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# STUDIES ON THE MECHANISM OF CAPACITATION

## II. EVIDENCE FOR LIPID TRANSFER BETWEEN PLASMA MEMBRANE OF RAT SPERM AND SERUM ALBUMIN DURING CAPACITATION IN VITRO *

BRIAN K. DAVIS, RICHARD BYRNE and BASALINGAPPA HUNGUND

*Long Island Research Institute and Department of Obstetrics and Gynecology, SUNY, Stony Brook, NY 11794 (U.S.A.)*

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## Summary

1. Evidence has been provided for the transfer of phosphatidyl[$^{14}$C]choline and [$^{3}$H]cholesterol between bovine serum albumin and cauda epididymal rat spermatozoa in Krebs-Ringer bicarbonate medium, which can promote sperm capacitation.

2. An analysis of the lipid composition in both albumin and spermatozoa revealed that phospholipid levels decreased in the protein and increased by roughly comparable amounts in sperm cells during incubation in vitro.

3. Cholesterol (free + ester) increased in albumin and decreased in spermatozoa. Changes in the amount of esterified cholesterol were solely responsible for the increase associated with albumin, whereas whole sperm cell extracts showed a significant decline in free cholesterol.

4. The composition of albumin-bound fatty acids did not alter appreciably as a result of incubation with spermatozoa.

5. Rates of [$^{14}$C]palmitic acid utilization by spermatozoa suggest that lipid synthesis accounted for less than 5% of the changes observed under the conditions of this study.

6. These results are interpreted as broadly supporting our previous proposal that lipid exchange between albumin and sperm cells is implicated in sperm capacitation in vitro. Specifically, the results are compatible with the idea that a decreased cholesterol/phospholipid ratio in the sperm plasma membrane facilitates this transformation.

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* For part I see Ref. 22.

## Introduction

Mammalian fertilization involves an intra-spermatozoan membrane fusion, the acrosome reaction, which may be regarded as an exocytotic process. The reaction causes release of hydrolytic acrosomal enzymes that facilitate sperm penetration through the cellular investments and outer coat (zona pellucida) surrounding the egg. In a previous communication [1], we reported a decrease in the plasma membrane cholesterol/phospholipid ratio among rat sperm cells incubated in a chemically defined medium containing serum albumin. Significance was attributed to this finding because the medium employed has the ability to promote expression of sperm-fertilizing capacity. It was suggested a decrease in membrane microviscosity may be expected to accompany this change in the lipid phase [2] of the sperm plasma membrane and that this should enhance the likelihood of fusion with the outer acrosomal membrane.

A decrease in the sperm plasma membrane cholesterol/phospholipid ratio apparently involves lipid exchange with serum albumin contained by the medium [3]. If an exchange of this kind occurred, it might be expected to produce a predictable, complementary change in albumin-bound lipids. As serum albumin binds various lipids and also transfers them to cells in culture [4], this possibility appeared worthy of investigation. In the present endeavor, an attempt was made to demonstrate the transfer of phospholipid and cholesterol between albumin and rat spermatozoa during incubation in vitro with a medium that allows capacitation of sperm and also fertilization of ova from the rats under chemically defined conditions.

