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]

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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.

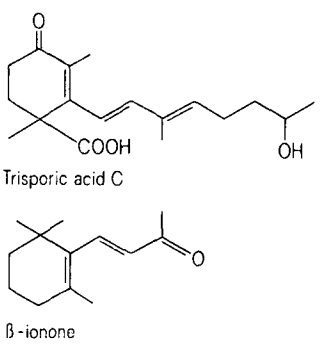

Fig. 1. Structures of trisporic acid C and β-ionone.

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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The (+) (NRRL 2895) and (−) (NRRL 2896) strains of B. trispora obtained from U.S. Dept. of Agriculture, Peoria, Illinois, were maintained as described by Anderson et al.[9] and grown on a Synthetic Mucor Medium (SMM)[10] containing 2% malt extract.

Carotenoids were estimated from the wet mycelia. Mycelia were homogenized in a Sorvall omni mixer and carotenes were obtained in acetone:diethyl ether (1:1) mixture. Acetone was removed by washing with cold distilled water and dried over anhydrous sodium sulphate. β-Carotene was estimated by using $E^{1\%}_{1\,cm}$ value of 2500 at 450 nm as described by Davies[11]. TA was extracted from the potato glucose thiamine medium of mated cultures according to the method described by Sutter[12]. The final TA extract was obtained in 0.1 M tris-sulphate buffer, pH 7.5, and estimated using $E^{1\%}_{1\,cm}$ value of 700 at 325 nm. A TA preparation from B. trispora contains mainly the C-13 alcohol trisporic acid C(80%) with the C-13 ketone, trisporic acid B (15%) and minute amount of the C-13 deoxy derivative trisporic acid A[13]. TA and β-ionone (filter sterilized) were added to 72-h-old minus cultures in 250 ml Erlenmeyer flasks containing 100 ml of SMM medium with 2% malt extract, which were further incubated for 48 h. Flasks were covered with black bags to avoid the degradation of TA due to light. Saturation curves of β-ionone and TA are shown in figure 2. Addition of 5.23×10^{-1} μM TA resulted in the maximum stimulation of carotene, whereas saturation level was reached with 4.92×10^{-1} mM β-ionone. As compared to β-ionone, TA gives a higher saturation level of β-carotene production.

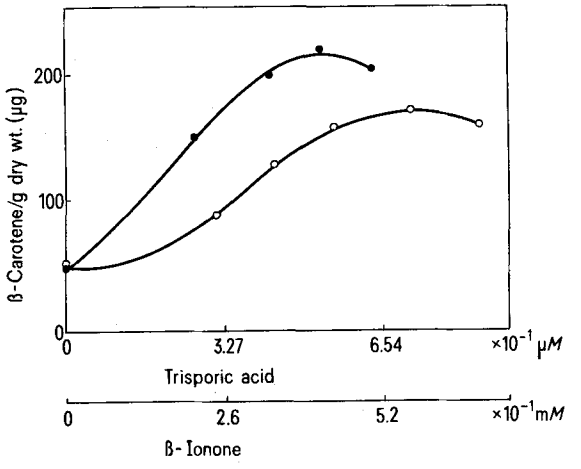

Fig. 2. Effect of trisporic acid and β-ionone on carotenogenesis in Blakeslea trispora (−). (●—●) Trisporic acid curve. (○—○) β-Ionone curve.

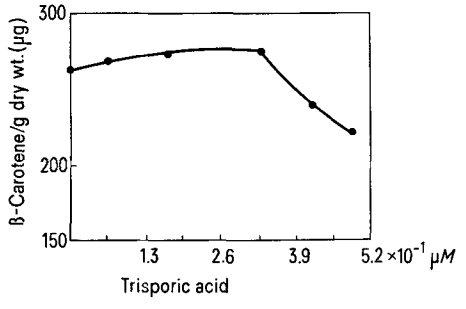

Fig. 3. Effect of trisporic acid on carotenogenesis in β-ionone supplemented Blakeslea trispora (−).

