has no appreciable effect upon the calculations shown above. But the elimination by an individual organ must be taken into account. We know that labelled cholesterol synthesized in the organs passes into the plasma and its radioactivity is equal to the sum of R_{PF} and R_{PE} of the whole animal (or R_{SP}) (Table II). A certain adjustment therefore had to be made, so we divided radioactivity R_{SP} in proportion to the synthesized sterols remaining behind in each organ. For intestine, for instance, we have

$$R_{SP}(\text{intestine}) = \frac{R_{SF} + R_{SE}(\text{intestine})}{R_{SF} + R_{SE}(\text{whole rat})} \times R_{SP}(\text{whole rat}).$$

It should be stressed that the shorter the experimental period, the smaller this correction (like the previous one) has to be, i.e. results obtained from the 3.5 h experiments are subject to the smallest margin of error.

Regardless of whether we take free sterol radioactivity (R_{SF}) or the sum of free and esterified sterol radioactivity ($R_{SF} + R_{SE}$), and whether we make the second adjustment (R_{SP}), it becomes apparent that the intestine is the main organ concerned in cholesterol synthesis (Table III). The liver contributes a mere 13.5% to the total. Finally, the part played by the colon and the stomach lies somewhere between 4 and 5% so that it is in the digestive tract that 55–56% of cholesterol synthesis in the rat takes place, a percentage which is close to the one proposed before[1]. So evidence of the major role of intestine in cholesterol synthesis in the adult male rat is furnished. Investigations are carried out for extending this conclusion to female and young male rats, or to various dietary conditions[9].

Table III. Sterols synthesized remaining in situ in free form in the liver and the intestine (m_{SF}). Synthesized sterols remaining in situ but detected in esterified form (m_{SE}). Sterols synthesized and passing into the plasma (m_{SP}). Sterols definitely synthesized ($m_S = m_{SF} + m_{SE} + m_{SP}$) expressed as a percentage of the cholesterol synthesis in the whole rat.

	m_{SF}	m_{SE}	m_{SP}	m_S
Liver	10.2 ± 2.5	2.6 ± 1.2	0.7 ± 0.2	13.5 ± 3.9
Intestine	46 ± 14.5	1.2 ± 0.5	3.5 ± 1.1	50.7 ± 16.1
Whole animal	74.6 ± 19.5	18.7 ± 14	6.7 ± 1.7	100

Summary. By a new in vivo method using 1-^{14}C-acetate, it becomes apparent that the intestine is the main organ concerned in cholesterol synthesis. The liver contributes a mere 13.5% to the total. These results challenge the traditional theory which considers the liver as responsible for producing most of cholesterol synthesized by the rat.

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The Oak Leaf Roller (*Archips semiferanus* Walker) Sex Pheromone Complex: Field and Laboratory Evaluation of Requisite Behavioral Stimuli

The tree defoliating insect *Archips semiferanus* Walker, better known as the oak leaf roller moth (OLR), has caused extensive damage in the forests of the Northeastern United States[1]. Attempts to control this pest began with a comprehensive study of the sexual behavior of the adult moth. In addition to determining that a sex pheromone was present in OLR females[2], over 20 principles[3] were identified in the attractant portion of female abdominal extracts. Previous reports[4] have dealt with chemical analyses and syntheses of the attractants and preliminary investigation of their activity. We wish to report here on the evaluation of these principles by laboratory electroantennogram studies and by field trapping in order to better understand the role of these agents in the sexual message of the oak leaf roller.

A series of 14 carbon monounsaturated acetate isomers which were found in the oak leaf roller female attractant fraction were tested in field traps in Pennsylvania forests. 17 of the 21 isomers tested caught a greater number of male insects than controls (Figure 1). The same set of isomers was tested in the laboratory using the electroantennogram technique; all of the isomers gave better electroantennogram responses than controls (Figure 2). A description of these experiments follow.

