

the mature spermatozoon, while all the cytoplasm of the spermatid is discarded except that which will later form the delicate membrane of the spermatozoon and the small

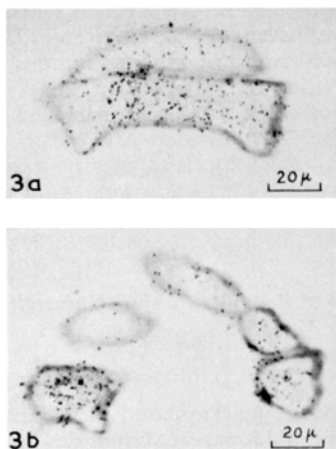


Fig. 3 a and b.—Autoradiograph of sectioned seminal receptacle of a female *D. melanogaster* showing labelled spermatozoa.

(3–5 μ) persisting tail remnant¹¹. An early spermatid after nuclear elongation has started is shown in Figure 4, and on average 10–15 grains associated with the nucleus were

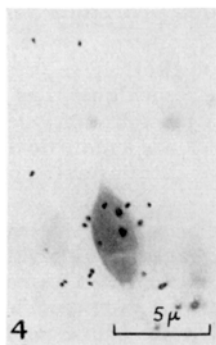


Fig. 4.—Autoradiograph of an early spermatid of *P. hirtellus* (smear).

observed. In more fully developed spermatozoa, in which the nucleus has greatly elongated, 20–25 grains were observed (Fig. 5 and 6). Though there has been an increase

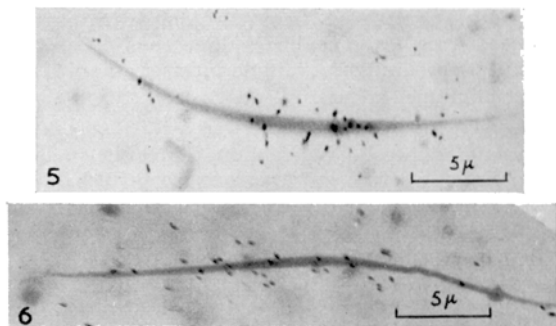


Fig. 5 and 6.—Autoradiographs of two stages of immature spermatozoa of *P. hirtellus* (smear).

¹¹ J. JACOB, *Cytologia*, in press (1958).

in nuclear content the amount of DNA has presumably not increased⁴ (the intensity of staining decreasing with maturation) and the RNA investment of the nucleus has become much thinner¹¹. The increase in tracer content may therefore indicate that the two generations of cells which gave rise to the less developed and more fully developed spermatozoa have incorporated different amounts of tracer in DNA, which could have been caused by the timing of the two injections.

We wish to thank Professor C. H. WADDINGTON, F.R.S., for his interest, the Royal Commission for the Exhibition of 1851 for a research award to one of us (J. J.), and the Medical Research Council for a grant made to Dr. ANN R. SANDERSON, Queen's College, Dundee.

J. JACOB and J. L. SIRLIN*

Institute of Animal Genetics, Edinburgh, June 18, 1958.

Résumé

La technique de marquage avec de l'adénine-8-¹⁴C des spermatozoïdes mûres du coléoptère *Ptinus hirtellus* et de *Drosophila melanogaster* est décrite. L'isotope était administré par injection dans les larves de *Ptinus* et par bouche dans celles de *Drosophila*.

* Research Worker of the British Empire Cancer Campaign.

What Constitutes a Network in the Acinar Cells of Amphibian and Mammalian Pancreas?

BOWEN¹, BEAMS², DUTHIE³, SUBRAMANIAM⁴, CHODNIK⁵, and NASSONOV⁶, all of whom have worked on vertebrate pancreas, described the so-called 'Golgi apparatus' as a reticulum consisting of solid strands. According to LACY⁷, however, it is in the form of canals. On the other hand, some modern cytologists like BAKER^{8, 9}, NATH¹⁰, and THOMAS¹¹ have denied categorically the existence of such net-like and canalicular 'Golgi apparatus' in both the somatic and germ cells. HIRSCH¹² and MORGAN¹³ have likewise stated that such structures are non-existent in mammalian pancreatic cells. The reasons put forward by the antagonists of the 'Golgi networks' for the appearance of such structures in pancreas can be summarized as follows:

(1) Silver and osmium, used in the classical 'Golgi' techniques, over-impregnate the lipid bodies or the neutral red granules and also fill up the spaces in between them^{8, 12, 13}.

