but in some epithelial cells from cultures exposed to 8 puffs, the whole of the apical cell area bulged out into the lumen (Figure 4). The cilia were also observed to have swollen in the same way, but the internal microtubular structure of the individual cilia was not affected (Figure 5). After exposure to 12 puffs of whole smoke, these effects were present in more of the epithelial cells and many of the columnar cells were found to have undergone lysis, leaving a layer of cellular debris and exposing the less differentiated basal cells (Figure 6). An increase in the number of secondary lysosomes, especially of the autophagic vacuole type, was noted in all types of cells including the basal cells.

Discussion. The maintenance of the basic morphology of 'positive' control preparations under the experimental conditions described demonstrates that this model system is potentially useful for studying the effects of in vitro exposure of a respiratory epithelium to cigarette smoke. The use of a standardized fixation following immediately upon exposure may visualize cellular changes which would subsequently be lost in a more extensive cell and tissue breakdown or, alternatively, in recovery and repair.

FRASCA et al.[11] described ballooning of the apical membranes of cells in the bronchial epithelium of dogs which had been exposed daily to up to 12 cigarettes for a period of 44 days. Our similar findings were produced after only 8 or 12 puffs, suggesting that the results observed in the dogs may have been due to the acute cytotoxicity of the smoke exposure preceeding fixation rather than to any long term cumulative effects. It is interesting to note that NIDEN[12] observed ballooning of the apex of bronchiolar Clara cells in adult mice exposed to ammonia. Exposure to smoke may inhibit the energy-dependent maintenance of osmotic pressure within the cells such that solutes will pass across the cell membrane along a concentration gradient. As the apical surface of the cell is the most exposed to the smoke, this might explain the development of ballooning at this site. Swelling of cilia could be an explanation for ciliostasis.

An effect of tobacco smoke on cell junctions in the respiratory epithelium has been noted by SIMANI et al.[13] in guinea-pigs exposed to more than 50 cigarettes. These workers used horseradish peroxidase to demonstrate the failure of the junctions to maintain an effective seal against the passage of larger molecules. Although in the present study there were no changes in the ultrastructure of the junctions, the development of widened intercellular spaces immediately adjacent to these structures may have been due to an impairment of their highly specific function.

In the foetal tracheae used in this study, no mature goblet cells could be observed. Thus the lack of a mucin layer overlying the epithelium might exacerbate the cytotoxic effects of the smoke. In the post-natal trachea, where goblet cells are present, such a layer would act, in short-term exposures, as a barrier to both the particulate and gas vapour phases of smoke. Work is continuing to determine if there are any differences in the response to smoke exposure between the foetal and the adult rabbit tracheal epithelium[14].

Summary. Foetal rabbit tracheal organ cultures were exposed under defined conditions to whole cigarette smoke and fixed immediately for electron microscopy. After an exposure to 4 or 8 puffs, epithelial intercellular spaces were enlarged, the apical portion of many cells bulged out into the lumen and many cilia were swollen. An exposure to 12 puffs produced a breakdown of the epithelium.

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The Sperm Bundles of Honeybee *Apis cerana indica* Fabr.

The sperm bundles are characteristics of the insect testes. They have been reported in Coleoptera, Orthoptera, Homoptera and Odonata[1–5]. The present work was undertaken to study the sperm bundles of the honeybee, *Apis cerana indica* Fabr.

The honeybee brood was reared at 34 °C in the laboratory. The testes, seminal vesicles, and accessory glands from living pink head pupae and newly emerged adults were used. For routine microanatomy, Zenker and Carnoy fixed material was sectioned and stained in haematoxylin/eosin.

Cytochemical localization of lipids was made on neutral formalin and gelatin embedded material postchromed according to BAKER[6]. Frozen 10 µm thick sections were cut and stained for lipids with Sudan black B in propylene glycol after the method of CHIFFELLE and PUTT[6]. Phospholipids were stained with acid haematein along with pyridine extracted controls according to BAKER[6], and neutral and acidic lipids with Nile blue after Cain[6]. Carbohydrates were localized in material fixed for 3–6 h after Zenker, or 1–4 h at 0–4 °C after Carnoy or Gendre's. The PAS reaction and Best's carmine along with acetylation and KOH reversal after MCMANUS[6] and diastase digestion were used as controls. Acid mucopolysaccharides were stained with alcian blue.

