Some preliminary experiments have been carried out in feeding large doses of exogenous cortisol to subjects in each of the 3 groups studied and measuring % of dose recovered as 6β-OH-F. There was 13.5 and 16% conversion to 6β-OH-F in 2 toxemics and 13.5 and 22% in 2 non-toxemic pregnant women, while 2 non-pregnant women excreted 3.8 and 6% as 6β-OH-F. These observations indicate that there is an increased conversion of cortisol to the 6-beta derivative in both toxemia and normal pregnancy.

The biological effects of 6β-OH-F have not been fully studied, but at present there is no evidence which would assign to it a causative role in toxemia. The likelihood is that 6β-hydroxylation represents an available pathway for cortisol degradation, rendering the steroid more water soluble and suitable for excretion without the necessity for A-ring reduction and glucuronide conjugation. If the enzymatic steps concerned with either of these 2 reactions are interfered with, as might occur in some conditions, then 6-beta-hydroxylation could function as an important alternate pathway of cortisol metabolism.

Summary. 6β-OH-cortisol has been found to be the most abundant unconjugated corticoid in human urine. Normal pregnancy is accompanied by elevated levels which are further increased in toxemia. The evidence to date suggests that in these conditions an altered metabolism of cortisol takes place in which 6β-hydroxylation becomes of greater quantitative significance.

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Immunological Differentiation of Human Testicular (Spermatocele) and Seminal Spermatozoa.* (26003)

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The seminal plasma and spermatozoa of man have powerfully antigenic material in common(1). These antigens are highly organ and species specific. The same is true for rabbit(2), guinea pig(3), bull(4), and buffalo(5). The fact that similar antigens are found in aqueous extracts of prostate and seminal vesicles, but not in those of testicle or epididymis, and that the ejaculate of azoospermic man contains these antigens, suggested that they are the product of the adnexal glands of the genital tract and that spermatozoa take up the antigens during their passage through these organs(1,2). Recently, direct evidence was obtained that spermatozoa from the rabbit's epididymis lack these antigens(6).

For men, similar evidence is difficult to obtain, because spermatozoa from testes or epididymis cannot be easily obtained in adequate numbers. An alternative source for spermatozoa, however, is occasionally available in

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spermatoceles. These cyst-like structures form when the epididymis becomes obstructed (7,8). Usually they contain very numerous spermatozoa, indistinguishable in morphology from seminal spermatozoa and often found motile at the time when a spermatocele is punctured or opened by surgical intervention. Inasmuch as these spermatozoa are excluded from contact with the adnexal glands, they can for our purposes be considered as equivalent to testicular spermatozoa.

The fluid content of spermatoceles is low in protein. Immunologically, these proteins are indistinguishable from those of the serum and do not cross-react with seminal plasma or spermatozoa(9).

We were fortunate in obtaining spermatocele contents through courtesy of Dr. John R. Herman. Thus we were able to procure direct evidence that in man also testicular spermatozoa lack the antigens present in seminal spermatozoa and in seminal plasma.

Materials and methods. Three specimens of spermatocele content were available for these experiments:

a) From a 56 year old man; 218 ml of spermatocele fluid was collected containing 17 x 10^9 spermatozoa (77 x 10^6/ml).

b) From a 45 year old man; 93 ml spermatocele fluid containing 5 x 10^9 spermatozoa (54 x 10^6/ml).

c) From a 40 year old man, 2 ml containing 19 x 10^6 spermatozoa.

A minority of the spermatozoa was found motile on examination immediately after collection.

The spermatozoa were removed from the spermatocele fluids by high speed centrifugation, washed thrice in physiological saline solution, and resuspended in the same diluent in a concentration of 10^8 cells/ml. Dilutions were made from this in saline solution as needed. Sterile precautions were observed. Merthiolate was added to a concetration of 1 10,000.

Immune sera against human seminal spermatozoa, seminal plasma, whole serum, or aqueous organ extract were available from previous work. New batches of immune sera were prepared as needed according to the methods previously described(1,2,5). Rabbits and guinea pigs were injected with spermatozoa from spermatoceles in Freund's adjuvant according to these procedures. In these publications, the methods of complement fixation test (Kolmer modification) and precipitation by the agar diffusion method of Ouchterlony used for the work presented here have also been given.

