completely removed by absorbing the antiserum to FSH with blood serum. Bovine LH gave two precipitin lines with unabsorbed and a single precipitin line with absorbed antiserum, indicating that one of the antigen was specific only to the bovine hormone preparation.

Experiments were also carried out with a specific rabbit antiserum obtained with sheep serum as the antigen. In the Ouchterlony plate the antiserum was placed against sheep serum, ovine FSH, ovine LH, bovine LH, ram serum and porcine LH to study the common antigens these hormone preparations have in common with sheep serum (Figure 4). The results indicated that sheep and ram sera had as many as 6 to 8 antigens of which one was common to ovine FSH and LH and bovine LH. Porcine LH did not show any reaction.

The studies reported here have shown the presence of both LH and sheep serum contaminants in ovine FSH. A common precipitin line between antiserum to sheep serum and FSH, LH and sheep serum (Figure 4) and also the common precipitin line between antiserum to sheep FSH, LH, FSH and sheep serum (Figure 1) strongly suggests the LH contaminant observed is also common to sheep serum. The observation that absorption of the antiserum to FSH with sheep serum selectively removes the antigen common to ovine FSH, LH and serum indicates that normal sheep and ram serum have LH activity which can be demonstrated in the agar diffusion test. Our results, as well as the observation of SEGAL et al.[1,2] that the specific antiserum to LH removes the LH contaminant in FSH and also gives a negative Weaver Finch test, strongly suggest that one of the antigen in ovine serum common to ovine FSH is due to LH. It would be worth while to prove that this particular contaminant is only due to LH. Biological experiments are under progress to prove this.

SEGAL et al.[1] have shown that removal of LH activity from ovine FSH causes loss of gonadotrophic potency of the material as studied by mouse uterine weight assay. It would be interesting to find out whether absorption of ovine FSH with antiserum to sheep serum would cause a similar loss of gonadotrophic activity of ovine FSH. Work now under progress will be presented in a later publication. The results reported here also indicate that ovine LH does not have antigens in common with ovine LTH. Preliminary experiments have indicated that the antiserum to sheep serum causes inhibition of testicular weights and those of the accessory sex organs in immature male rats. The results of the *in vivo* experiments and other immunological work will be reported in a detailed publication[6].

Résumé. L'hormone folliculaire ovine (FSH) purifiée révèle la présence d'antigènes d'hormone lutéinare (LH) et de ceux du sérum ovin. L'antigène LH fait partie de ces derniers. L'absorption des antigènes d'antisérum de l'FSH avec le sérum ovin, élimine l'antigène qui accompagne ceux de l'hormone folliculaire ovine, de l'hormone lutéinaire et du sérum ovin. Ce fait suggère que le sérum ovin agit comme l'hormone lutéinaire. L'antisérum d'hormone lutéinaire est capable d'éliminer l'antigène d'hormone lutéinaire et les antigènes d'hormone folliculaire ovine. Cela signifie que l'un des andigènes du sérum ovin qui est présent aussi dans l'hormone folliculaire ovine, provient de l'hormone lutéinaire. Nos études en cours tendent à vérifier ce fait.

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[6] Ovine FSH and LH used in these studies were a gift from the Endocrinology Study Section, National Institute of Health. Human, bovine and porcine FSH and LH were a gift from Dr. A. E. WILHELMI, Emory University, Atlanta (Georgia). We are grateful to them for making these hormones available. It is a pleasure to acknowledge the help and encouragement given by the Director, Dr. V.R. KHANOLKAR. – This investigation is supported by a grant from the Indian Council of Medical Research.

