the other hand, the cytochromes bound to the inner membrane need both intra- and extramitochondrial protein synthesis for their formation[12]. After birth intramitochondrial protein synthesis is rate-limiting for the formation of cytochrome aa_3[5]. The changes in the proportions of the respiratory chain cytochromes during development possibly reflect a difference in the factors that control the formation of these components (but see ref. 1). It is also possible that this phenomenon is due to differences in the assembly of various cytochromes. The latter alternative would support the view that mechanisms that regulate the rate of both intra- and extramitochondrial protein synthesis are somehow coupled to each other.

Zusammenfassung. Der Gehalt an Cytochromen ($c+c_1$, aa_3 und b) in Herz, Leber, Gehirn und Niere der Ratte wurde bestimmt. Ihre Vermehrung nach der Geburt erwies sich in den Geweben als relativ individuell. Die Vermehrung des ausserhalb der Mitochondrien synthetisierten Cytochromen c ging den übrigen, von der intramitochondrialen Proteinsynthese unabhängigen Cytochromen voraus.

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Absence of Naturally Occurring Coronary Atherosclerosis in Squirrel Monkeys (*Saimiri sciurea*) Treated with Chondroitin Sulfate A

Previous studies indicate that administration of chondroitin sulfates, which are widely distributed in mammalian, fish and fowl connective tissues, have an inhibitory effect on the development of experimentally induced atherosclerosis. KURITA[1] in 1955 reported that i.v. injections of 5 mg/kg of body weight daily of chondroitin sulfate C inhibited atherosclerosis in cholesterol-fed rabbits. OHDOI[2] found that sodium chondroitin sulfate inhibited the formation of atheromatous aortic lesions in cholesterol-fed cockerels when administered orally at a level of 20 mg/kg of body weight per day. MURATA[3] noted that daily i.v. injections of 5 mg/kg of body weight of a chondroitin polysulfate prepared by sulfation of chondroitin from shark cartilage reduced the severity of cholesterol-induced atherosclerosis in rabbits. MORRISON et al.[4] observed that when chondroitin sulfate A (CSA) was administered for 9 months at a level of 10 mg daily by s.c. injection to squirrel monkeys (*Saimiri sciurea*) fed a diet consisting of 1.5% cholesterol, 20% butter and 78.5% ground Purina Monkey Chow, the severity of atheromatous aortic lesions was substantially less than that of animals fed a similar diet which did not receive the chondroitin sulfate A treatment. More recently, MORRISON et al.[5] found that orally administered chondroitin sulfate A was highly effective in reducing the incidence and severity of coronary atherosclerosis in x-irradiated cholesterol-fed rats and in preventing the occurrence of aortic and coronary athero-arteriosclerotic lesions in rats fed a hypervitaminosis D atherogenic diet[6].

Available data indicate that the adult squirrel monkey as a sub-human primate is unique among a number of animal species studied for its development of a high incidence of naturally occurring aortic and coronary atherosclerotic lesions similar in many respects to those in adult man[7]. These lesions although macroscopically rare in younger animals, occur with increasing frequency and severity with advancing age as in humans. They are especially pronounced and most marked in severity and number in the coronary arteries of the basal section of the heart as has been found in human subjects at necropsy[8,9]. In the present experiment it was observed that coronary atherosclerotic lesions were completely absent both microscopically and macroscopically in the basal section of the heart of $7^1/_2$- to 8-year-old squirrel monkeys administered daily i.m. injections of CSA for 90 days preceding necropsy in contrast to their presence in squirrel monkeys of similar age administered physiologic saline solution.

Eight squirrel monkeys of approximately $7^1/_2$ to 8 years of age were employed in the present experiment. The males averaged 727 g (range 649 to 818 g) and the females 586 g (range 534 to 675 g) in body weight. The animals were obtained at approximately $2^1/_2$ to 3 years of age as judged by their weight, sexual development and dentition and were flown from a monkey compound in Leticia, Columbia, to our laboratory where they were fed a diet of Purina Monkey Chow and water ad libitum. Twice weekly they were fed fresh fruit such as grapes, apples, oranges and bananas. For 5 years they were kept on the above regime during which they maintained a good state of health with minimal changes in body weight. The animals were weighed once monthly during this period.

At the outset of the experiment the squirrel monkeys were divided into 2 groups: Group I controls consisted of 3 females and 1 male; Group II consisted of 2 males and 2 females. Weights were comparable. Group I was administered daily i.m. injections (6 days per week) of sterile physiologic saline solution; Group II was administered daily i.m. injections (6 days per week) of a sterile solution of 20 mg chondroitin sulfate A (CSA) dissolved in physiologic saline solution and with the pH adjusted to 7.0. Each injection consisted of 0.5 ml of solution, i.e., either physiologic saline solution or CSA solution. The identical dietary regime was followed as in the past 5 years.

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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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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

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