style of work.") Despite this concern of the party leaders about China's scientific manpower resources, it would be difficult for Chinese scientists not to be affected by the recent violence.)

The current uproar in China has made congressmen and Executive branch officials somewhat wary about pushing for further changes in China policy. American officials also believe that this commotion has not helped the case for seating Peking at the United Nations. This appears to be one of the reasons why the United States still seems ready to advocate continued exclusion of Peking from the world body in debates and voting on seating this autumn. Whether the United States will drop its opposition to Peking's admission to the U.N. in the 1967 session remains to be seen. Earlier this year administration officials, including Rusk, began to emphasize keeping Nationalist China in the U.N. rather than keeping the Peking government out.

In the Executive branch there is some reluctance to move much farther on a change in China policy until Peking shows some signs of reciprocating. But China seems in little mood to respond, especially when the U.S. Seventh Fleet still patrols the Formosa Strait and when American military forces battle along China's southern boundary in Vietnam. In addition to the aggravation of Vietnam, the State Department has, in effect, admitted that American warplanes have violated Chinese airspace. Chinese leaders act as if they must prepare for an attack from the United States, and U.S. leaders will probably shy away from giving absolute assurance that no attack is forthcoming.

In a British House of Lords debate on China earlier this year, Lord Kennet argued that both America and China seemed to pay attention only to one part of the other's behavior. The Americans, he said, pay attention to China's militant words but ignore the fact that Chinese soldiers are not employed outside China. The Chinese ignore America's peaceful words but concentrate on the great American military effort in Vietnam.

For any reconciliation to take place, both sides will probably have to do more to bring together the seeming disparity between national words and deeds. As another observer, A. Doak Barnett, warned in his testimony before the Senate Foreign Relations Committee, "it is essential that the United States exercise great restraint in the use of its power, especially in North Vietnam, and demonstrate by deeds as well as words that we are determined to avoid provoking any direct American-Chinese conflict."

There may be those who would argue that Washington has done little to change its policy toward Peking. Although this may be technically true, this view does not take into account the significant change, toward conciliation, in the language American officials now use and, more importantly, in the attitudes which are the basis of that language. While the policy may not have changed markedly, the willingness to discuss the need for a more flexible China policy has increased sharply in Washington. Chinese officials who make some attempt to actually test American responses on different proposals probably will be shocked by the cordiality of the U.S. response. Washington is increasingly aware that attitudes toward China which were useful in 1950 are of limited relevance in today's world.

United States officials, however, have little hope that the Chinese will seek out greater contact with America in the next few months or even the next few years. In their opinion, America is still too useful to the Peking government in the role of villain to mobilize the energies of the Chinese people. Washington does not expect the old revolutionary cadre, composed of men like Mao and Defense Minister Lin Piao, to change attitudes about the United States. However, official Washington does hope for an eventual change in China similar to the relaxing of attitudes which occurred in the Soviet Union after Stalin's death. In the past few months the U.S. government has been trying to communicate its potential goodwill to the post-Mao generation of Chinese leaders. A main concern of Washington today is the possibility that a future dialogue with China will be permanently forestalled by a direct Chinese-American military clash originating in the Vietnam war.—BRYCE NELSON

REPORT FROM EUROPE

Conference on Insect Endocrines

Brno, Czechoslovakia. Recent findings about insect hormones, of interest for both fundamental biology and insect control, were discussed here from 22 to 26 August. The occasion was a symposium on insect endocrines, sponsored by the Entomological Institute of the Czechoslovak Academy of Sciences. About 130 scientists from 20 countries attended the meeting, at which about 80 papers were presented.

A central theme of the symposium was the importance of insect endocrine systems as models of the role of hormones in the differentiation of complex animals.

In the announcements and discussions at Brno, five main areas received particular attention.

1) It was announced that pure samples of juvenile hormone, which prevents maturation of insects, had been isolated in the United States from cecropia moths.

2) There was discussion of the juvenile-hormone-like effect of the so-called "paper factor," from balsam trees of North America, which affects only one group of insects, the Pyrrhocoridae—a group which does not occur in North America.

