The animals were sacrificed after 90 days of treatment, and frozen sections were prepared of the basal portion of the heart. 10 sections, cut at 15 μm in thickness, were selected at random from each animal and were stained with Oil-Red-O and hematoxylin. These were examined without knowledge of the groups from which they were obtained. The severity of coronary atherosclerotic lesions was graded on a scale of 0 to 4 with 0 indicating no lesions; 1, slight; 2, moderate; 3, marked; and 4, most severe. Histologic changes in early or slight atherosclerosis of the coronary arteries were characterized by localized lipid deposits mostly in the intima, corresponding to grade 1, as described by the authors in a previous report of pathologic studies in squirrel monkeys[4]. With the advance and increase of degree of coronary atherosclerosis, corresponding increases in lipid deposition, fibrous proliferation and hyalinization infiltrated through the subintima into the medial layers of the coronary arteries. With further advance of the atherosclerotic process, increases in numbers of foam or phagocytic cells were seen.

Three of the 4 monkeys in control group I exhibited atherosclerotic lesions averaging 2 or moderate in severity. One control animal (female) was without such lesions. In contrast, no lesions were found following histologic sections of the base in any of the animals of group II treated by CSA. The medial part and apex of the heart were similarly sectioned as above, revealing a significant reduction of coronary atherosclerotic lesions in CSA treated animals as compared to control monkeys.

This preliminary report is based upon previous studies demonstrating prevention of experimental atherosclerosis and suggesting regression of atherosclerotic lesions in the squirrel monkey[4], the rat[5,6] and the rabbit[3]; our present findings further suggest that the absence of atherosclerotic lesions in group II may have been due to the regression of such lesions induced by administration of chondroitin sulfate A.

Extensive, long-range similar studies are now programmed.

Zusammenfassung. Die überraschende Atheromatose-hemmende Wirkung von Chondroitin-Sulfat konnte nun auch bei *Saimiri scurea*, einem Primaten mit hoher Inzidenz von Atheromatose, nachgewiesen werden.

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With the technical assistance of
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The Male Reproductive System of the Spruce Budworm, *Choristoneura fumiferana*. 3. Incorporation into Seminal Components of Leucine Released During Apolysis

During some of our earlier investigations on sterilization of the spruce budworm it became necessary to develop a reliable method for determining mating success[1]. Each female moth was caged with a male that had received L-leucine-^{3}H or ^{14}C as a 6th instar larva. After collecting the eggs, the abdomen of the female was assayed for radioactivity, the presence of which indicated successful insemination. Since leucine was injected into 6th instar larvae where excepting for the testes none of the male accessory structures that secrete the seminal fluid and spermatophore are developed, it was postulated that only the sperm proteins would acquire the label. In this communication the results of experiments conducted to test this hypothesis are presented.

Materials and methods. The spruce budworm was reared on a meridic diet after the method of Grisdale[2,3].

The labelling pattern of the seminal components was determined by injecting 1 μl of an aqueous solution containing either 1 μCi of L-leucine-4,5 ^{3}H (specific activity: 50 Ci/m*M*) or 0.2 μCi of L-leucine-^{14}C (uniformly labelled; specific activity: 250 mCi/m*M*) into male, 5th instar or 6th instar larvae. After the insect reached the adult stage each male was allowed to mate with an untreated female and the sperms, seminal fluid, and spermatophore were collected. The spermatophore was rinsed in distilled water and transferred to a vial containing 1 ml of NCS® solubilizer. The vial was incubated at 50 °C for 30 min, after which scintillator was added, and counted. The seminal receptacle from the female and the two seminal vesicles from the male were dissected and transferred into a cavity slide containing 0.5 ml of Ringer-Locke solution[4]. The sperms were expressed into the medium and the empty receptacle and vesicles were removed. The sperms and the seminal fluid suspended in saline were passed through a Swinny holder containing a Millipore filter (pore size: 0.45 μm). The filtrate consisting of the seminal fluid and insect saline was collected into a vial containing 1 ml of NCS solubilizer and counted as

Table I. Distribution of radioactive leucine in some seminal components

Stage and isotope used	Radioactivity as cpm in		
	Seminal plasma	Spermatophore	Sperms
6th instar, ^{3}H-leucine	533	319	357
	912	43	391
	1,437	242	73
5th instar, ^{3}H-leucine	253	48	419
	286	58	793
	1,170	80	541
6th instar, ^{14}C-leucine	181	295	289
	114	355	287
	189	296	364
5th instar, ^{14}C-leucine	353	566	174
	240	676	261
	194	441	177

[1] A. Retnakaran, J. econ. Ent. *64*, 578 (1971).
[2] McMorran, Can. Ent. *97*, 58 (1965).
[3] D. Grisdale, Can. Ent. *102*, 1111 (1970).
[4] R. C. Parker, *Methods of Tissue Culture* (Paul B. Hoeker Inc., 1961), p. 358.

before omitting the incubation step. The Millipore filter containing the sperms was washed by passing distilled water through the Swinny unit, transferred into a vial containing scintillator and counted.

