THE TRANSFER OF [4-¹⁴C]CHOLESTEROL FROM BLOOD TO SEMEN IN MAN

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Cholesterol has been found in both spermatozoa and seminal plasma of various species (Goldblatt, 1935; Scott, 1945; Mann, 1964; Eliasson, 1966). However, its origin has not been determined: it could be formed by synthesis from acetate in cells of the genital tract or it could be transferred to semen from blood plasma. This question is relevant not only to the biological mechanism but also with regard to possible hazards resulting from radiation exposure to the germinal cells when investigations are carried out with ¹⁴C-labelled cholesterol or its precursors.

In the course of an investigation aimed at establishing a relative isotopic steady state in man, 1 μ Ci of [4-¹⁴C]cholesterol in 5 ml of peanut oil was given daily in the breakfast for 51 days to a single human subject. During this period and subsequently for a total of 134 days, the blood serum and semen were analysed at intervals for cholesterol content and for radio-activity by methods previously described (Connor & Lin, 1967a, b).

After liquefaction, the semen was centrifuged at 4000 rev/min in order to separate spermatozoa from the seminal plasma, and the spermatozoa were washed three times with 0.9% NaCl. Seminal cholesterol was measured and identified by gas-liquid chromatography and thin-layer chromatography. The measured radio-activity was largely confined to the sterol nucleus. This was confirmed by thin-layer chromatography of the lipids of the specimens and the determination of their radio-activity.

After the oral administration of labelled cholesterol to the subject, a relative isotopic steady state was attained in the blood plasma from the 17th to the 66th days of the study with serum specific activities (disintegrations/ml/mg) from 393 to 640 and then to 355. [4-¹⁴C]Cholesterol was readily transferred from the blood plasma to the semen (Table 1). As might be expected, there was a delay of build-up of this isotope in the semen as compared to the blood plasma. This delay in the build-up of seminal [4-¹⁴C]cholesterol is a characteristic feature of all product-precursor relationships. At 10 days, the specific activity of blood serum was 340 but that of seminal plasma and spermatozoa only 34 and 39, respectively. This suggested that the cholesterol newly entering the genital organs mixed with a large pool of unlabelled cholesterol and that the rate of entry was slow. Yet, by 35 days, the specific activities of spermatozoa and seminal plasma were 52 and 53% of serum cholesterol specific activity, indicating the existence of a considerable equilibrium of the two cholesterol

pools and suggesting that much of the cholesterol of spermatozoa was derived from that of blood serum. The highest percentage of spermatozoal cholesterol derived from blood cholesterol was 82. The specific activity of seminal plasma tended to be somewhat lower than that of spermatozoa, especially from Day 39 to Day 60.

After the administration of [4-¹⁴C]cholesterol was discontinued on Day 51, the specific activity of blood serum gradually declined. However, the specific activity of semen declined more slowly than that of the serum, with the result that the values in spermatozoa and seminal plasma were up to 160% greater than in serum. This again confirmed the impression that the cholesterol pool of both spermatozoa and seminal plasma slowly exchanged with the blood plasma pool and that cholesterol synthesis by the genital tract into semen was

TABLE I
THE SPECIFIC RADIO-ACTIVITIES OF THE CHOLESTEROL OF BLOOD
SERUM, SEMINAL PLASMA AND SPERMATOZOA

Days after start of [4- ¹⁴ C]cholesterol administration	Specific activity in dpm/mg of cholesterol			Ratio of the specific activities of seminal cholesterol (%)	
	Blood serum	Seminal plasma	Sperma- tozoa	Seminal plasma	Sperma- tozoa
3	167	—	—	—	—
6	(248)*	15	62	6	25
10	340	34	39	10	11
17	393	39	135	10	34
29	500	218	194	44	39
35	(545)*	284	290	52	53
39	575	394	472	69	82
43	635	242	400	38	63
51†	640	280	288	44	45
66	355	238	320	67	90
87	217	—	—	—	—
107	147	237	184	160	130
109	(143)*	176	223	123	156
134	97	120	118	120	120

* Extrapolated specific activities in blood serum used in the calculation of ratios between seminal plasma and spermatozoa.

† No further [4-¹⁴C]cholesterol given after this day.

sluggish compared with those organs which synthesized cholesterol into the blood (i.e. the liver and intestine). This is illustrated again by the more rapid decline of cholesterol specific activity in blood plasma than in semen. The cholesterol specific activity declined because the cholesterol newly entering the body pool was non-labelled. Such cholesterol may have been derived from the dietary cholesterol or synthesized within the body.

If all of the seminal cholesterol was derived from blood serum and a perfect isotopic steady state had been attained, then the ratio of the specific activities of semen and serum should have been unity. A percentage ratio of over 100 occurred in the semen samples only after the specific activity of the blood plasma was declining. However, the isotopic steady state was imperfect in the

subject under study, and precise calculations are, therefore, not valid. Furthermore, the collection of semen samples in a free living subject is difficult, and we obtained only a portion of his seminal output.

The average percentage of spermatozoal [4-¹⁴C]cholesterol from Day 29 to Day 66 relative to that of blood serum (the period of relative isotopic steady state in the blood serum) was roughly 62. The assumption may then be made that spermatozoal cholesterol synthesized in the cells of the genital tract itself, and therefore, non-isotopic, must constitute about 38% of the total cholesterol of spermatozoa. The comparable figures for the cholesterol of seminal plasma would be about 52% derived from blood plasma, and 48% from local synthesis in tissues of the reproductive tract.

These findings indicate that a certain amount of radio-activity in the testes resulting from the administration of a ¹⁴C-isotope, capable of being incorporated into cholesterol, may conceivably find its way into the very matrix of spermatozoa. Presumably, the mammalian ovum can also incorporate [4-¹⁴C]cholesterol from the blood since the egg yolk of the laying hen readily takes up labelled cholesterol from blood plasma (Connor, Osbourne & Marion, 1965). [4-¹⁴C]Cholesterol is also transferred from blood plasma across the placenta into the rabbit and guinea-pig foetus and into the milk of the rabbit and guinea-pig (Connor & Lin, 1967a, b).

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