Histochemistry of the Cytoplasmic Droplet in the Mammalian Spermatozoon

The cytoplasmic droplet invests the neck of young mammalian spermatozoon. By employing light and phase-contrast microscopy and classical methods of technique, some workers have described certain inclusions in the cytoplasmic droplet. According to GATENBY and WOODGER[1], the cytoplasmic droplet in the spermatozoon of *Cavia* contains a number of 'argentophil platelets or rods', which impregnate exactly like the 'Golgi apparatus' of younger spermatogenic cells. Later GATENBY and WIGODER[2] show its 'Golgi apparatus' as a reticulum. But SHARMA et al.[3] state that in *Cavia* its 'Golgi elements' are in the form of granules. Late Miss DHILLON (quoted from NATH[4]), working with the phase-contrast microscope, demonstrated 'Golgi elements' in the form of granules in the cytoplasmic droplet of spermatozoa of rats. GRESSON[5], while reviewing his previous observations on the spermatogenesis of mammals, states that 'a number of granules and irregularly shaped bodies' are present in it. NATH[4], however, is of the opinion that the 'irregularly shaped bodies' of GRESSON are artefacts. From the previous literature[6] it seems that no attempt has been made to study the histochemistry of its cytoplasmic inclusions.

The cytoplasmic droplet appears to play some significant role in the physiology of mammalian spermatozoon. Therefore, it was considered useful to describe here the results of a study of its histochemistry in certain mammals. For this investigation, the testicular material of the goat, sheep and buffalo was used. It was treated with various histochemical techniques[7]. Some classical 'Golgi' techniques, such as those of AOYAMA and KOLATCHEV, were also employed.

As the spermatid of the goat, sheep and buffalo differentiates into spermatozoon, most of its cytoplasm and cytoplasmic inclusions are gradually sloughed off through the posterior regions of the spermatozoon tail. However,

[1] J. B. GATENBY and J. H. WOODGER, Quart. J. micr. Sci. *65*, 265 (1921).
[2] J. B. GATENBY and S. B. WIGODER, Proc. Roy. Soc. B *104*, 471 (1929).
[3] G. P. SHARMA, G. C. CHAUDHURI, and V. S. SATTEE, Res. Bull. Panjab Univ. *38*, 157 (1953).
[4] V. NATH, Res. Bull. Panjab Univ. *95*, 1 (1957).
[5] R. A. R. GRESSON, Cellule *54*, 81 (1951).
[6] M. W. H. BISHOP and A. WALTON, in MARSHALL's *Physiology of Reproduction* (ed. by A. S. PARKES, 1960), 3rd edition.
[7] S. S. GURAYA, Cellule *62*, 95 (1961).

some of the cytoplasm containing certain inclusions persists in the region of the manchette. This residual cytoplasm constitutes the cytoplasmic droplet in the present material. It is a pear-shaped body and invests the neck of early spermatozoa seen in the testicular tubules. It is also known by various other names[6], such as protoplasmic bead, kinoplastic droplet, equilibrateur and acidophile body. Following three categories of cytoplasmic inclusions having diverse nature are identified in it: (1) sudanophil rods and granules, (2) sudanophobe bodies and (3) mitochondria (Figure 1, 2).

(1) *Sudanophil rods and granules.* Some sudanophil rods and granules are present in the cytoplasmic droplet of the present material (Figure 1, 2). Some of them seem to lie in association with a sudanophobe vacuole which gives a negative reaction with the various tests used. The histochemical reactions of the rods and granules are very similar to those of the rods and granules described by the author[8] in the spermatids of the goat and buffalo. They are also composed of phospholipids and some proteins. They are argentophil and osmiophil. This shows that they correspond with the osmiophil and argentophil 'plates' or 'rods' or 'granules' or 'irregularly shaped bodies' described by earlier workers under the names of 'Golgi apparatus' or 'Golgi granules' etc. The 'reticulum' described by GATENBY and WIGODER is not seen in the present material. It is, therefore, an artifact formed by the excessive deposition of silver and osmium on and in between the closely placed cytoplasmic inclusions of the droplet.

