in inhibiting mitosis when added to cultures within a period of approximately 100 min prior to mitosis (Figure 1). Before this time, incubation on caffeine for increasing time appeared to result in a delay of mitosis which was approximately a linear function of time. Cultures similarly incubated with theophylline at the same concentration (3 m*M*) showed an almost identical pattern of delay of mitosis (Figure 1).

When cultures were starved for a long time, some asynchrony appeared to develop. Usually it was possible to evaluate the time of mitosis if the smear was taken from the growing edge of an advancing lobe[15]. Cultures starved for a period of time longer than 180 min showed a delay of mitosis which increased for longer periods of starvation (Figure 1). Between about 100 min prior to mitosis and 180 min, a small but consistent acceleration of mitosis by starvation occurred.

Preliminary experiments have shown a marked increase in cyclic AMP levels in cultures starved for 6.5 h prior to the expected time of mitosis, which is accompanied by marked delays in mitosis. Other cultures at shorter times of starvation, in which mitosis is not inhibited, showed a small spike of cAMP (only double normal levels at its maximum) at approximately 75 min prior to mitosis. These data are consistent with a model in which the peak in cAMP is a determining step in mitosis.

Cultures which were dipped in salts medium appeared to gain water and when scraped off the papers appeared more slimy than control cultures. When such cultures were compared to those of controls not exposed to salts medium, in 5 different experiments the weight of the frozen starved plasmodia was 86.1 mg/mg protein, while that of controls was 67.9 mg/mg protein, a 27% increase in wet weight as a result of the starvation treatment. The effect was noticeable already after 15 min.

No consistent delay of mitosis was observed in several preliminary experiments using dibutyryl cyclic AMP in the medium. It is possible that endogenous levels of the cyclic nucleotide phosphodiesterase are high enough in G2 to prevent significant accumulation of cyclic AMP under these conditions. It was observed, however, that dibutyryl cyclic AMP at high levels, when added to cultures near mitosis, appeared to speed up the overall process of mitosis and reconstruction (Figure 2). The stimulation of the overall process appears to be a linear function of the log of the concentration of cAMP (Figure 2). In caffeine (10 m*M*), an even greater acceleration of reconstruction after mitosis was found than that shown by db-cAMP.

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Mode of action of Acorus calamus L. oil vapours on adult male sterility in red cotton bug

O. Koul, K. Tikku and B. P. Saxena

Regional Research Laboratory, Council of Scientific and Industrial Research, Jammu Tawi 180 001 (India), 1 March 1976

Summary. In normal freshly moulted insects Spermotozoa are present and pass into the vas deferens after 24 h. They show morphological changes when inside the female genital tract. Sterility caused by Acorus calamus oil vapours is due to agglutation, malformation and immobility of sperms, which makes the sperms unable to undergo morphological differentiation in the female tract.

Acours calamus oil vapours have been found to sterilize male house flies[1] (Musca domestica L.); females of Thermobia domestica (Packard)[2]; Dysdercus koenigii, F[3]; and females of five stored product beetles[4]. Unlike classical chemosterilants, Tepa and related compounds which have high mammalian-toxicity and phytotoxicity[5], the oil is a non-toxic compound. The investigations into the mode of male sterilization have been made by taking D. koenigii as the test insect.

Material and methods. The bugs were reared as described earlier[6]. Male and female adult insects were separated immediately after moulting into separate jars and provided with food and water. 0–4-hold males were exposed to oil vapours for 4 days by the method already described[3]. Random samples containing 10 insects were taken from the control and the thrice replicated experiments each day. For various studies, Aceto orcein stained squashes of testes, vas deferens and oviduct were prepared.

Results. In normal insects, the spermatogenesis was maximum in freshly moulted adult insects, where the meiotic figures were quite apparent. The number of sperms varied in different testes; but no individual lacked such structures. No sperm was found in the vas deferens in such individuals. In 24–48-h-old insects, the bundles of sperms were seen in the testes and the vas deferens. Numerous sperms were seen separating from each other (figure 1), with their bodies in complete swirling waves (figure 2). The sperms entered the vas deferens only 24 h after moulting and their maximum number was found in 72-h-old adults. Copulations were observed 24 h after moulting. The maximum number of sperms traced in the female tract was found 24 h after copulation. In the female tract, the sperms showed definite morphological changes, particularly in the head or nuclear region. This end becomes broad and flattened (figure 3) where the body shows normal swirling waves.