## Materials and Methods

Mature Sprague-Dawley rats (400—500 g) were used in these experiments. They were kept under constant temperature (21 ± 1°C) and light (0700—1900), and fed Purina chow ad libitum. To analyze for lipid changes in spermatozoa and albumin during incubation, sperm cells obtained from the cauda epididymis immediately after autopsy, were incubated at a concentration of about $2 \cdot 10^6$ spermatozoa/ml in 50 ml of a modified Krebs-Ringer bicarbonate medium [1] and, when present, 4 mg/ml crystallized bovine serum albumin (Sigma). Sperm numbers were determined with a hemocytometer. All the sperm suspensions used in these experiments showed motility, when examined microscopically (X100), after incubation; motility was consistently improved by addition of serum albumin to the medium. The incubation required 5 h and was usually performed in a sterile plastic flask (Falcon) at 37°C with a humidified atmosphere of 5% $CO_2$ in air, while a layer of mineral oil (Squibb) covered the suspension. Following incubation, the mineral oil layer was removed with a separatory funnel, the sperm were sedimented at $3000 \times g$ for 30 min, resuspended in 50 ml of Krebs-Ringer bicarbonate buffer and recentrifuged; after resuspension in 2 ml Krebs-Ringer bicarbonate buffer, the sperm cells were extracted. The supernatant was concentrated seven fold in an Amicon ultrafiltration apparatus using a PM-10 filter. Albumin was isolated by gel filtration on a column (length, 32 cm; diameter, 2.5 cm) of Sepharose 2B and then on a similar column with Sephadex G-200 employing a 0.1 M phosphate-

buffered saline solution, pH 7.2, as eluant. The column effluent was assessed for absorbance at 280 nm by continuous flow through a Gilford spectrophotometer attached to a chart recorder, prior to collection of 5-ml fractions in an LKB fraction collector. The fractions containing serum albumin were pooled and concentrated by ultrafiltration. Lipids associated with the protein and spermatozoa were extracted by the method of Bligh and Dyer [5]. Individual phospholipids were identified by co-migration with standard phospholipids (Sigma) on thin-layer silica gel plates (Kontes) developed in chloroform/methanol/water (65 : 25 : 4) followed by petroleum ether/ethylether/acetic acid (80 : 20 : 1) and quantitated after assaying for phosphorus [6]. These determinations were made on eluates obtained by twice extracting with chloroform/methanol (2 : 1) zones scraped from the silica gel plates. The cholesterol zone was also eluted, and the sterol applied to another silica gel plate and chromatographed with hexane/ethyl ether/acetic acid (70 : 30 :1). Both free and esterified cholesterol were then eluted from well-separated zones and quantitated fluorimetrically [7]. Phospholipid and cholesterol levels in albumin, pre- and postincubation, and in spermatozoa incubated in the presence and absence of the protein are presented in the next section. Albumin fatty acid concentrations were also determined in extracts obtained after incubation in the presence and absence of spermatozoa, under comparable conditions to those described above. Fatty acids in these extracts were identified by their retention times after injection into a gas chromatogram (Hewlett Packard) with a 10% DEGS column at 180°C and an integrator to quantitate peak areas.

Direct evidence of phospholipid transfer was obtained with albumin bearing radioactively labeled phosphatidylcholine. To presaturate the protein, 2 mg of delipidated albumin [8] was dissolved in 1 ml of Krebs-Ringer bicarbonate buffer and a suspension formed by addition of 30 mg of Celite (Johns Manville) coated [8] with 1 $\mu$Ci phosphatidyl[$^{14}$C]choline (0.32 Ci/mol). After incubation with constant agitation for 14 h at 22°C, the suspension was sedimented at 1000 $\times g$ for 10 min to remove Celite and a clear supernatant was passed over a small Sephadex G-200 column. Subsequently, albumin was found to have bound 0.12 $\mu$Ci phosphatidyl[$^{14}$C]choline/mg; where the protein concentration was estimated by absorbance at 280 nm and the radioactivity measured in a liquid scintillation counter (Nuclear Chicago) using Aquasol (New England Nuclear) as phosphor. Sperm cells ($3 \cdot 10^6$ spermatozoa/ml) were then incubated in 4 ml Krebs-Ringer bicarbonate medium containing 0.1 mg albumin bearing phosphatidyl[$^{14}$C]choline for various intervals at 37°C. At the termination of incubation, each sperm suspension was chilled in an ice bath and centrifuged at 1000 $\times g$ for 30 min at 4°C in a 6 ml plastic tube (Falcon) with a zone of 1 ml 60% (w/v) sucrose dissolved in 0.1 M KCl and 0.01 M Tris, pH 7.2, to arrest the sedimenting spermatozoa. After dispersion of the sperm layer that formed on sedimentation with 4 ml of 0.01 M Tris, pH 7.4, and 0.1 M KCl buffer, the cells were suspended in Aquasol and appraised for radioactivity. A control preparation containing no sperm cells was included to allow correction for possible contamination by non-bound phosphatidyl[$^{14}$C]choline.