3) It was announced that the active principle of a terpenoid compound which had been synthesized at Harvard and found to be very active against a wide range of species had been identified in Czechoslovakia. Workers in Prague showed that the active material is a dihydrochloride of methyl farnesoate.

4) More data were presented in support of the idea that changes in the ion concentration in the nuclei of cells of the *Chironomus* midge can mimic the effect of the hormone ecdysone (which promotes moulting of insects

from one stage of development to the next) in inducing the chromosome "puffs" observed in the salivary glands of this insect.

5) There was extensive discussion of how and when hormones could interact with the insect's genome to promote or retard development.

The symposium was held at an important time for the field of insect physiology, which appears to be growing in general importance for biology. There is a growing general interest in the systems with which insect physiologists work, systems which may produce significant insights into genetic regulation in higher organisms.

Insects undergo differentiation in sharply delineated phases, or moults, in which massive reorganizations of cells are accompanied by distinct changes in appearance. In the development of insects from the egg, through larval and pupal phases, to adulthood, large blocks of the insect's original endowment of genetic information, embodied in deoxyribonucleic acid, appear to be opened up for expression —that is, derepressed—while other large blocks of information used in previous phases may be repressed.

Of the four insect hormones associated with differentiation, juvenile hormone was the focus of interest at Brno. Juvenile hormone can hinder the metamorphosis of insects from immature stages of development to adulthood. Indeed, the hormone can induce insects to moult into extra stages, or instars, of larval development. Discovered in the 1930's by V. B. (now Sir Vincent) Wigglesworth of the University of Cambridge, juvenile hormone is produced in the corpora allata, endocrine glands located near the insect's brain.

The action of juvenile hormone is opposite to that of ecdysone, which stimulates metamorphosis. The action of ecdysone was discovered in England in 1935 by Gottfried Fraenkel, now of the University of Illinois.

Ecdysone normally is produced in the insect's prothoracic glands, under the stimulus of the third main hormone associated with differentiation, the so-called brain hormone (whose production is influenced, in turn, by factors such as day length and temperature).

The fourth main hormone, bursicon, is produced in the same intercerebral region of the brain as the brain hormone. Bursicon, discovered by Fraenkel and C. B. Cottrell in 1962, has recently been found to have a molecular weight of about 40,000 (*Science*, 7 January 1966). Bursicon is responsible for the darkening and hardening, or "tanning," of the insect cuticle which occurs when the insect emerges from the pupal phase to adulthood.

Interest in juvenile hormone was high at Brno because of (i) the possibility that the hormone may affect the way in which the information in the genes is expressed, and (ii) the possibility that tailored synthetic juvenile hormones might be highly specific insecticides.

Such specificity is a main goal of current insecticide research, which follows such paths as the isolation of sex-attractant substances, the development of chemical sterilants, and the use of radiation to sterilize males which then are released to mate unproductively with females of their species.

These studies have been stimulated by uneasiness about broad-attack control of insect species with DDT and other chlorinated hydrocarbons, and about the speed with which many species develop resistance through selection.

Students of juvenile hormone, notably Carroll M. Williams of Harvard, maintain that it would be extremely difficult for insect species to develop resistance against substances nearly identical to their own developmental hormones. They are confident that substances of this type can be manufactured in large amounts, and that such substances in very low concentrations may be highly effective insecticidal agents, operating through primary contact and through contact of one insect with another.

There was skeptical questioning of this thesis at Brno, particularly from such experts in insect control as J. de Wilde of the Dutch agricultural research center at Wageningen. De Wilde noted that there are many ways in which resistance can arise, including deviant behavior. Williams and others acknowledged that much remains to be done in working out ways to apply such insecticides economically. Thus there was much interest at Brno in papers on the chemistry of juvenile hormone and its analogs.

P. Schmialek of the Free University of Berlin reviewed his studies of the compound farnesol and its derivatives. In 1960 Schmialek found that a juvenile-hormone-like activity in the excreta of the tenebrionid mealworm was due to farnesol and its aldehyde, farnesal.

The discovery of the juvenile-hormone-like activity of farnesol, which is an open-chain terpene alcohol composed of three isoprene units, was significant. Howard Schneiderman of Western Reserve University and Lawrence Gilbert of Northwestern University noted, in a review of work on insect hormones (*Science*, 24 January 1964), that farnesol "was the first substance of known structure to act like an insect hormone, and its isolation was a major achievement in comparative endocrinology."