In treated insects, after 24 h of the treatment, the meiotic divisions were normal but the sperms appeared to be unable to move. In 48-h-old treated insects, the sperms in the testes and vas deferens were very elongated structures (figure 4). Most of them were adhered to each other. The head was either highly elongated (figure 5) or com-

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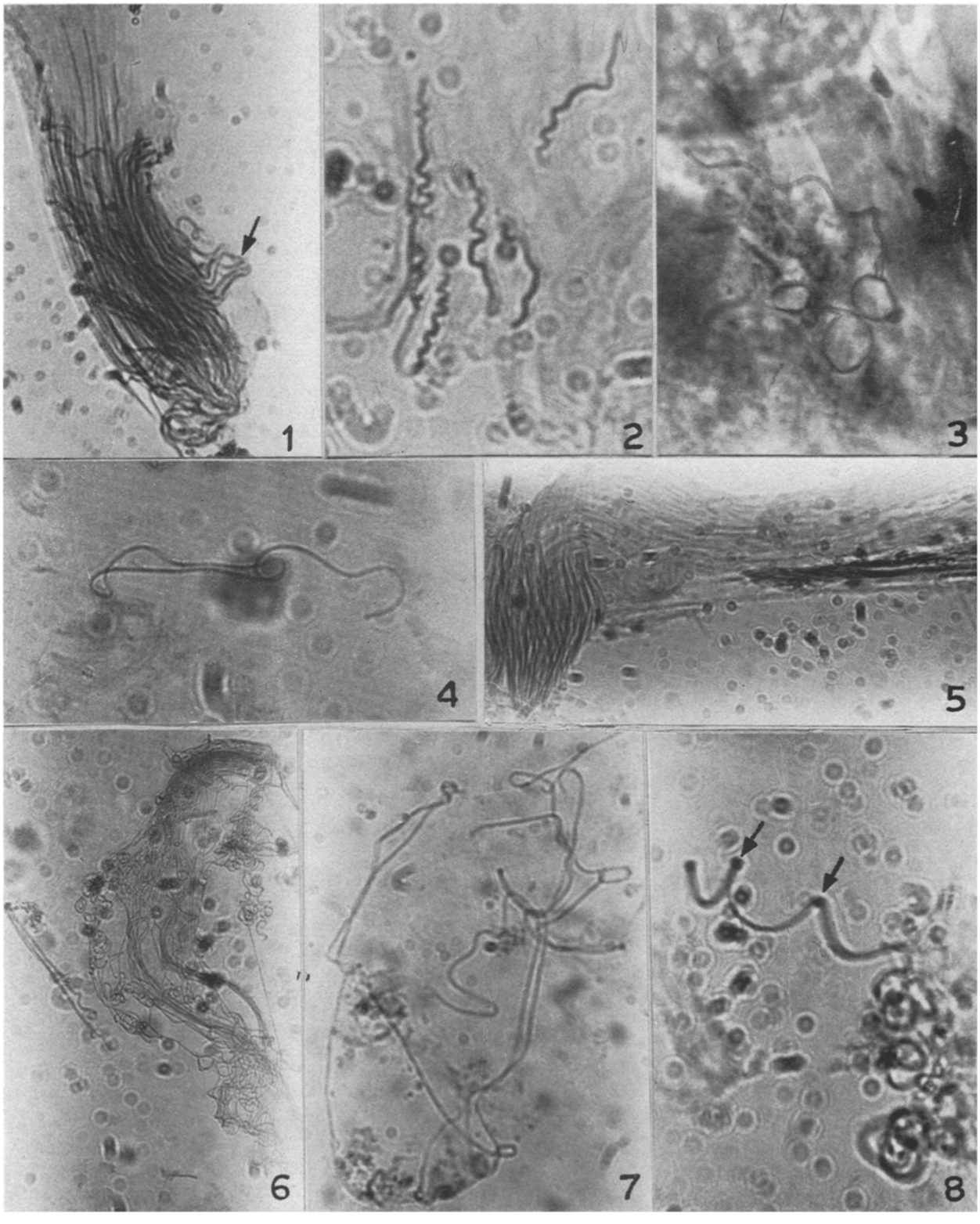