Twenty-one Z and E tetradecenyl acetates having double bonds in the 2–5 and 7–13 positions were synthesized, purified by $AgNO_3$ thin layer chromatography and assessed for purity by computerized gas chromatography-mass spectrometry (GC-MS) aided by mass fragmentography[3]. All isomers were found to be better than 97% pure[5]. Field evaluation of the attractancy of these isomers to OLR males was conducted in Moshannon State Forest, Pa. (USA), an area of heavy OLR defoliation, from July 5 to July 16, 1974. 48 vane traps were constructed as previously described[6] and arranged in a square block design with 50 m between each trap. 500 nanograms of each isomer were spotted in a trap beginning July 5 and samples were replenished with the same amount every 2 days until July 16; duplicate traps

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were set for each isomer and 6 traps were used as controls. The number of insects caught in each trap was counted everyday until the end of the experiment[7]. Results of the field trapping are shown in Figure 1. The bars represent the total number of insects caught per trap for each isomer minus the number of insects caught in the controls during the flight period of the oak leaf roller males[8]. The daily number of males caught for the 5 isomers which trapped the most OLR males is represented in Figure 3. Electroantennogram (EAG) analyses were performed on all isomers using the technique described by ROELOFS[9]. 10 nanogram samples of each isomer were freshly prepared before each set of tests. Antennae were excised from virgin male OLR which were raised from field pupae. The results which are recorded in Figure 2 represent the average of 20 replicates. In every test, Z-11-tetradecenyl acetate gave the best male antennal response.

It is clear from Figure 1 that 17 of the 21 isomers tested caught greater numbers of insects than blanks in the field; 5 isomers were highly attractive. The EAG data presented in Figure 2 indicates that all isomers were EAG active. Comparison of the isomer activity as determined by field trapping and EAG shows a poor correlation between the two techniques in this instance[10].

Apparently the oak leaf roller is not very specific in utilizing chemical signals for its sexual behavior. Moreover, since oak leaf roller females contain a complex mixture of similar isomers, it is possible that the recipient rather than the emitter of the chemical, in this case OLR males, are responding to only a few of the chemical messengers. It is also plausible that the apparently homogeneous oak leaf roller infestation may be evolving subgroups in which males are responding to separate chemicals. The observation that the highly attractive isomers are active on different days would also support this postulate. (To our knowledge, this is the first report which suggests a temporal difference in the activity of isomeric sex attractants within a particular species). Alternatively, it is possible that during maturation of the adult moth, the males respond to different chemical signals at different stages of development. However, none of these theories are supported by the electroantennogram analyses. The discrepancies in these two methods of analysis are not clearly understood. Efforts to further elucidate the parameters that are

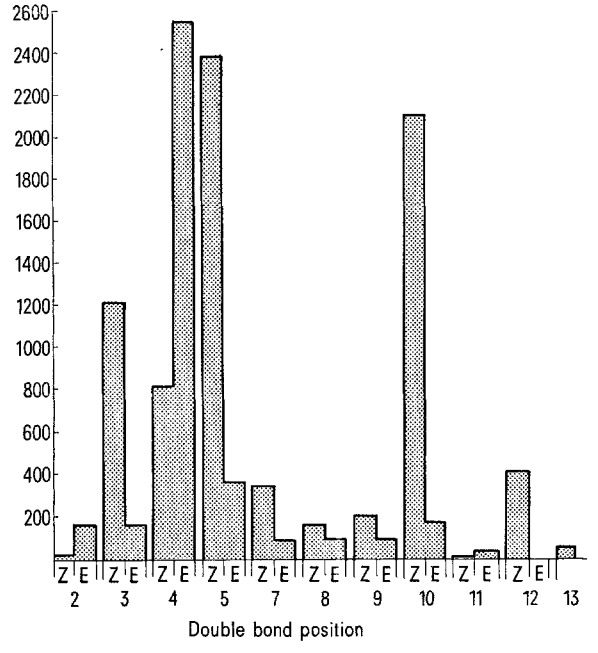

Fig. 1. Total number of oak leaf roller males caught in duplicate traps baited with various tetradecenyl acetates minus the number caught in blank traps from July 5–16, 1974.