(2) Golgi network may be formed simply by the deposition of silver or osmium between crowded secretion granules⁸.

¹ R. H. BOWEN, *Quart. J. micr. Sci.* 70, 75 (1926).

² H. W. BEAMS, *Anat. Rec.* 45, 137 (1930).

³ E. S. DUTHIE, *Proc. R. Soc. (London) [B]* 114, 20 (1933).

⁴ M. K. SUBRAMANIAM, *Proc. Indian Acad. Sci.* 9, 271 (1939).

⁵ K. S. CHODNIK, *Quart. J. micr. Sci.* 89, 75 (1948).

⁶ D. N. NASSONOV, *Arch. mikr. Anat.* 97, 136 (1923).

⁷ D. LACY, *J. R. micr. Soc.* 74, 226 (1953).

⁸ J. R. BAKER, *Nature* 172, 617 (1953).

⁹ J. R. BAKER, *Symp. Soc. exper. Biol.* 10, 1 (1957).

¹⁰ V. NATH, *Nature* 180, 967 (1957).

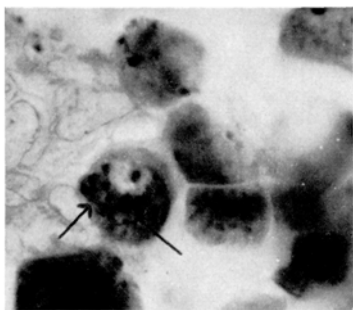
¹¹ O. L. THOMAS, *Quart. J. micr. Sci.* 89, 333 (1948).

¹² G. C. HIRSCH, *Form- und Stoffwechsel der Golgi-Körper* (Berlin 1939).

¹³ W. S. MORGAN, *Quart. J. micr. Sci.* 94, 269 (1953).

(3) WORLEY¹⁴ is of the opinion that the 'Golgi reticulum' is produced by the ruptured and elongated Golgi vesicles, and sometimes even the 'puddingstone' masses, as he observed in the active cells of frog pancreas, may appear in the form of a 'reticulum'.

I have studied the acinar cells of frog, toad, common house rat, and white rat pancreas by employing Aoyama, formaldehyde-osmium, Champy/iron-haematoxylin, formaldehyde-calcium/Sudan black, Lewitsky-saline/Sudan black¹⁵ or iron-haematoxylin and Regaud/iron haematoxylin or Sudan black techniques. To me it appears that in addition to the above reasons, the 'Golgi networks' may be formed in the amphibian pancreatic acinar cells due to the following causes also:



(1) When the zymogen synthesis starts in the cells, the medulla of the lipid vesicles, inside which the proteins are being synthesized, grows, and the outer sudanophil, osmiophil, and argentophil cortex, which is ultimately reduced to a thin crescentic cap, is seen attached to the maturing zymogen granules. These residual lipid crescents detach from the extruding zymogen granules and aggregate haphazardly in the supra-nuclear region. Such an aggregation of the lipid remnants, if superficially observed, gives the appearance of the so-called disintegrated 'Golgi apparatus', said to have been observed during the resting phase or early discharge phase^{1,4}.

(2) With the onset of the discharge phase, there appear in the supra-nuclear zone, popularly known as 'Golgi zone' (free from zymogen granules), filamentous mitochondria which lie loosely interwoven and interspersed with the lipid granules and spheres. These mitochondria are stainable with acid fuchsin after Regaud, whereas the lipid bodies are not. These filamentous mitochondria, as well as the lipid bodies, are blackened in osmium tetroxide preparations to give the appearance of a typical 'Golgi network'. But the truth is revealed when such preparations are bleached with saturated solution of potassium persulphate and coloured afterwards with Sudan black B. This dye colours only the lipid bodies whereas the mitochondria remain uncoloured and hence there is no 'Golgi network'.