Fig. 1. Note the sperm separation (arrow) from a sperm bundle. × 500.
Fig. 2. The normal sperm movement in the testis. × 500.
Fig. 3. Sperms in the oviduct. Note the morphological differentiation and the movement. Phase × 500.
Fig. 4. Elongation of sperms in trated males. × 500.
Fig. 5. Head elongation and adherance of the sperms in treated males. × 500.
Fig. 6. Curled head condition in the sperms. × 500.
Fig. 7. Sperms from a treated male in the oviduct showing no morphological change. × 500.
Fig. 8. Same as in figure 7. Note the adherence of the head region. × 1250.

pletely curled up (figure 6). These features were very prominent in 72-h-old insects, where they were seen in irregularly jumbled groups in the vas deferens particularly. The spermatids in the testes were highly shrunken. The sperms of such treated insects, when traced in females, were fewer in number with no apparent morphological change vis-à-vis the control insects (figure 7). The nuclear parts of the sperms adhered to each other (figure 8) and the bodies had not a normal outline.

Discussion. Reviewing the effects of chemosterilants on the reproductive organs of insects, Campion has discussed sperm inactivation caused by nitrogen mustard, apholate, tepa and several sulphonic acid esters[5] but the phenomenon has been only related to the fertility or unfertility of females without assigning any inhibition in sperm morphology. However, the sperm immobility has been reported by several workers[7–9].

In the present study, the main cause of the male sterility is the agglutination, malformation and immobility of the sperms. Sperm bundles are present at the time of exposure and they pass into the vas deferens next day. From 24-h onwards the sperms show all above features and it appears that the inactivation is because of the imbalanced secretions of the vas deferens and to some extent of the testicular tissue[10]. Tremendous changes in sperm morphology have been observed in the female tract after copulation of normal insects. Thus in concurrence with the observation of Johnson et al.[11]. The sperms of the treated males, when traced in the female tract, did not show any morphological change and lacked motility, whereas in the case of classical chemosterilants the sperm does not lack the normal appearance, motility and ability to enter the ovum[12].

On the whole, it is difficult to say whether the transfered sperms are dead or living. Their inactivation may be due to some imbalance in the interplay of hormonal and testicular mechanisms. However, to bring about any concrete generalization, more investigations are needed[13].

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Carotenogenesis: Possible mechanism of action of trisporic acid in Blakeslea trispora[1]

Satya Rao and V. V. Modi

Department of Microbiology, Faculty of Science, M. S. University of Baroda, Baroda 390002 (India), 29 March 1976

Summary. Stimulatory effect of trisporic acid and β-ionone on carotenogenesis seems to be of a competitive nature in the minus strain of Blakeslea trispora, which suggests the same site of action. Trisporic acid may be derepressing the enzyme(s) involved in the conversion of 5-phosphomevalonate to dimethyl allyl pyrophosphate.

Trisporic acid (TA), a well-known sex hormone of the Mucorales, was first isolated from mated fermentations of Blakeslea trispora[2], which produced much higher yields of carotene than unmated fermentations[3]. This rise in carotene production was attributed to the formation of TA. TA regulates the sexual reproduction in heterothallic Mucorales[4] and conspicuously activates carotenogenesis in minus but not in the plus strain of B. trispora[5]. Goodwin et al.[6] have demonstrated that TA acts as a derepressor of an enzyme in carotene pathway which is normally rate limiting. But the exact site of TA action has not been established so far. Knowledge of the mechanism of TA action in carotene pathway is fundamental for the understanding of the regulation of carotenogenesis. In this report, some experiments aimed at the elucidation of the exact site of action of TA are described. β-Ionone is known to stimulate carotenogenesis. Reyes et al.[7] reported that the site of action of β-ionone lies in the biochemical pathway of terpenoid synthesis between the conversion of 5-phosphomevalonate to dimethyl allyl pyrophosphate. There is a significant structural similarity between TA and β-ionone as shown in figure 1. Therefore, it was worthwhile investigating whether β-ionone might compete with TA for the site of action[8]. Minus strain of B. trispora was chosen as the test organism because it does not produce TA but the carotene synthesis is stimulated by TA.

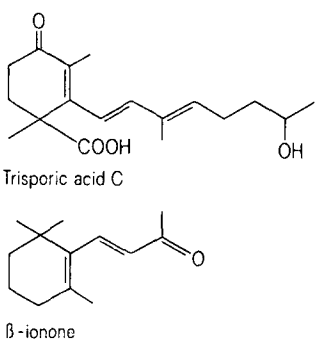

Fig. 1. Structures of trisporic acid C and β-ionone.

1 Acknowledgment. The investigation was supported by a PL-480/FG-IN-509 grant from the Agricultural Research Service of the United States Department of Agriculture.
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