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.

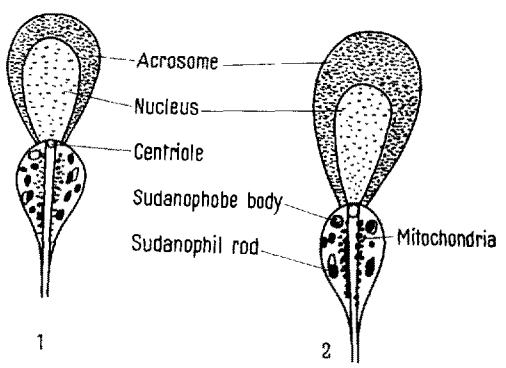

Fig. 1. Cytoplasmic droplet in the spermatozoon of the goat.
Fig. 2. Cytoplasmic droplet in the spermatozoon of the buffalo.

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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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

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[2] H. GÄNSHIRT und A. MALZACHER, Naturwissenschaften *47*, 279 (1960).
[3] Amberlite Ionenaustauscherpapier WA-2, Carboxylgruppenharz.

reines Wasser. Bei absteigender Chromatographie (im Dunkeln) wandert die Lösungsmittelfront in 120 min um ca. 20 cm. Die Rf-Werte einiger Vitamine betragen unter diesen Bedingungen:

Thiamin 0; Pyridoxin 0,04; Riboflavin 0,12; Cyanocobalamin 0,14; Nicotinsäureamid 0,18; *p*-Aminobenzoesäure 0,70; Folsäure 0,74; Panthenol 0,85; Na-riboflavinphosphat 0,88; Calcium-pantothenat 0,96.

Summary. A simple new method is described for the separation of water-soluble vitamins, using ion exchange-papers.

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The Effect of Sodium Nitrite on Red Cell GSH[1]

Introduction. In 1946 KEILIN and HARTREE[2] reported that nitrite resulted in the oxidation of red cell glutathione (GSH). It has been generally accepted that this occurs, and similar observations have formed the foundation of several other studies of red cell GSH[3,4]. The investigations reported briefly in this communication demonstrate that nitrite does not oxidize glutathione intracellularly, but that the apparent disappearance of GSH after incubation with nitrite is probably due to *in vitro* interaction of nitrous acid with GSH during the procedure for determining GSH.

Methods. These studies were carried out using human red cells obtained from a polycythemic donor. GSH determinations were carried out by a modification of the method of ELLMAN[5]: Proteins were precipitated with metaphosphoric acid (MPA) and salt and the filtrate added to a cuvette containing 0.5 *M* phosphate buffer pH 7.5. Color was then developed by the addition of 5, 5'-dithiobis (2-nitrobenzoic acid).

Results. It first became apparent to us that nitrite does not actually result in intracellular oxidation of GSH when we observed that repeatedly washing red cells in physiologic salt solution (without glucose) resulted in return of the GSH level to a concentration equal to that before treatment with nitrite. It is evident from Table I that this effect depends upon the number of washings and the volume of washing solutions. Identical results were obtained when the nitroprusside method[6] for determining GSH was employed. It is undoubtedly because of the large number of washings required that other investigators have not noticed this effect.

Tab. I. GSH measured in RBC after nitrite treatment and saline washing (% of initial value)

	Volume of washing solution 1–2	6	10
Washings	%	%	%
1	5	0.5	5
2	0.5	7	—
3	5	53	86
4	1	82	—
5	—	—	110
6	—	—	—
7	—	—	97

When nitrite was mixed with GSH and the analytic procedure, including the addition of metaphosphoric acid (MPA), was carried out in the usual manner, virtually all of the GSH had disappeared. However, if nitrite was added to the cuvette containing GSH and buffer, there was no loss of GSH and no interference with the GSH determination. The experiment depicted in Table II was therefore carried out. A solution approximately 25 mg of GSH per 100 ml of 0.5 *M* phosphate buffer, pH 7.0 was mixed with an equal volume of freshly prepared solutions of 0.25%, 0.12%, 0.06%, 0.03%, and 0.015% sodium nitrite. A control aliquot of GSH was incubated with physiological saline. Two-tenths of a milliliter of each nitrite-GSH mixture was then added to each of two tubes containing 1.8 ml of distilled water. To one of these tubes 3 ml of NaCl saturated 1.67% MPA was added, as is done in our modification of the Ellman technique for GSH determination. To the duplicate tube 3 ml of 0.5 *M* phosphate buffer, pH 7.5 was added instead of MPA. After standing for 5 min, GSH determinations were carried out on each of the mixtures in such a way that the final contents of each cuvette with respect to salt, MPA, and buffer were identical. As indicated in Table II, it was found that when a mixture of nitrite and GSH had been treated with MPA, virtually all of the GSH had been destroyed. In contrast, when phosphate buffer, pH 7.5 had been substituted for MPA the GSH concentration was unaffected by the presence of nitrite.

Preliminary studies have suggested that only slightly more than 1 μM of nitrous acid was required to destroy 1 μM of GSH. It is probable that the products of the reaction are NH_2OH and NH_3[7].

Discussion. These studies demonstrate that the apparent destruction of red cell GSH by sodium nitrite is an artifact produced during the protein precipitation step in the determination of GSH. The results emphasize the difficulty in removing nitrite from red cells, a phenomenon which we have observed previously in studies involving methaemoglobin reduction. The fact that high concentrations of methaemoglobin can exist in erythrocytes with-

Tab. II. GSH remaining after incubation with nitrite

	Mg% sodium nitrite in incubation mixture 120	60	30	15	$7^1/_2$	0
Incubated in acid	1.2	2.3	0.5	0.2	0	100
Incubated in buffer	115.4	98.2	98.5	94.5	95.4	100

[1] Supported in part by U.S.P.H.S. Grants H-5313 and H-5292.

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