A very striking example of the filamentous mitochondria, appearing in the form of a 'network' (Golgi), is shown in the Figure, which is of a cell in recovery phase from Lewitsky/iron-haematoxylin preparation of white rat pancreas. Though this reticulum resembles closely the typical 'Golgi network', yet it is certain that it is composed of only the mitochondria, as no previous worker has ever described the 'Golgi network' in Lewitsky/haematoxylin preparations. If the fibrillar mitochondria can give the appearance of a 'network' in such *harmless* techniques,

one can very well imagine the appearance which this 'loose network' of the mitochondria would give, when drastic techniques like KOLATCHEV, AOYAMA, DA FANO etc., involving long metallic deposition, are employed.

My observation on the acinar cells of anurans and rat pancreas have thoroughly convinced me that most of the 'Golgi networks', appearing in osmium techniques, in the acinar cells during the late discharge and early recovery phases, are nothing but over-impregnated mitochondrial fibres.

Similar reasons for the appearance of the 'Golgi network' in neurones have been given by THOMAS¹¹ and CAIN¹⁶.

K. C. KANWAR

Department of Zoology, Panjab University, Hoshiarpur (India), June 25, 1958.

Résumé

Il a été montré que l'appareil réticulaire classique de Golgi dans les cellules pancréatiques des amphibiens et des mammifères est un produit artificiel. Un certain nombre de raisons sont proposées pour expliquer ce fait.

¹⁶ A. J. CAIN, *Quart. J. micr. Sci.* 89, 421 (1948).

Sur l'interaction de la Protamine avec des protéines sériques

De nombreux polyélectrolytes présentent des interactions avec les protéines sériques. Les plus connues de ces interactions sont la précipitation sélective des β -lipoprotéines par l'héparine ou le sulfate de dextrane en présence de Ca^{++} ou bien par certains autres polysaccharides sulfatés¹.

Ces mêmes polyélectrolytes présentent une interaction notable avec le complexe haptoglobine sérique-hémoglobine, dont elles empêchent la formation et inhibent son activité peroxydasique². Comme l'haptoglobine est un mucoïde α_2 de pH isoélectrique 4,1, on comprend que l'interaction électrostatique ne soit pas le seul facteur déterminant l'affinité des polyélectrolytes pour les protéines sériques³.

Deux d'entre nous ont montré que la salmine ajoutée à un sérum humain normal ou pathologique produit une opacification initiale, suivie (pour une certaine concentration de salmine, 2 mg/ml) à 37°C d'une clarification⁴. L'étude physiopathologique de cette réaction fut effectuée par DE GENNES et TRUFFERT⁴. D'autre part, nous avons entrepris des expériences dont nous présentons ici les résultats préliminaires afin de préciser au point de vue physicochimique la nature de l'opacification et de la clarification.

Nous avons cherché tout d'abord à préciser quelles sont les protéines du sérum qui seraient susceptibles de former avec la salmine un complexe insoluble dans les conditions

¹ P. BERNFELD, V. M. DONAHUE et M. E. BERKOWITZ, *J. biol. Chem.* 226, 51 (1957).

² L. ROBERT, V. BAJIC et M. F. JAYLE, *C. R. Acad. Sci.* 242, 2868 (1957).

³ M. F. JAYLE et G. BOUSSIER, *Exposés annuels de Biochimie Médicale* (1956).

⁴ J. L. DE GENNES, *Presse méd.* 65, 263 (1957). – J. L. DE GENNES et J. TRUFFERT, *C. R. Soc. Biol.* 148, 1361 (1954); *C. R. Soc. Biol.* 149, 648 (1955).

¹⁴ L. G. WORLEY, *J. Morph.* 75, 261 (1944).

¹⁵ J. R. BAKER, *Quart. J. micr. Sci.* 97, 621 (1956).