The persistence of radioactive leucine during metamorphosis was investigated using 3-day-old, sixth instar larvae that were similar in size. Each larva was injected with 1 µl of an aqueous solution containing 0.2 µCi of L-leucine-^{14}C. Each day after the day of injection, at least 3 insects were homogenized individually in 2 ml of cold (4 °C) 10% trichloroacetic acid in an Elvehjem homogenizer. The homogenate was passed through a glass fiber filter and the clear liquid was re-filtered through a Millipore membrane. The final filtrate was mixed with NCS solubilizer and scintillator then counted.

Labelling the RNA of the sperm was achieved by injecting 1 µl of an aqueous solution containing either 1 µCi of uridine-6-^{3}H (specific activity: 10 Ci/m*M*) or 1 µl of an aqueous solution containing 0.2 µCi of uridine-2-^{14}C (specific activity: 50 mCi/m*M*) into male, 5th instar larvae. Mating success was ascertained as described earlier[1].

Results and discussion. Male moths that had received labelled leucine as either 5th or 6th instar larvae were allowed to mate with females after which the sperms, spermatophore, and seminal fluid were collected and assayed for radioactivity. It was found that all 3 components had incorporated the radioactive material (Table I). Variations in spermatophore size, seminal fluid volume, and sperm quantity between replicates made quantitative comparisons impossible.

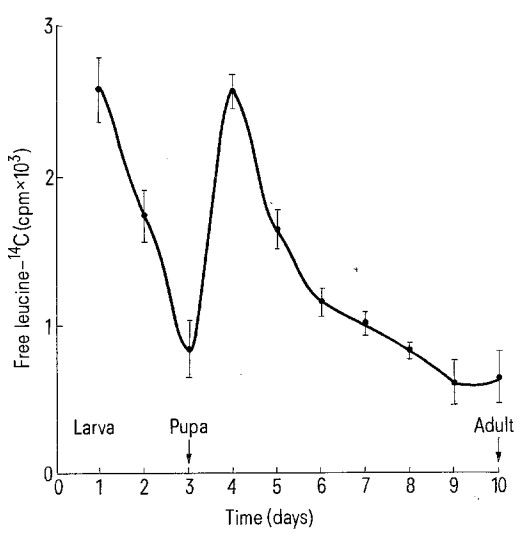
The present results are contrary to our earlier report that it was unlikely that any radioactive leucine injected in the larval stage would be available for incorporation in the male accessory secretions that occur in the adult[1]. In order to explain these findings it was postulated that radioactive leucine became available during pupal apolysis by breakdown of proteins that had incorporated the labelled material. The released radioactive leucine was later incorporated in the accessory glands and their secretions. This hypothesis was tested by following the titer of free, radioactive leucine during metamorphosis after administering the radioactive material to 6th instar larvae. The results indicate that radioactive leucine is rapidly incorporated into proteins in the 6th instar larva as a result of which the free leucine level drops drastically. During the early pupal stage, the titer of free radioactive leucine increases significantly indicating the release of the labelled material. As metamorphosis progresses the level of free leucine drops again suggesting its incorporation into proteins (Figure). The labelled leucine released during apolysis becomes available in part for incorporation into precursors of accessory secretions.

The results shown in the Figure can also be interpreted to mean that during the larval, late pupal, and adult stages there is rapid protein synthesis whereas during the early pupal stage there is very little protein synthesis as in *Plodia interpunctella*[5].

When leucine-labelled males are used for determining mating success, a positive result can indicate either transfer of sperms, spermatophore and seminal fluid, or the accessory material alone without the sperms. The latter possibility arises when a chemosterilant causes aspermia[6]. In such intsances the RNA of the sperm can be labelled by injecting radioactive uridine into male, 5th instar larvae as shown in Table II[7,8]. Since RNA is present only in the cellular material, only the sperms will be labelled[9,10].

Table II. Determination of mating success using uridine labelled males

Isotope used	Eggs	Hatch	Hatch (%)	cpm/♀ abdomen	Mating success
^{3}H-uridine	88	72	81.8	1,642	+
	3	0	0.0	4	—
	117	103	88.0	1,248	+
	65	0	0.0	4	—
	109	104	95.4	807	+
^{14}C-uridine	129	95	73.6	223	+
	31	0	0.0	0	—
	121	98	81.0	117	+
	14	0	0.0	2	—
	107	103	96.3	70	+



Leucine-^{14}C titer in the spruce budworm during morphogenesis. The isotope was injected on 'day 0' into 6th instar larvae. Each vertical bar represents 1 standard deviation above and 1 below the point.

Résumé. Durant l'apolyse de la nymphe de *Choristoneura fumiferana* la leucine radio-active, qui faisait corps avec la proteine, est mise en liberté. L'amino-acide libre s'y infiltre dans les composants séminaux tels que le spermatophore, la semence et autres. On recommande l'uridine radio-active pour détecter la semence seule.

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[8] The radioactive isotopes were obtained from New England Nuclear, Dorval, Quebec, Canada; NCS solubilizer was from Amersham/Searle, Des Plaines, Illinois, USA; the Swinny holders and filters were from Millipore Ltd., Montreal, Canada. All chemicals used were of reagent grade.
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