

It should be noted that some people have suggested to me that the two forms of young in the two broods were both hybrids, one sort intermediate and the other exactly resembling one parent. A. P. Gray assures me that it is very unlikely that hybrids would exactly resemble one parent, and knows of no such cases. Silver<sup>1</sup> states that aviary-bred male and female hybrids are different, although both are intermediate in their characters. Both the recaptured hybrids in my case were male-type in plumage after the moult, as are the tree sparrows by definition. But I think it is much more reasonable to assume from my data that polyptaternity occurred rather than that the hybrids are polymorphic, one morph exactly resembling one of the parental species.

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## Multiple Sex Pheromones of the Mealworm Beetle, *Tenebrio molitor* L.

AMONG insects known to use a sex pheromone, it is most common for one sex to respond to the chemical signal produced by the opposite sex<sup>1</sup>. Yet in some species, apparently both sexes respond to the pheromone<sup>2,3</sup>, and recently it has been shown, in a few cases at least, that the two sexes may produce distinct pheromones<sup>4,5</sup>. *Tenebrio molitor* has previously been regarded as a classic case: females excite and attract males by means of a sex pheromone<sup>6,7</sup>. There are several pheromones which mediate the reproductive behaviour and physiology of *Tenebrio*, and we have found that the males as well as the females produce sex pheromones. Furthermore, the male pheromones are of two distinct types: (1) an excitant which attracts females and (2) an anti-aphrodisiac which inhibits the response of other males to female scent.

To demonstrate that both sexes produce attractants, groups of ten beetles of the same sex were confined in a small chamber. While unscented air passed through the chamber, the beetles aggregated near the air-outflow. When the airstream was laden with an attractive scent, the beetles rapidly moved to the air-inflow<sup>8</sup>. As Table 1 shows, each sex produces an attractant, and only the opposite sex responds.

When similarly bioassayed, homogenates of either sex yielded analogous results; for example, extracts of males

Table 1. RESPONSE OF MALE AND FEMALE *Tenebrio* TO SCENTS OF LIVE BEETLES

Scent source	Males responding* (per cent)	Females responding* (per cent)
Live male	5	62
Live female	60	9
No scent	6	8

\* Each percentage represents pooled results of at least twenty replicates. All beetles were virgin and more than 3 weeks old.

were fifty times more potent towards the females than were extracts of their own sex<sup>9</sup>. But the responses to a mixture of male and female extracts were surprising. When tested on females, the mixture was fully as attractive as its male component, but when tested on males, the mixture was less than one-tenth as potent as the pure female extract. The male component of the mixture somehow seemed to mask the effectiveness of the female component.

Experiments with live beetles showed that males emit an anti-aphrodisiac. One male and one female were used as simultaneous scent sources for the bioassay. The two sexes were inserted into the airstream in three sequences: in parallel to each other; in series with the male upwind from the female; and in series with the female upwind from the male. Each female was tested alone on four chambers of males and then she was tested in combination with the male on four more chambers. (A single female could be used as a source of scent for twenty consecutive tests with no loss of effectiveness.) Only when the female was upwind did the addition of male scent affect the results (Table 2). In such cases, the male in the influent was exposed to female scent; often he was observed to be extruding his genital segments. The male emitted the inhibitory pheromone only after stimulation by female scent. The longer his exposure to female scent, the greater was the inhibitory effect of any given male. In the first, second, third and fourth tests with the female upwind, the male responses (pooled) were 62, 57, 42 and 40 per cent respectively.

Table 2. RESPONSES OF MALE *Tenebrio* TO THE SCENT OF A LIVE FEMALE AND TO THE COMBINED SCENTS OF A LIVE FEMALE AND A LIVE MALE

Sequence	Female alone* (per cent)	Female + male* (per cent)
In parallel	67 (not significant)	73.5
In series		
Male upwind	70 (not significant)	71
Female upwind	59 ( $P < 0.001$ , $\chi^2 = 19.0$ )	45.2

\* Pooled results from at least five replicates.

A final series of experiments indicated that the male transfers some of his anti-aphrodisiac to the female during mating. Live virgin females were tested for attractiveness in the bioassay, then allowed to mate, and were immediately re-tested. Before mating, 60 per cent of the males responded; after mating, only 47 per cent responded, and this difference is significant by the  $\chi^2$  test.

The adaptive significance of the attractant produced by males of *Tenebrio molitor* is obvious: it brings the sexes together for mating. This pheromone may well have additional significance after mating. Females which rushed to the inflow in response to male scent often extruded their ovipositors, suggesting that male scent promotes rapid oviposition. *Tenebrio* females mate many times; as many as six spermatophores may be transferred to a single female in a few hours. Such repeated matings have been reported in other tenebrionids. In one species, *Tribolium castaneum*, Schlager has used a genetic marker (black body colour) to compare the relative utilization of sperm from successive matings. In terms of their tendency to fertilize eggs, sperm transferred in later copulations took precedence over sperm previously sequestered in the spermatheca<sup>10</sup>. A similar situation probably holds for *Tenebrio*. If so, then the excitant which releases oviposition behaviour and the anti-aphrodisiac play complementary parts; both increase the likelihood that a mated female will utilize the freshly transferred sperm before another male chances on her.