Transfer of cholesterol from sperm cells to albumin was also determined. The spermatozoa from a freshly excised rat cauda epididymis were preincubated for 2 h with [$^3$H]cholesterol-bearing vesicles. Phospholipid vesicles con-

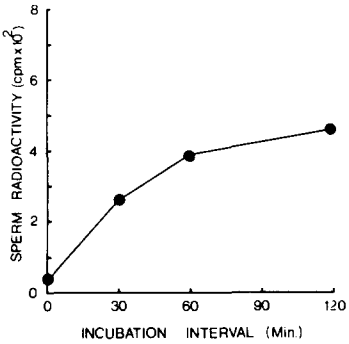
taining the sterol were prepared by ultrasonication [9] of a homogeneous mixture consisting of 38 mg egg phosphatidylcholine (purified by Dr. L. Dawidowicz), 2 mg phosphatidyl-L-serine (Nutritional Biochemicals) and 0.9 μg [$^3$H]cholesterol (55 mCi/μmol) suspended in 5 ml Krebs-Ringer bicarbonate buffer with $Ca^{2+}$ and $Mg^{2+}$ omitted. After preincubation of the sperm cells ($4.4 \cdot 10^6$) with the vesicles, they were sedimented into 2.4 ml of Krebs-Ringer bicarbonate medium containing 10 mg/ml albumin, and incubated for approximately 9 h. To isolate albumin, the suspension was filtered through a millipore membrane (0.45 μm porosity), centrifuged over a 3 ml zone of 20% (w/v) sucrose in a Ti 50 rotor using a Beckman L65 ultracentrifuge operated at 165 000 × *g* for 4 h. The supernatant was concentrated in a collodian tube (Schleicher and Schuell) to a volume of 1 ml, applied to a Sephadex G-100 column (length 25 cm, diameter 1.25 cm) and eluted with Tris/KCl buffer. A single ultraviolet-absorbing peak, corresponding to albumin, was obtained, assayed for radioactivity, and extracted. The sedimented sperm cells were also extracted. Radioactivity attributable to [$^3$H]cholesterol in these extracts from albumin and sperm cells was determined after chromatography on thin-layer silica gel plates (Kontes).

Lipid synthesis by spermatozoa under the conditions used in this investigation was assessed from incorporation of a radioactively labeled fatty acid. For this purpose cauda epididymal sperm cells were incubated in Krebs-Ringer bicarbonate medium for 7 h at 37° C in the presence of 1 μCi of [$^{14}$C]palmitic acid (approx. 400 Ci/mol). Prior to extraction, the sperm cells were sedimented to reduce the level of unincorporated [$^{14}$C]palmitic acid which remained in the supernatant. Inclusion of the fatty acid into various lipids was subsequently assessed from the distribution of radioactivity following chromatography on a silica gel-coated plate using chloroform/methanol/water (65 : 25 : 4) followed by petroleum ether/ethylether/acetic acid (80 : 20 : 1) as moving phase. The locations of glycolipid and ganglioside zones on the plate were established by reactivity to periodic acid-Schiff reagent and resorcinol, respectively, using extracts from non-radioactive spermatozoa.

## Results

Fig. 1 clearly indicates that phosphatidylcholine can be transferred from albumin to cauda epididymal rat spermatozoa during incubation with Krebs-Ringer bicarbonate medium. In 2 h, almost 1 μg phosphatidyl[$^{14}$C]choline (0.32 Ci/mol) was taken up by a suspension containing $4.4 \cdot 10^6$ spermatozoa. It may be noted, for subsequent reference (cf. Table II), that this is equivalent to 23 μg phospholipid/$10^8$ sperm cells.

