

A similar study was made of mated and virgin females. Extracts were made of the spermathecal region which also contained the seminal receptacle, accessory gland, uterus, rectum, associated adipose tissue, and external genitalia. It was found that the male lipid was definitely present in the spermathecal region of mated females but absent from the same region of virgin females. Extracts of the remaining parts of both mated and virgin females did show a faint streak between positions 6 and 8 of Fig. 1.

There is a mutation in *Drosophila* which causes karyotypic females to develop into phenotypic males. For example, karyotypic females homozygous for *transformer* (*tra*) (9) develop into normal-looking, sterile, adult males. The gonads of such an individual are abnormal, but accessory glands and an ejaculatory bulb are present. A TLC analysis of *tra* "females" indicated that they are typically masculine with respect to the male lipid. Since the karyotype of the *tra* "female" used was XXY, it was thought that perhaps the Y chromosome mediated the synthesis of the male lipid. However, a TLC analysis of normal males lacking a Y chromosome (XO) showed the male lipid to be present. Alternatively, normal females with an XXY karyotype did not have the male lipid. It appears, then, that the formation of the male lipid is not regulated by genes on the Y chromosome.

The function of the lipid is unknown. The fact that it is present in the male genital tract and appears to be transmitted to the female suggests that the compound may serve a reproductive function.

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## New Antheridiogen from the Fern *Onoclea sensibilis*

**Abstract.** *An antheridium-inducing hormone (antheridiogen) derived from the fern species Onoclea sensibilis (Polypodiaceae) is chromatographically distinct from the native antheridiogen of Pteridium aquilinum (Polypodiaceae). It also differs from the native antheridiogens of Lygodium japonicum and Anemia phyllitidis (Schizaeaceae).*

Prothalli of *Pteridium aquilinum* elaborate a hormone [antheridiogen (1)] which induces antheridia in many if not all species of the fern family Polypodiaceae (2, 4, 9). The isolated hormone is active at a concentration of less than one part in ten billion, and it contains a carboxylic group that is necessary for activity (1). The antheridiogen of *P. aquilinum* failed to induce male sex organs in *Anemia phyllitidis* and *Lygodium japonicum* (Schizaeaceae) (3).

The prothalli of *Anemia phyllitidis* also elaborate an antheridiogen that is highly active toward the prothalli of this species but fails to induce male sex organs in *Onoclea sensibilis*, the species used to assay for the hormone elaborated by *P. aquilinum*. This, and other results, led to the conclusion that the antheridiogens of *A. phyllitidis* and *P. aquilinum* are different molecular entities (3). In turn, the prothalli of *Lygodium japonicum* elaborate an antheridiogen which differs both from the antheridiogens of *A. phyllitidis* (4), and of *P. aquilinum* (4, 5). More recently, Schraudolf (6, 7) demonstrated that known gibberellins are capable of inducing antheridia in tested species of the fern family Schizaeaceae. Like the native *Anemia* antheridiogen (3, 5), these phytohormones fail to hasten antheridium formation in other fern families (6, 8, 9). The native antheridiogen of *A. phyllitidis* does not seem to be identical with any of the several tested gibberellins (9).

In undisturbed cultures of *O. sensibilis* an antheridiogen could be detected at most in traces. However, clear-cut activity was detected after the prothallial extract or the medium from 16-day-old cultures was heated. Low pH increased the yield. It could not yet be decided conclusively whether heating converts an inactive precursor to an antheridiogen or whether it destroys a heat-labile inhibitor of antheridiogen action (10). An attempt was made to learn whether this antheridiogen differs from those demonstrated earlier.

The methods used in spore inoculation and in the culture of the prothalli

have been described (11). Descending chromatography on filter paper (Whatman No. 1) with an isopropanol, water, and ammonia system (8:1:1) was performed. Chromatography was discontinued when the solvent front was 20 cm from the origin. After drying, the chromatogram was cut into ten sections, each section being eluted with water. Antheridium-inducing activity of the eluates was detected by bioassay (11). To obtain active antheridiogen, agar-solidified medium from 16-day-old cultures of *O. sensibilis* was harvested and frozen. After thawing, the liquid was filtered off and its pH adjusted to 2.0. The liquid was then boiled for 10 minutes; upon cooling the pH was readjusted to that of the fresh culture medium (5:8).

This activated preparation induced antheridia in *O. sensibilis* to a dilution of 1/100 as observed 12 days after spore inoculation. The active preparation was extracted twice with half the volume of ethanolate for chromatography. The combined extracts were evaporated in a vacuum, and the residue was dissolved in an amount of water 1/200 that in the original preparation. Fifty microliters of this concentrate was spotted. Of the *Pteridium* antheridiogen 25  $\mu$ l of an ethanol extract was spotted which induced antheridia in *O. sensibilis* to a dilution of about 1/60,000. The antheridiogens of *O. sensibilis* and *P. aquilinum*

Table 1. Antheridium-inducing activity of *Onoclea* antheridiogen in *Onoclea sensibilis*, *Anemia phyllitidis*, and *Lygodium japonicum*. Values are averages of antheridium-bearing individuals in two samples of 30 prothalli 12 days (*O. sensibilis*) or 20 days (*A. phyllitidis*, *L. japonicum*) after spore inoculation.