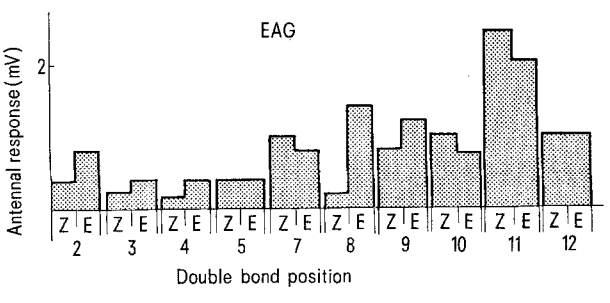

Fig. 2. Electroantennogram responses of oak leaf roller male antennae to 10 ng of various tetradecenyl acetates. Each bar represents the average responses of 20 male antennae.

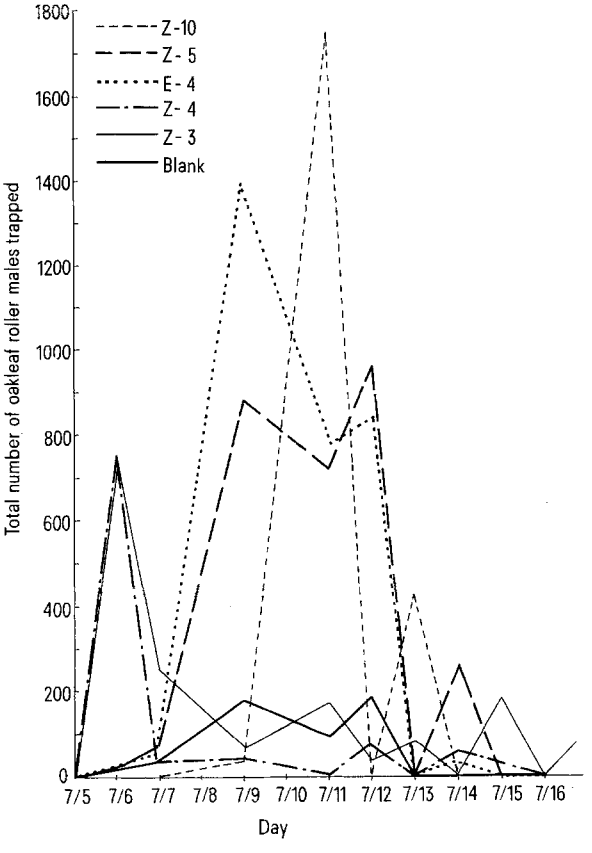

Fig. 3. Daily number of oak leaf roller males caught in duplicate traps baited with the 5 most active tetradecenyl acetates. The average number of males caught in 2 controls is represented as Blank.

[7] Poor weather conditions prevented trap counts on July 8 and July 10, 1974.

[8] Light traps were used to monitor the oak leaf roller moth population; few moths were trapped before July 5 or after July 16, 1974.

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[10] A similar phenomenon has been reported for another Lepidopteran, see M. S. MAYER, J. Insect Physiol. *19*, 1191 (1973).

essential for the communication of discrete chemical messages and the evolutionary mechanisms which are responsible for these phenomena are currently underway and will be the basis of subsequent reports.

In order to apply these results to survey and detection of the oak leaf roller infestations or to the eventual control of this pest, a mixture of several isomers might prove to be essential. In any case, it is evident that sexual communication in the oak leaf roller and perhaps other insect species is exceedingly complex and presents an interesting challenge in this area of science[11].