Evidence of cholesterol transfer from spermatozoa to albumin was also obtained. Thus, Fig. 2 shows a peak of radioactivity associated with albumin in the effluent from a Sephadex column after addition of medium, which had been used to incubate [$^3$H]cholesterol-bearing spermatozoa. Moreover, this radioactivity migrated with the protein during gel electrophoresis (unpublished result). The data in Table I reveal that albumin bound nearly 5% of the radioactivity in spermatozoa, which had been preincubated with phospholipid vesicles containing [$^3$H]cholesterol. 84% of albumin-associated radioactivity



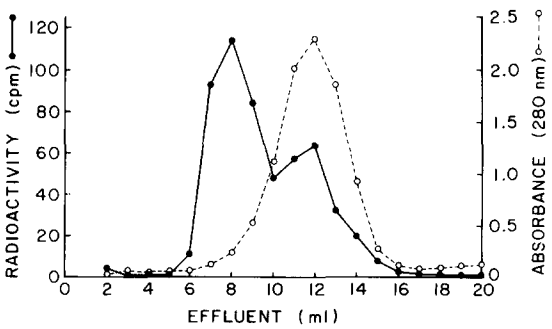




Fig. 1. Uptake of albumin-bound phosphatidyl[$^{14}$C]choline by rat epididymal sperm cells during incubation in vitro. The spermatozoa ($4.4 \cdot 10^6$ sperm/ml) were incubated in 4 ml Krebs-Ringer bicarbonate medium containing $13 \cdot 10^3$ cpm of albumin-bound phosphatidyl[$^{14}$C]choline at 37°C in a humidified atmosphere of 5% $CO_2$ and air. At indicated intervals, the sperm cells were chilled. sedimented, and then assayed for radioactivity.

Fig. 2. Distribution of tritium after gel filtration chromatography of an albumin-containing medium in which [$^3$H]cholesterol-bearing rat spermatozoa had been incubated. There is an obvious peak of radioactivity coincident with albumin whose location is indicated by the absorbance (280 nm) profile. Another peak occurs in the void volume of the Sephadex column. Eluant, 0.1 M KCl and 0.01 M Tris, pH 7.4.

was identified as [$^3$H]cholesterol after chromatography on a silica gel plate. Whereas, only 34% of sperm cell radioactivity co-migrated with cholesterol. After correcting for this heterogeneity, 12% of sperm cholesterol appears to have been bound by albumin. Conversely, 8% of the tritiated sterol was transferred to epididymal rat spermatozoa ($2.5 \cdot 10^6$) during incubation in Krebs-Ringer bicarbonate medium (1 ml) having albumin (2.2 mg) loaded with 0.05 $\mu$Ci [$^3$H]cholesterol (unpublished result).

Large, complementary variations occurred in the phospholipid levels of albumin and spermatozoa during incubation. Specifically, a depletion of

TABLE I

DISTRIBUTION OF RADIOACTIVITY FOLLOWING INCUBATION OF [$^3$H]CHOLESTEROL-BEARING RAT SPERMATOZOA IN MEDIUM CONTAINING ALBUMIN

Epididymal rat spermatozoa ($4.9 \cdot 10^6$ sperm/ml), incubated with phospholipid vesicles containing [$^3$H]-cholesterol (55 mCi/$\mu$mol), were incubated for almost 9 h at 37°C in 2.5 ml of Krebs-Ringer bicarbonate medium containing 4 mg/ml of bovine serum albumin. The suspension was centrifuged at 3000 × *g* for 30 min to sediment sperm cells following incubation. Two peaks of radioactivity were observed after elution of the supernatant from a Sephadex G-200 column (Fig. 2); one peak, in the void volume, apparently represented membrane fragments, and the other peak coincided with bovine serum albumin. The fraction of radioactivity representing [$^3$H]cholesterol in sperm cells and albumin was determined after chromatography of chloroform/methanol extracts from each source on a thin layer of silica gel. n.d., not determined. These data are means of triplicate determinations.