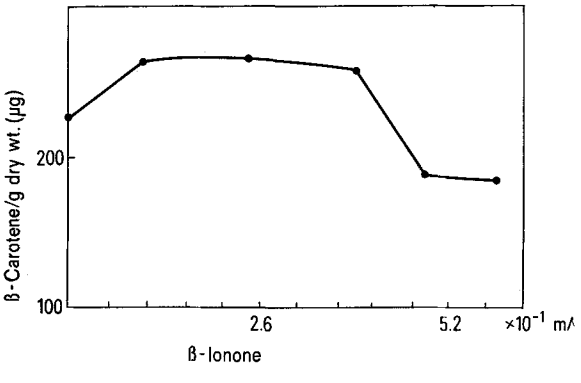

Fig. 4. Effect of β-ionone on carotenogenesis in trisporic acid supplemented Blakeslea trispora (−).

The effect of the addition of TA to the medium containing the minus cultures supplied with saturating concentrations of β-ionone was studied. Figure 3 shows that the stimulatory effect of TA was not detectable in the presence of saturating concentration of β-ionone, that is 4.92×10^{-1} mM. Similarly, in the presence of saturating concentration of TA, a stimulatory effect of β-ionone on carotene synthesis was not observed. Cultures were supplemented with 4.2×10^{-1} μM TA, which is slightly lower than the saturating concentration. There was a slight rise in the level of carotene on the addition of 1.0×10^{-1} mM β-ionone. Further addition did not make any difference. The results show that the presence of TA/β-ionone interferred with the stimulatory effect of the other stimulator β-ionone/TA. The competitive nature of TA and β-ionone mediated stimulations suggests that they may be acting at the same site in the carotene biosynthetic pathway.

To verify this assumption, experiments were carried out to see the effect of different combinations of β-ionone and TA (where concentrations of both the stimulators were lower than the saturation level) on minus cultures. Results (table) show the stimulation of carotene production of the addition of different combinations of TA and β-ionone, as well as the stimulators alone. In the case of combination (1.05×10^{-1} μM TA + 3.92×10^{-1} mM β-ionone), stimulation of carotene production was 8.14 units. If it had been the case of additive effect, stimulation should have been 13.2 units (combined values of stimulatory effect on addition of both stimulators singly). Another combination (2.61×10^{-1} μM TA + 2.45×10^{-1} mM β-ionone) also showed a similar pattern. If β-ionone and TA were acting at two different sites in carotene biosynthetic pathway, presence of one stimulator should not interfer with the stimulatory activity of the other stimulator. But, if they are acting at the same site, as happens in this case, presence of one stimulator would inhibit the other stimulator depending upon the concentrations, as they compete for the same site. The effect of these combinations of TA and β-ionone shows that the stimulatory

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Effect of different combinations of β-ionone and TA on carotenogenesis in B. trispora (–)

Addition		Carotene/ g dry wt. (μg)	Increase compared to control (units)*	
β-ionone (mM)				
2.45×10^{-1}		87.50	3.85	
2.95×10^{-1}		110.00	6.10	
3.92×10^{-1}		145.00	9.6	
TA (μM)				
1.05×10^{-1}		85.00	3.6	
2.09×10^{-1}		125.00	7.6	
2.61×10^{-1}		147.5	9.85	
TA (μM)	+ β-ionone (mM)			Probable value if additive effective
2.61×10^{-1}	2.45×10^{-1}	151.52	10.25	13.7
2.09×10^{-1}	2.95×10^{-1}	217.11	16.81	13.7
1.05×10^{-1}	3.92×10^{-1}	130.41	8.14	13.2

* A unit is defined as an increase of 10 μg of β-carotene per g dry wt.

activity of one of the stimulators was inhibited by the other stimulator which confirmed the competitive nature of β-ionone and TA mediated stimulations. Only in the case of one combination (2.09×10^{-1} μM TA + 2.95×10^{-1} mM β-ionone), stimulators did not inhibit each other's activity. A possible explanation could be that the concentrations of β-ionone and TA were so low that both together made saturation level.