The sperm bundle of honeybee *A. cerana indica* Fabr. is comprized on an average of 72 sperms arranged in a definite hexagonal geometric array (Figure 2b). Each sperm bundle has a hyaline cap at its anterior region (Figure 1). This is formed by a double walled nutritive sac, covering most of the anterior portion of the bundle

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(Figure 2, a, b, c). The sac is filled up with enormous amount of phospholipids and carbohydrates. As the sperms gain their entry into the seminal vesicle, the hyaline cap disappears and the sperms are no longer seen in bundles. They are mostly present with their heads oriented towards the secretory epithelium, lining the seminal vesicle (Figure 3). Individual spermatozoa have also been recorded in the secretion of male accessory gland (Figure 4).

The present studies reveal that the sperm bundles of the honeybee *A. cerana indica* Fabr. have a hyaline cap at its anterior region formed by a double walled nutritive sac. Such type of hyaline caps have also been reported in

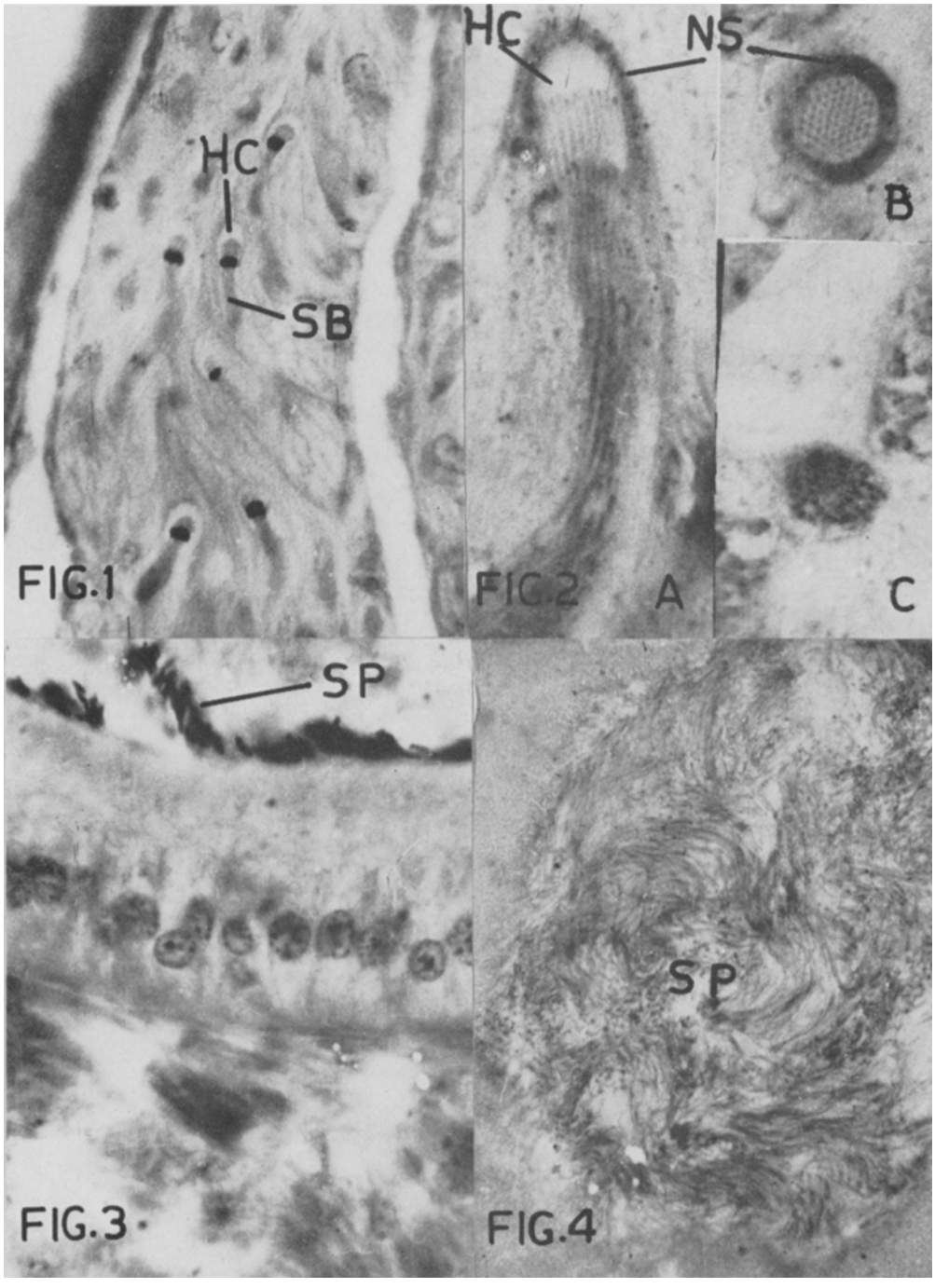

Fig. 1. Pupal testis: longitudinal section of testicular tubule of honeybee, showing sperm bundles (SB) with hyaline cap (HC) at the anterior region. Haematoxylin-eosin, ×1000.

Fig. 2. Sudan black B-preparations of sperm bundles. a) Longitudinal section of a sperm bundle with a hyaline cap (HC) at its anterior region. ×2500. b) Transverse section from the anterior half of the sperm bundle showing the presence of double walled nutritive sac (NS). ×2500. c) Transverse section from the posterior half of the sperm bundle showing the absence of double walled nutritive sac (NS). ×2500.

Fig. 3. Adult seminal vesicle of male, iron haematoxylin. Note the sperms have lost their hyaline cap and are lying free with their heads mostly oriented towards the secretory epithelium of the seminal vesicle. ×2500.