Zusammenfassung. Die früher als Bestandteile der von Abdominalextrakten des weiblichen Eichenblattrollers, *Archips semiferanus* Walker, festgestellten 21 Isomeren von Tetradecenyl-Acetat wurden mit der Elektroantennogram- (EAG) und der Fallenfang-Methode auf ihre Wirkung bei Männchen von *A. semiferanus* geprüft. Während alle 21 Isomere sich als EAG-aktiv erwiesen, vermochten nur 17 Männchen anzulocken. Aktivitätsvergleiche der Isomeren haben gezeigt, dass zwischen den Resultaten der beiden Test-Methoden nur eine geringe Beziehung existiert.

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[13] Note added in proof: For a possible explanation of these results see L. B. HENDRY, J. K. WICHMANN, D. M. HINDENLANG, R. O. MUMMA and M. E. ANDERSON, Science *188*, 59 (1975).

Effect of Spermine on Adenyl Cyclase Activity of Spermatozoa

The presence of large quantities of spermine (2–15 m*M*) in human seminal plasma has been reported[1–3]. However, the role of polyamine, if any, in spermatozoal motility and metabolism still remains problematical. TABOR and ROSENTHAL[4] have reported that addition of high concentration of spermine to spermatozoa obtained from the vas deferens of mice, rats, guinea-pigs and rabbit, produced an unusual hyperactivity characterized by rapid vibrations without forward motion. However, the specificity of the effects of spermine on sperm cell movement still remains to be clearly understood.

Recently, we have reported that maltase activity of human seminal plasma increased on addition of spermine (up to 3 m*M*)[5]. Further, it was observed that spermine decreased the utilization of fructose by spermatozoa. Studies carried out by GARBERS, FIRST and LARDY[6] have demonstrated that motility can be induced and prolonged in spermatozoa of several mammalian species, by cyclic nucleotides. The purpose of the present investigation was to study whether polyamines-spermine, spermidine and putrescine would affect the spermatozoa adenyl cyclase activity and consequently sperm motility.

Activation of adenylate cyclase of human spermatozoa by spermine expressed in terms of cyclic AMP produced (pmoles/mg sperm protein/10 min)

Concentration of spermine (m*M*)	Semen samples from fertile donors							
	A	B	C	D	E	F	G	H
–	38	165	20	13	16	49	134	75
2.9	38	185	38	48	22	78	140	[a]
7.6	105	195	53	57	29	71	[a]	222
13.4	130	215	[a]	127	[a]	88	174	258

[a] As the determinations were carried out in duplicate and at 3 levels of added spermine, some semen samples did not have sufficient number of spermatozoa for the test to be carried at all dose levels.

(^{3}H) adenosine 3′-5′-cyclic monophosphate (specific activity. 20–30 Ci/m*M*) was purchased from Radiochemical Centre (Amersham, England). Spermine tetrahydrochloride was obtained from Sigma Chemical Co. (USA). Semen samples were obtained from 8 fertile donors (samples A to H mentioned in the Table). After liquefaction of the semen samples, the seminal plasma was separated from spermatozoa by centrifuging the semen at 800 *g* for 30 min. Seminal plasma was drained off and sperms were washed with 3 ml of *Tris*-HCl buffer, centrifuged and resuspended in the buffer. Protein content of sperm suspension was determined according to the method as described by LOWRY et al.[7].

Determination of adenyl cyclase activity was carried out as follows. The incubation mixture contained sperm (adjusted to mg of sperm protein) suspended in 0.04 *M* *Tris* HCl buffer (pH 7.1), 0.033 *M* $MgSO_4$., 10 m*M* theophylline, 1 m*M* adenosine triphosphate, and different concentrations of spermine as indicated in the Table. The total volume of the reaction mixture was 0.4 ml and the incubation was carried out at 37 °C for 10 min. The cAMP was assayed by the competitive binding assay as described by TSANG et al.[8], using bovine adrenocortical receptor protein. All the determinations were carried out in duplicates.

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