Nonetheless, Schneiderman and Gilbert went on to say, "farnesol does not appear to be the juvenile hormone, for in most test systems pure farnesol is required in milligram or, at best, tenths of a milligram amounts, while purified preparations of the juvenile hormone of insects are active in millimicrogram amounts."

Schmialek's work opened up a major search for similar activity in hundreds of terpene compounds and also made even more urgent the isolation of a really pure juvenile hormone from cecropia (Williams made the first crude extract in 1956).

Success in this latter task was reported at Brno by a group from Madison, Wisconsin, where H. Röller, J. S. Bjerke, and D. W. Norgard have isolated about 90 micrograms of pure juvenile hormone from cecropia. Bjerke told the meeting that the substance was purified about 125,000 times. Physicochemical experiments to characterize the substance are to start this fall.

Bjerke described purification methods involving low-temperature precipitation followed by molecular distillation and modified gas-liquid chromatography. He also described a number of checks to insure that the material producing the characteristic peak on the final chromatogram was truly homogeneous.

The Madison group also has compared the chromatograms of the substance it purified from the abdomens of adult cecropia moths with other substances which have shown juvenile-hormone-like activity. Among the substances examined were some of the farnesol derivatives investigated by Schmialek and a highly active substance synthesized by Williams and

John H. Law of the University of Chicago. All these substances gave distinct peaks on the chromatogram.

Another stimulus to the search for terpenoid compounds with juvenile-hormone activity was the discovery in late 1964 of a "paper factor" which affected maturation of pyrrhocorid insects. The discovery was made by Karel Sláma (of the insect physiology department of the Entomological Institute in Prague) while he was working with Williams at Harvard. Some 1500 individuals of *Pyrrhocoris apterus* which Sláma had brought with him to Harvard had failed to mature, and many were pushing beyond the normal five stages of larval development to perfect sixth and seventh stages. Seeking the cause, Sláma and Williams traced it to the presence of paper toweling in the jars that contained the insects (in which Canadian balsam fir was a major constituent). They made an extract of the toweling and found that this "paper factor" affected only *Pyrrhocoris*.

But at what stage did it act? Sláma and Williams found that it had no effect during the first four stages of larval development. The action occurred in the first 3 days of the fifth stage—just the time at which normally developing insects stop manufacturing juvenile hormone, which they make throughout the first four stages. Later, Sláma and Williams found that the paper factor could also prevent the hatching of *Pyrrhocoris* eggs, while failing to affect the embryonic development of eggs of related *Rhodnius* and *Oncopeltus*. The paper factor had this effect whether it was placed directly on freshly deposited eggs or was put on freshly moulted adult females who later were fertilized [*Nature* **210**, 329 (1966)].

Williams and Sláma argued that this prevention of the metamorphosis of *Pyrrhocoris* eggs into larvae was analogous to the prevention of metamorphosis of the larvae into adulthood, and so was consistent with the idea that "nearly all the effects of juvenile hormone can be accounted for in terms of its ability to block the derepression, transcription, or utilization of fresh genetic information."

The paper factor also affects other Pyrrhocoridae. At Brno, K. N. Saxena of Delhi University described success in using the paper factor to prevent fifth-stage larvae of the red cotton bug, *Dysdercus koenigii* F., from emerging from their cuticles. In many of Saxena's experiments the dose was 4 micrograms of paper factor, applied directly to the larvae, but, even when the dose was cut to 0.05 or 0.01 microgram, only a few insects became normal adults. Others, although they emerged from the larval cuticles, failed to develop normal adult wings [*Nature* **210**, 441 (1966)]. At Brno, Saxena said that he had also tried a variety of extracts from *Cedrus deodara*, which showed activity, although they were not stable.

The red cotton bug is of considerable economic importance in India and North Africa, where it pierces the bolls, leaving behind a fungus which stains the fibers. Some of the inhibitory extracts have already been tried in the field in India as insecticides, Saxena reported.