(2) *Sudanophobe bodies.* Besides the sudanophil rods and granules, there are also present one to two, generally one, sudanophobe bodies (Figure 1, 2). They are osmiophobe and argentophobe. Iron-haematoxylin stains them blue after fixation with Zenker, Carnoy and Bouin. In such preparations, they are not corroded. They are basiphil and stain pink with the methyl green/pyromin G technique. The ribonuclease and trichloroacetic controls show that they are rich in RNA combined with proteins.

(3) *Mitochondria.* The mitochondria are in the form of sudanophobe granules. They are aggregated adjacent to the axial filament (Figure 1, 2). Their histochemical reactions reveal their usual lipoprotein nature—the lipids in them being phospholipids.

All the three categories of cytoplasmic inclusions identified in the cytoplasmic droplet are the residual inclusions of spermatogenic cells. In the light of the present investigations, nothing can be said authentically about their role in the physiology of the spermatozoon. They probably constitute the endogeneous source of energy for the movement of the spermatozoon through the accessory ducts.

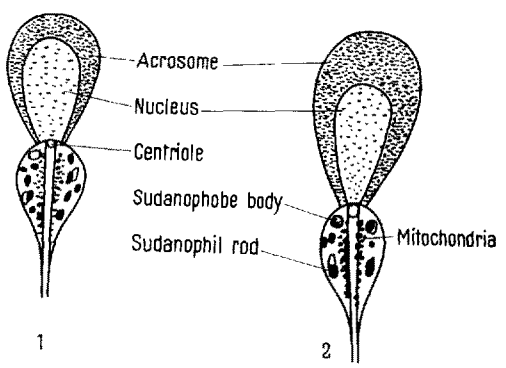

Fig. 1. Cytoplasmic droplet in the spermatozoon of the goat.
Fig. 2. Cytoplasmic droplet in the spermatozoon of the buffalo.

Résumé. Chez le bouc, le mouton et le buffle, la gouttelette cytoplasmique du spermatozoïde précoce contient des inclusions cytoplasmiques de différents genres: (1) des bâtonnets et granules sudanophiles constitués pars des phospholipides et des protéides, (2) un ou deux corps sudanophobes constitués par de l'acide ribonucléique et des protéides, et (3) des granules mitochondriés constitués par des lipoprotéides.

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[8] S. S. GURAYA, Exper. *18*, 167 (1962).
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Vitamintrennungen an Ionenaustauscherpapieren

Für die Untersuchung von Oligo- und Polyvitaminpräparaten besitzen einfache und zuverlässige Analysenmethoden besondere Bedeutung. In den letzten Jahren sind die Papierchromatographie[1] und Dünnschichtchromatographie[2] mit Erfolg in dieses Gebiet eingeführt worden.

Neue Möglichkeiten in der Vitaminanalyse bieten sich bei der Anwendung von Ionenaustauscherpapieren. Wir fanden, dass Gemische wasserlöslicher Vitamine und ihrer Zersetzungsprodukte durch eine solche Austauschchromatographie im Mikromaßstab besonders schnell und einfach scharf getrennt werden können. Für die Einstellung optimaler Adsorptionsverhältnisse kann man die Imprägnierung des Papiers variieren und das Harz in verschiedener Weise vorbehandeln. Zur Detektion eignen sich übliche Nachweisreaktionen der Vitamine und ihre Auswertung im sichtbaren und UV-Licht.

Kombinationen des Vitamin-B-Komplexes lassen sich gut an einem schwach sauren Ionenaustauscherpapier[3] trennen. Das Papier wird zunächst mit einer Standardacetatlösung vom pH 4,62 gepuffert. Die Vitamine trägt man in Mengen von 20 bis 100 µg auf, als Laufmittel dient

[1] J. A. BROWN und M. M. MARSH, Analyt. Chem. *24*, 1952 (1952).
[2] H. GÄNSHIRT und A. MALZACHER, Naturwissenschaften *47*, 279 (1960).
[3] Amberlite Ionenaustauscherpapier WA-2, Carboxylgruppenharz.