Dilution	Activity toward		
	<i>O. sensibilis</i>	<i>A. phyllitidis</i>	<i>L. japonicum</i>
1/10	25.5	0.0	0.0
1/30	19.0	0.0	0.0
1/100	4.5	0.0	0.0
1/300	0.0	0.0	0.0
Co*	0.0	0.0	0.0

\* Control, with no *Onoclea* antheridiogen.

were spotted on the same filter strip in two experiments and on separate ones in three others.

The relative flow rate of *Onoclea* antheridiogen was between 0.8 and 0.9 (much closer to the latter value), and that of *Pteridium* antheridiogen was approximately 0.2. The results were the same whether the two antheridiogens were spotted on the same filter strip or on different ones. Clearly, the antheridiogens derived from *O. sensibilis* and *P. aquilinum* are not identical. In another experiment, *Onoclea* antheridiogen was tested for antheridium-inducing activity in *Anemia phyllitidis*, *Lygodium japonicum*, and *Onoclea sensibilis* (Table 1). The *Onoclea* preparation was active to a dilution of 1/100 in *O. sensibilis* but inactive even at the highest concentration in *A. phyllitidis* and *L. japonicum*. The antheridiogen of *Onoclea sensibilis* differs not only from the native antheridiogen of *P. aquilinum* but also from those of *A. phyllitidis* and of *L. japonicum*. In addition the relative flow rate of the *Onoclea* antheridiogen reported here

differs from those reported for *Anemia* and *Lygodium* antheridiogens with the same solvent system (5).

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## Alcohol and Recall: State-Dependent Effects in Man

**Abstract.** Male volunteers performed four memory tasks either while sober or under effects of alcohol. Twenty-four hours later they were tested under the same or different conditions. In tasks measuring recall and interference, learning transfer was better when the subject was intoxicated during both sessions than when he was intoxicated only during the learning session. In a task measuring recognition, transfer was not significantly affected by changing state. Thus, alcohol appears to produce "dissociated" or state-dependent effects in man, but not all forms of memory are equally sensitive to the phenomenon.

Animals trained in a drugged state may "remember" their training better if tested in a comparable drugged state than in a nondrugged state (1). Similarly, learning acquired in a nondrugged state transfers better to the same state than to a drugged state. This "dissociation of learning" has been demonstrated primarily with anesthetic agents (2). Given in sufficient quantities these drugs impair performance, and it could be expected that animals would manifest learned responses better in a nondrugged state than when drugged. The observation, however, that performance may actually improve in a drugged state, provided that original learning was in the same state, cannot be attributed to the drug's depressant effect on acquisition, retention, or performance. Thus, to some extent, learning is

apparently state-dependent, that is, it depends for optimum expression on restoration of the original condition in which learning was acquired.

That alcohol produces dissociation has been demonstrated in animals (3) and in man (4). In studying this phenomenon in man we used a higher dosage of alcohol than was previously used and a wider range of learning tasks to determine whether interaction effects are more evident in some tasks than in others.

Forty-eight male medical students, paid to participate in a training session (day 1) and a testing session (day 2) separated by 24 hours, were randomly assigned to four groups of 12 subjects each (Table 1). One group (SS) was sober both days. A second group (AA) was intoxicated

both days. A third group (AS) was intoxicated on day 1 and sober on day 2. The fourth group (SA) was sober on day 1 and intoxicated on day 2. Intoxicated subjects, depending on body weight, consumed between 8 and 10 ounces (250 and 300 ml) of 80-proof vodka, diluted in a soft drink, over 1 hour, after which testing began. Concentrations of alcohol in blood, as determined by breath analyses (5), varied from 80 to 140 mg/100 ml, with a mean of 111 mg/100 ml. All subjects drinking this amount showed signs of intoxication. Equivalent amounts of the soft drink were given to non-drinkers. Subjects knew in advance that they might receive alcohol, but had no other knowledge of the experiment.

Tests were administered in the same order to all subjects over a 40-minute period. They included an avoidance task to measure interference and latency of response, a verbal rote-learning task to measure recall, a word-association test to measure recall of "self-generated" learning, and a picture task to measure recognition. A motor task with a pursuit rotor also was used, but proved so easy to master, regardless of state, that the resultant data were unusable.

In the avoidance task, four patterns of lights were randomly presented. Each pattern could be extinguished by a specific switch that could be controlled by hands or feet. An incorrect response or failure to respond resulted in presentation of a noxious tone. Criterion was 20 correct responses, with number of errors to reach criterion taken as the measure of performance. The task was identical on both days, except that on day 2 the pattern-switch relation was altered. Thus, performance of day 2 was assumed to reflect interference; that is, the greater the number of errors on day 2, the greater the degree of interference (6). Latency of response also was recorded.

The rote-learning task involved memorizing four five-word "sentences" of varying meaningfulness (normal sentence, anomalous sentence, anagram, and word list) (7). On day 2 subjects were asked to recall the sentences memorized on day 1, after which a relearning session was conducted. Performance was measured in terms of errors of sequence and omission.

For the word-association test, ten words of low association value (8) were presented. Subjects were instructed to respond to the stimulus words with