| Source | Radioactivity (cpm) | [$^3$H]Cholesterol (%) |
|---|---|---|
| Spermatozoa | 2710 | 34 |
| Albumin | 156 | 84 |
| Leading peak | 243 | n.d. |

TABLE II

COMPARISON OF THE CHANGE IN PHOSPHOLIPID AND CHOLESTEROL LEVEL IN RAT SPERMATOZOA AND ALBUMIN WITH INCUBATION

The incubation was performed for 5 h at 37°C using 50 ml Krebs-Ringer bicarbonate medium, which had 4 mg/ml of bovine serum albumin when present and approximately $2 \cdot 10^6$ rat sperm/ml. Data presented represent means of 3—6 determinations.

| Lipid | Albumin (μg/200 mg protein) | | | Sperm cells (μg/$10^8$ sperm) | | |
|---|---|---|---|---|---|---|
| | Pre-incubation | Post-incubation | Δ | Control | Albumin | Δ |
| Phospholipid | 44.0 | 10.6 | −33.4 | 96.4 | 140.0 | +43.6 |
| Lecithin | 7.3 | 1.7 | −5.6 | 33.1 | 33.6 | +0.5 |
| Lysolecithin | 1.8 | 1.0 | −0.8 | 2.3 | 4.0 | +1.7 |
| Phosphatidyl-ethanolamine | 7.9 | 1.8 | −6.1 | 13.7 | 19.7 | +6.0 |
| Phosphatidyl-glycerol | 2.2 | 1.2 | −1.0 | 15.2 | 22.1 | +6.9 |
| Phosphatidyl-serine | 16.7 | 2.8 | −13.9 | 5.3 | 21.1 | +15.8 |
| Sphingo-myelin | 2.2 | 0.3 | −1.9 | 5.8 | 10.1 | +4.3 |
| Unidentified | 6.0 | 1.8 | −4.2 | 21.0 | 29.4 | +8.4 |
| Cholesterol | 8.8 | 17.6 | +8.8 | 58.5 | 52.1 | −6.4 |
| Free | 5.3 | 0.1 | −5.2 | 40.7 | 34.9 | −5.8 |
| Ester | 3.4 | 17.6 | +14.1 | 17.8 | 17.2 | −0.6 |

albumin-bound phospholipid by 33.4 μg/200 mg protein matches well with an increase of 43.6 μg phospholipid/$10^8$ spermatozoa (Table II). Furthermore, the data in Table II display a reasonable match for individual phospholipids. It is interesting to note that sperm levels of the fusigenic phospholipid, lysophosphatidylcholine, also showed an increase in the presence of albumin. Results relating to cholesterol in Table II have less agreement. A complementary rela-

TABLE III

COMPOSITION OF ALBUMIN-BOUND FATTY ACIDS

After incubation with Krebs-Ringer bicarbonate medium for 9 h at 37°C, in the presence or absence of epididymal rat spermatozoa, albumin was isolated from the medium, extracted with a chloroform/methanol mixture and an analysis of its fatty acid composition performed with gas chromatography. The results are means of duplicate measurements. n.d., not detected.

| Fatty acid | Amount (μg/200 mg albumin) | |
|---|---|---|
| | Sperm cells omitted | Sperm cells present |
| Total | 174.8 | 173.7 |
| 14:0 | n.d. | 5.7 |
| 16:0 | 55.2 | 47.2 |
| 18:0 | 36.8 | 45.3 |
| 18:1 | 51.5 | 60.4 |
| 18:2 | 31.3 | 15.1 |