Results. Spermatozoa from spermatoceles did not react with immune sera against seminal spermatozoa or seminal plasma (Table 1). They also did not react with anti-human serum immune sera.

Seminal spermatozoa remove antibody both from anti-seminal plasma and anti-seminal spermatozoal sera. Absorption of antibody can be demonstrated in the agar diffusion test, if a suspension of spermatozoa in proper concentration(or seminal plasma) is added to a trough containing immune serum. Spermatocele spermatozoa do not inhibit formation

TABLE I. Fixation of Complement by Rabbit Immune Serum (Diluted 1/10) and Antigens (Human).

	A		B		C		D		E	
	Spermatocele spermatozoa 2×10^6/0.1 ml		Seminal spermatozoa 2×10^6/0.1 ml		Seminal plasma 1/200		Spermatocele fluid 1/100		Blood serum 1/100	
Antigen dilution	Anti-sem.	Anti-sperm.	Anti-sem.	Anti-sperm.	Anti-sem.	Anti-sperm.	Anti-sem.	Anti-sperm.	Anti-sem.	Anti-sperm.
1/1	2+	2+	4+	4+	4+	4+	—	—	—	—
1/2	—	—	4+	4+	4+	4+	—	—	—	—
1/4	—	—	4+	4+	4+	4+	—	—	—	—
1/8	—	—		—	4+	4+	—	—	—	—
1/16	—	—		—	-	4+	—	—	—	—
1/32	—	—	—	—	—	—	—	—	—	—

Antiserum and antigen controls, also antigens plus normal rabbit serum negative.
Anti-sem. = Anti-seminal plasma serum; Anti-sperm. = Anti-seminal spermatozoal serum.
4+, no hemolysis; 2+, moderate (approx. 50%) hemolysis; —, complete hemolysis.

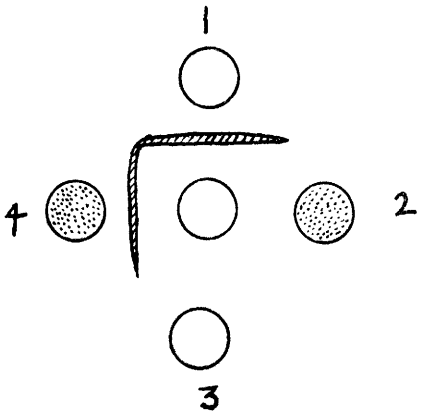

FIG. 1. Tracing of Gel Diffusion Test. Center trough: Human seminal plasma. Peripheral troughs all contain anti-seminal plasma immune serum; (1) no addition; (2) 10^8 seminal spermatozoa (in 0.05 ml saline) added; (3) 0.05 ml seminal plasma added; (4) 10^8 spermatocele spermatozoa (in 0.05 ml saline) added.

of a line of precipitation between the trough containing immune serum and the one with antigen (seminal plasma) (Fig. 1); seminal spermatozoa or seminal plasma inhibit precipitation. Thus the first agent lacks the antigenic material present in the two others.

We have not been able thus far to obtain evidence of antibody formation in sera of rabbits or guinea pigs treated with spermatozoa from spermatoceles. However, immunization experiments with this antigen are being continued in rabbits, guinea pigs and mice.

Discussion. Immunological evidence is presented showing that spermatozoa from spermatoceles ("testicular" spermatozoa) lack the antigens which characterize seminal plasma and seminal spermatozoa. Similar data have been previously presented regarding the difference between testicular and seminal spermatozoa of the rabbit(6). Thus the highly antigenic material present on spermatozoa from semen must be taken up during the passage through the adnexal glands. Though we have failed hitherto to obtain evidence of antigenicity of spermatozoa obtained before they come into contact with the secretions of the adnexal glands (collected from spermatoceles), the data of Henle(10.11) of Voisin (12) and Freund(13) show that testicular spermatozoa are not devoid of antigenicity, and those of Pernot(3,4) show that such antigenicity may still be discerned in seminal spermatozoa. At the present time, it remains unknown whether the antigenic material acquired by spermatozoa during their passage through the adnexal glands plays any role in the physiology of reproduction.

Summary. Spermatozoa from spermatoceles lack the antigenic material present on seminal spermatozoa, which these latter cells share with the seminal plasma. This provides direct evidence that human spermatozoa acquire antigenic material during their passage through the adnexal glands of the genital tract.

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