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### Lethal Effect of Feeding Rats on Galactose-Ethanol

GALACTOSE is a physiological substance which is usually well tolerated provided its breakdown is normal. In patients with galactosaemia, who lack the enzyme galactose-1-phosphate uridyl transferase, galactose is toxic, causing cataract and brain and liver damage, presumably because of the accumulation of galactose-1-phosphate<sup>1</sup>. The exact mechanism involved is not known, however, and extensive studies have been hampered by the lack of an experimental model.

The elimination of galactose can be depressed experimentally by ethanol, which inhibits uridine diphosphate galactose 4-epimerase by increasing the ratio of NADH<sub>2</sub> to NAD<sup>2</sup>. The transferase reaction which precedes the epimerase step is reversible<sup>3</sup> and the phosphorylation of galactose is irreversible<sup>4</sup>, so that inhibition of the epimerase by ethanol may have the same consequences as the lack of transferase activity. We have investigated this question by giving rats a diet rich in galactose and ethanol.

Albino rats of identical breed, weighing about 100 g, were kept in separate cages and received nothing but a liquid diet containing 1 calorie/ml. given freely. The body weight and the quantity of diet consumed were recorded daily. Four types of liquid diet (Table 1) were used and each was given to five male and five female rats. Vitamins were added to all the diets. Animals surviving 29 days on the diet were decapitated. Liver, brain and eyes were removed for histological examination in all animals as soon after death as possible.

Table 1 shows that animals receiving ethanol or galactose lost weight, but those on the combined ethanol-galactose diet lost far more weight. After a few days on the ethanol-galactose diet they looked sick and apathetic, and they all died between the tenth and the twenty-second day. Histological examination of liver and brain revealed no marked differences between groups. The eyes from all animals receiving galactose had cataract; the changes were most pronounced in the ethanol-galactose group.

The results indicate a toxic effect of combined ethanol-galactose feeding in rats. Ethanol given in equal amounts

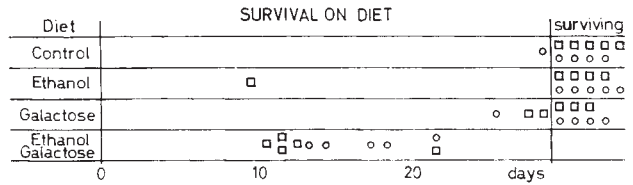


Fig. 1. The effect of liquid diets on the survival of rats. For composition of diets see Table 1. □, Male rat; ○, female rat.

to rats<sup>5</sup> has produced fatty infiltration in the liver during 24 days. Rats receiving a diet containing 81 per cent galactose died in a week<sup>6</sup>, and in experiments with a 50 per cent galactose diet slight degenerative changes were seen in the liver after 23 days, small necroses after 50 days, and after 108 days degenerative, sudanophilic material was present in the white matter of the cerebellum<sup>7</sup>.

The rather acute lethal effect of combined ethanol-galactose feeding cannot be explained by the addition of an ethanol effect and a galactose effect. One of the substances can be assumed to modify the metabolism of the other in a harmful way. The established inhibitory effect of ethanol on galactose metabolism is the most probable clue to the problem. If that is so, ethanol-galactose feeding may provide a much needed experimental model of the toxic effects of galactose in patients with galactosaemia.

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### Australia Antigen detected in the Nuclei of Liver Cells of Patients with Viral Hepatitis by the Fluorescent Antibody Technique

OUR suspicion that the Australia antigen associated with acute and chronic hepatitis is a virus and is the cause of the disease has been supported by examination of material from affected patients. Au(1) was first detected in the serum of an Australian aborigine<sup>1</sup>, and the geographic distribution, disease association, genetics and physical and chemical characteristics of this unusual antigen have been described<sup>2-4</sup>. One of the most startling findings in

Table 1. EFFECT OF LIQUID DIETS ON BODY WEIGHT AND INTAKE OF CALORIES (MEAN AND S.E.M.) OF MALE (M) AND FEMALE (F) RATS

Casein	Diet Percentage of calories			Sex	Body weight at start (g)	Average change in weight (g/24 h)	Average intake of calories (cal/24 h)	Body weight at end (g)	
	Fat	Sucrose	Ethanol						
14	26	60	0	0	M	106 (±1.0)	0.59 (±0.23)	48 (±3.0)	118 (±6.5)
					F	98 (±2.5)	0.00 (±0.13)	39 (±1.0)	98 (±4.2)
14	26	30	30	0	M	107 (±2.6)	-0.49 (±0.34)	36 (±1.8)	99 (±5.3)
					F	103 (±2.6)	-0.16 (±0.05)	33 (±1.0)	93 (±4.1)
14	26	30	0	30	M	104 (±2.5)	-0.26 (±0.20)	51 (±2.2)	96 (±4.4)
					F	98 (±1.2)	-0.37 (±0.11)	39 (±1.3)	86 (±3.5)
14	26	0	30	30	M	102 (±2.6)	-2.88 (±0.13)	30 (±1.5)	67 (±1.7)
					F	99 (±1.9)	-2.37 (±0.18)	25 (±1.2)	58 (±2.5)

Each group consisted of five animals.