TABLE IV

INCORPORATION OF [$^{14}$C]PALMITIC ACID INTO LIPIDS BY EPIDIDYMAL RAT SPERMATOZOA DURING INCUBATION IN VITRO

Cauda epididymal rat spermatozoa ($2.5 \cdot 10^6$ sperm) were incubated in 1 ml of medium containing 1 μCi [$^{14}$C]palmitic acid for 7 h at 37°C in a humidified atmosphere of 5% $CO_2$/95% air. The radioactivity of various lipids in a chloroform/methanol extract of postincubated spermatozoa was determined after chromatography on a silica gel plate. These determinations were performed in duplicate.

| Lipid | Radioactivity (cpm) |
|---|---|
| Cholesteryl ester | 167 |
| Ganglioside | 63 |
| Glycolipid | 197 |
| Lecithin | 129 |
| Lysolecithin | 18 |
| Phosphatidylethanolamine | 194 |
| Phosphatidylglycerol | 24 |
| Phosphatidylserine | 65 |
| Sphingomyelin | 44 |
| Unidentified | 125 |

tionship did occur between sperm and albumin cholesterol (free + ester) levels; the former having a decrease of 6.4 μg/$10^8$ sperm cells and the latter an increase of 8.8 μg/200 mg protein. It can be seen that the increase in albumin-sterol is entirely attributable to a large increase in esterified cholesterol. In contrast, the spermatozoa had virtually constant amounts of cholesteryl ester. It is worth mentioning, however, that these results refer to whole cells and not simply to plasma membranes.

Table III shows, as expected, that fatty acids are a major lipid component of the albumin preparations used in these experiments. From the results presented, however, fatty acid levels seem to be unchanged after incubation with spermatozoa. Stearic, palmitic and oleic acids were the three fatty acids present in the highest amounts in albumin, irrespective of the presence of spermatozoa during incubation. Nevertheless, rat spermatozoa in Krebs-Ringer bicarbonate medium can actively incorporate available [$^{14}$C]palmitic acid into various lipids including phospholipids and cholesteryl ester (Table IV). It was observed in the course of this study that fatty acid-free albumin competed with spermatozoa for [$^{14}$C]palmitic acid causing a seven-fold reduction in incorporation under the conditions used. From the specific activity of [$^{14}$C]palmitic acid (400 Ci/mol) and the levels of lipid in rat spermatozoa (Table II), it appears that synthesis accounted for less than 5% of the changes observed with incubation, judging by the data presented in Table IV. When [$^{14}$C]acetate was used as precursor there was no apparent synthesis of free cholesterol by rat spermatozoa.

## Discussion

We have previously proposed a molecular model, implicating the lipid binding property of albumin in the expression of fertilizing capacity in vitro by

spermatozoa from rats, hamsters and, by inference, from other mammals [3]. Induction of the acrosome reaction, which, as noted, is a precondition for fertilization of mammalian ova, was suggested to involve destabilization of the sperm plasma membrane through an action on the membrane lipid phase by albumin. According to the model, destabilization of this membrane is associated with increased permeability to $Ca^{2+}$, which chelates anionic phospholipid molecules in adjacent membrane lipid bilayers to promote [10] fusion between the plasma membranes and outer acrosomal membrane [3,11,12]. Although the details are far from being proven at present, the simple molecular model has displayed impressive heuristic value. Consistent with the model it has been discovered for example that a modification in albumin-bound lipid alters its sperm capacitation ability [13,14], synthetic phospholipid vesicles containing cholesterol, which stabilizes the plasma membrane, reversibly inhibit sperm-fertilizing capacity [9], elution of lipids from seminal plasma membrane vesicles (decapacitation factors) abolishes their inhibitory effect on fertilization [15], induction of the acrosome reaction by a $Ca^{2+}$ ionophore [16,17] various detergents [18,19] and lysophosphatidylcholine [20], and a lowered cholesterol/phospholipid ratio in the rat sperm plasma membrane following incubation with albumin [1].

