more active than BUTENANDT's substance.

This study was aided by a grant from the scientific research fund of the Ministry of Education of Japan. The authors wish to thank Dr. K. TAKAHASHI, the director of the Prefectural Sericultural Experiment Station, Kumamoto, for supplying the experimental materials used in this study.

KATASHI MAKINO
Department of Biochemistry, Jikei Medical School, Tokyo (Japan) KIYOO SATOH
Prefectural Sericultural Experiment Station, Kumamoto (Japan) KAORU INAGAMI

Received November 23rd, 1955

Activation of cytochrome c reductases by a lipid bound to crystalline bovine serum albumin*

It has already been shown that the marked decrease in activities of diphosphopyridine (DPN)- and succinate-cytochrome *c* reductases resulting from *iso*octane extraction can be completely reversed by the addition specifically of tocopherol[1]. The naturally occurring lipid material which is removed from the purified enzymes by *iso*octane extraction can also reactivate, although it contains no free tocopherol[1]. During a study of these systems it was observed that crystalline bovine serum albumin in relatively high concentrations could substitute for tocopherol in