Sláma then described work he has been doing with Miroslav Romaňuk of the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, and with František Sorm, head of the institute and president of the academy. The entomological and organic chemistry institutes have been collaborating not only in work on substances which mimic the effect of juvenile hormone but also in work on substances which are similar in structure to the moulting hormone, ecdysone, but which inhibit its action in insects.

The most notable outcome of this work so far is the identification of a dihydrochloride of methyl farnesoate as the active principle of a substance synthesized at Harvard by J. H. Law, Ching Yuan, and Williams [*Proc. Nat. Acad. Sci. U.S.* **55**, 576 (1966)]. The substance had been synthesized by reacting acid with ethanolic or methanolic hydrogen chloride gas. Williams had found that this substance was highly active in all species on which it was tested, ranging from the most primitive wingless Thysanura to the most advanced forms, such as flies and Hymenoptera [*Science* **152**, 677 (1966)]. This finding is in contrast to the specificity of the paper factor.

Meanwhile, in Prague, Sláma and his collaborators were synthesizing and testing various other farnesol derivatives. After the paper by Law, Yuan, and Williams had been published, however, the Prague workers also synthesized the substance reported by those authors and found that it was much more active than the derivatives they had been working with. They went on to identify the active principle.

The dihydrochloride of methyl farnesoate is about 100,000 times as active as farnesol and about 10,000 times as active as farnesyl methyl ether. Sláma reported that a dose as small as 0.001 microgram of the compound was sufficient to produce "adultoids" (imperfect adults) when it was applied directly to fifth-stage larvae of *Pyrrhocoris*. A dose of 0.01 microgram was enough to induce the insect to moult to a perfect sixth stage of development. Even 1 microgram was not toxic to the insect, Sláma reported.

Williams noted later that the active methyl farnesoate "proves to be something of a 'super-hormone' because the introduction of the two chlorine atoms makes it far more stable in the insect than any previously known material. This was a fine piece of work by Sláma and his collaborators; it opens the door to the preparation and use of this synthetic material for the control of insects of all types. Its use in protecting stored products is an immediate possibility, since it is non-toxic even for the insects themselves. It is merely a bogus endocrine signal which forces the eggs and the larvae into abnormal and non-viable development."

As mentioned earlier, the Brno meeting gave much attention to the ways in which hormones might interact with insect genomes to influence development. There were numerous papers bearing on this problem, and there was a special informal discussion on the role of juvenile hormone.

A number of participants, among them Sláma and H. Kroeger of the Federal Technical University of Zurich, argued strongly that the influence of the hormones was less direct than had been supposed. Sláma called attention to the need for more information about which types of cells are the targets for various hormones, and which regions in these cells are first affected by the hormone. He said there was not enough evidence to permit one to say whether hormones acted by affecting the cells' permeability, the mitochondria, the synthesis of enzymes or other proteins, or the synthesis of DNA, or whether, as Kroeger argued, they acted by loosening the bonds between the nucleic acids in the chromosomes and certain of the histones.

Williams, Sláma, and others spoke often of a "program" of differentiation which hormones normally influenced by promoting or delaying the "readout," rather than by altering the substance of the message contained in the program.

Lynn Riddiford of Harvard presented evidence bearing on this point. She had studied the effect of juvenile hormone on embryonic development in two ways: (i) by applying cecropia extract or hormone analogs to freshly laid eggs of *Hyalophora cecropia* and *Antheraea pernyi*; and (ii) by injecting females of these two species, either before or after mating, with the hormone or its analogs.

The two studies produced similar effects. The sensitivity of the freshly laid eggs was greatest during cleavage and progressively weaker afterward. Yet, even in those eggs which did not hatch, differentiation had proceeded far enough to produce recognizable first instar larvae, even though blastokinesis had been imperfect. In many of the eggs laid after females had been injected, development was arrested earlier, even before the beginning of blastokinesis.

The studies clearly indicated that application of juvenile hormone after blastokinesis affects neither the hatching of eggs nor subsequent larval development.

If the hormone was applied at the stage of germ-band formation, some larvae hatched, but their potential viability and growth were severely affected. Even so, larvae from most of the cecropia eggs which did hatch survived to the end of the fourth larval instar, and 30 to 50 percent pupated.