Additional support has been given to the model by our present observations. Thus, it is now clear that lipids are exchanged by albumin and spermatozoa during capacitation in vitro. Sperm capacitation is usually performed in smaller volumes than those employed in this investigation; however, it is improbable this would influence the validity of our conclusion regarding the occurrence of lipid transfer during sperm capacitation. A depletion of phospholipid and an elevation of cholesteryl ester bound to albumin, after incubation with spermatozoa, are in accord with our previous report [1] showing that complementary changes occur in the sperm plasma membrane following exposure to albumin. The smaller lipid variations observed for plasma membrane (—3.2 μg cholesterol, +7.4 μg phospholipid in plasma membrane vs. —6.4 μg cholesterol, +43.6 μg phospholipid in whole cells/$10^8$ epididymal rat spermatozoa) presumably involve incomplete isolation of the membrane. A possibility that these alterations arise through synthesis within the sperm cell is discounted because of the low levels of [$^{14}$C]palmitic acid incorporation observed (Table IV). From the results of a chemical analysis (Table II), serum albumin apparently exchanges free cholesterol for esterified sterol. However, cholesteryl ester levels in these spermatozoa did not significantly change during incubation with the protein, and since cholesterol esterification by serum albumin preparations employed in these experiments can be ignored (unpublished data), this implies compensating ester formation occurred in these cells.

The present findings indicate that the cholesterol/phospholipid ratio decreases in epididymal rat spermatozoa during incubation with serum albumin under conditions broadly comparable to those used for achieving capacitation. It seems reasonable to expect, therefore, that depletion of the sterol, as induced by incubation with phospholipid vesicles, might promote sperm capacitation. As already noted, cholesterol-bearing liposomes have been demonstrated to reversibly inhibit fertilizing capacity in rabbit sperm cells. Although liposomes lacking cholesterol failed to appreciably enhance fertilization rates in

these experiments [13], it may be pointed out that capacitation of rabbit sperm cells, unlike those from the rat, has not been reliably obtained by means of a serum albumin-containing medium. Possibly this results from a lower lipid-exchanging propensity in rabbit spermatozoa.

Around 12% (131 cpm/921 + 131 cpm) of [$^3$H]cholesterol in epididymal rat sperm cells apparently transferred to serum albumin during incubation (Table I) and this agrees with the result (11%; 6.4 $\mu$g/58.5 $\mu$g) obtained by chemical analysis (Table II). On the other hand, sterol transfer from liposomes, which were used to introduce radioactively labeled cholesterol into these spermatozoa, that may have adhered to the sperm surface is a possibility that cannot be directly excluded in this experiment. However, we have obtained direct evidence for the transfer of isotopically labeled cholesterol and phosphatidylcholine from liposomes to sperm plasma membrane fractions [21]. In addition, there was a reduced level (34%) of tritium in cholesterol among treated spermatozoa (Table I) and this can be interpreted as evidence that the sterol underwent modification following uptake of essentially pure cholesterol (the nature of these alterations in cholesterol are obscure at present, although the results in Table IV reveal a conversion to cholesteryl ester). Finally, the proposed transfer of cholesterol from sperm cells to albumin is supported by the close agreement obtained between the chemical analysis data and the extent of [$^3$H]cholesterol uptake by this protein. Parenthetically, delipidated serum albumin was used in the latter experiment and this may account for the comparatively low amount (less than 26%) of bound cholesteryl ester.

Requiring additional study is the significance of cholesteryl ester in sperm plasma membrane fractions. Our findings also suggest there is more to be learned about the way cholesterol is metabolized in the sperm cell. The mechanism of capacitation in utero remains obscure. There is also much additional information to be acquired concerning the biochemistry of sperm capacitation and induction of the acrosome reaction, even if the present viewpoint is valid.

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