Similarity between β-ionone and TA mediated stimulation of carotenogenesis is that there is an increase in the production of sterol as well as carotenoids in minus and mated cultures indicating that biosynthesis of intermediates of isoprenoid pathway is stimulated[14]. In each case, this effect is inhibited by cycloheximide[6,8]. As the site of β-ionone action is known, TA might be derepressing enzyme or enzymes involved in conversion of 5-phosphomevalonate to dimethyl allyl pyrophosphate. These steps result in the formation of isoprene unit, the building block of sterols and carotenoids.

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Effect of the growth-promoting alpha-globulin (GPAG) on in vitro incorporation of exogenous DNA into mammalian cells

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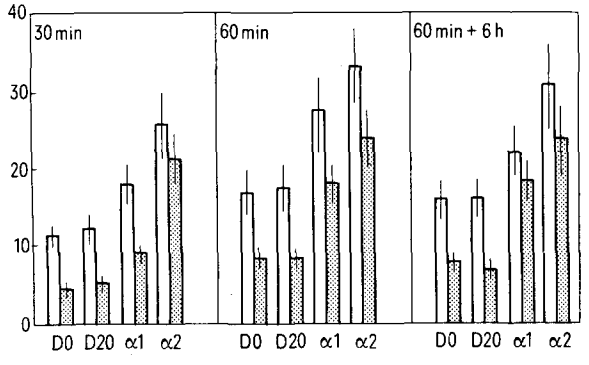
Summary. The uptake of exogenous ^{3}H-DNA by mammalian cells is increased in the presence of a specific serum protein complex – growth-promoting alpha-globulin (GPAG). ^{3}H-DNA is retained in the cell nucleus in a quantity 3 times higher than for control cultures without GPAG even after 47 h of additional cultivation in the medium without ^{3}H-DNA.

The great interest in the incorporation of exogenous DNA into mammalian cells and their nuclei[1] is stimulated by the potential possibility of DNA utilization for introduction of new genetic determinants into the cells. One of the problems is to find proper agents which enhance the incorporation of exogenous DNA and protect it from digestion in the cells. Incorporation of DNA into host cells is stimulated by polyornithine, polyarginine, polylysine, spermine, DEAE-dextran, latex particles, $CaCl_2$ and amphotericin B[2–4]. However, many of these substances may damage the cells as they are not natural components of the cell environment in the organism.

The present study was carried out on a specific serum protein complex – the growth-promoting alpha-globulin (GPAG) – which has been demonstrated to enter the cells by pinocytosis; GPAG has simultaneously a strong binding capacity for some precursors of cell macromolecules. During a co-incubation of GPAG and ^{3}H-thymidine, ^{3}H-uridine, ^{3}H-lysine or $^{32}PO_4'''$, gradual binding of the precursor to protein takes place; subsequently the complex of the 2 components is taken up from the medium by cultivated cells. High pinocytic activity is specifically connected with GPAG and the substitution of serum albumin for GPAG results in a sharply reduced formation of pinosomes[5]. We have therefore studied, in analogous experiments, incorporation of exogenous DNA pre-incubated with GPAG into the cells.



Average number of grains over L-cells (empty column) or their nuclei (solid column) after incorporation of exogenous ^{3}H-DNA or ^{3}H-DNA+GPAG in dependence on incubation period of exogenous ^{3}H-DNA with cells (30 or 60 min) and postincubation without ^{3}H-DNA (60 min incubation + 6 h postincubation).
DO, ^{3}H-DNA not preincubated (control); D 20, ^{3}H-DNA preincubated for 20 h at 37 °C; α_1, ^{3}H-DNA+GPAG (calf); α_2, ^{3}H-DNA+GPAG (sheep). Standard errors of the mean are given for each value.

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