Depending on the dosage and the time of application, then, the hormone and its analogs prevented the hatching of the eggs or caused abnormal development of the larvae. But the effects of such applications were often delayed weeks or months, as though the hormone had affected only "programming" of differentiation.

Fotis Kafatos of Harvard reported studies of the function of the labial gland of *Antheraea pernyi*, which may represent another such "program" of development. In the caterpillar, the cells in the back part of the gland secrete and accumulate silk for the cocoon that will surround the pupa. When pupation occurs, these cells degenerate completely.

Complete degeneration does not occur in the case of cells in the forward part of the gland, which form the thick, rigid cuticle through which the silk fluid passes. At pupation these forward cells start to degenerate, but the process stops when all but a thin layer of cytoplasm has disappeared. The nuclei are intact. When the *Antheraea pernyi* pupa metamorphoses into an adult and prepares to emerge from the cocoon, these "naked" nuclei begin to synthesize new cytoplasm. The cells undergo a spectacular change into glandular cells which secrete a salt solution that dissolves the enzyme which makes an escape hatch in the cocoon. In all this cellular renovation, Kafatos reported, there is no new synthesis of DNA.

G. R. Wyatt of Yale (working at Mill Hill, England, during 1966–67) reported studies of synthesis of DNA, RNA, and protein in the wing epithelial cells of cecropia pupae induced to end their diapause through increase of temperature or injection of ecdysone. Wyatt chose the wing cells for study after preliminary work had shown that they responded more vigorously than other cells to injection of the pupa with ecdysone.

The first notable effect, Wyatt found, is synthesis of all types of RNA, including ribosomal RNA. This response first occurs 8 to 12 hours after injection of the hormone. The incorporation of tritiated uridine, indicating synthesis of new RNA, remains high for about 48 hours and then drops rapidly to a level typical of diapause. Nonetheless, the net content of RNA in the wing cells continues to rise, suggesting that much of the early-formed RNA is turning over, and that the RNA formed later is more stable.

"Measurement of uridine incorporation and of template activity . . . suggests," Wyatt said, "that there is first a selective synthesis of template RNA followed by [selective synthesis] of ribosomal RNA."

The rate of synthesis of protein and DNA rises some hours after the rise in rate of RNA synthesis. Hence, Wyatt noted, it appears that new RNA synthesis is much closer to the primary action of ecdysone than DNA synthesis is. "From work with this system," said Wyatt, "it is not possible to draw conclusions about the primary action of ecdyson, but we begin to get some picture of the subsequent sequence of syntheses."

Kroeger studied the puffing of salivary gland cells of the midges *Chironomus thummi* and *C. tentans* in an effort to throw light on the primary action of hormones like ecdysone. He is convinced, he said, that one of ecdysone's earliest effects is that of changing the permeability of the cell or the nuclear membrane so that the concentration of potassium ions can build up. Such a buildup may loosen the bonds between DNA and the surrounding histones, bonds which are differentially sensitive to changes in ion concentration. Thus the synthesis of messenger RNA, which would guide the synthesis of proteins specified by genes in the region of the puff, could cause the puff (although it is still not proved that all the RNA of the puffing regions is messenger RNA).

Kroeger said it is possible to mimic many of the effects of ecdysone by administering large amounts of potassium without ecdysone. He said that similar effects could be produced on other regions of the *Chironomus* chromosome by administering sodium and magnesium. All these effects occur only in the first puffs that appear after stimulation (there is a whole sequence of later puffs which, apparently, cannot occur in the absence of an intermediary phase of protein synthesis, as investigations by Ulrich Clever and Wolfgang Beermann at Tübingen have shown).

Experiments with isolated nuclei have demonstrated, Kroeger said, that these effects can occur in the absence of the cytoplasm and even in the absence of parts of the nucleus. Hence it appears that the cell can operate a "program" of development with only a few components of the nucleus.

Kroeger also discussed experiments in which, by changing the ion balance of a cell, it had been found possible to restore to the chromosomes the appearance characteristic of earlier stages of development. Kroeger was cautious about interpreting this finding, because of the evident irreversibility of differentiation. He was adding one more unanswered question to those already posed concerning the time, site, and precise mode of action of insect hormones.

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