Fig. 4. Adult accessory gland stained with Sudan black B. Observe the sperms (SP) in the secretion of male accessory gland. ×1000.

grasshoppers, mealy bugs and even in Tettigonids[1–4]. The significance of the phospholipids and carbohydrates in the nutritive sac cannot be acertained at present. It is very likely that the oxidation of the large quantity of fatty acids liberated by the hydrolysis of phospholipids might help the honeybee spermatozoa in endogenous respiration during their storage in the testis. The experimental evidence is still lacking. The additional advantage of the sperm bundles in the testis seems to bring well-coordinated and synchronous beating of the spermatozoa during their migration towards the seminal vesicle.

The presence of the sperms in the male accessory glands which has not been reported so far, is still more intriguing. This might act as an additional reservior, for the storage of spermatozoa before ejaculation.

Zusammenfassung. Die Spermiozeugmen in den Hoden von Puppen und jungen Drohnen der indischen Honigbiene *Apis cerana indica* Fabr. enthalten durchschnittlich 72 Spermien, deren Köpfe in einer Ebene regelmässig hexagonal nebeneinander angeordnet sind und von einer hyalinen Kappe umgeben sind. Diese besteht aus einem doppelwandigen Nährsack, der mit Phospholipiden und Kohlehydraten gefüllt ist. Spermiozeugmen und hyaline Kappen lösen sich auf, wenn sie die Samenblasen der Adulten erreicht haben.

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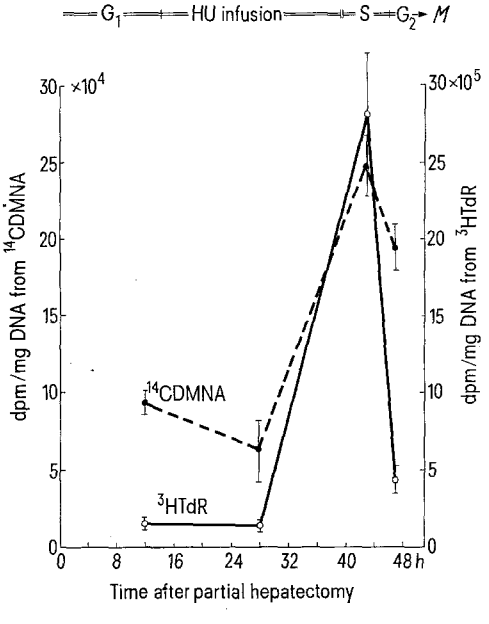
Synchronized Liver Cells: A New Tool for Analysis of Cell-Cycle Dependent Carcinogen-Binding to DNA in vivo

Alkylation of DNA is considered relevant to the induction of tumors by alkylating agents[1,2]. It is not definitely established in vivo if the physical state of DNA determines its sensitivity to alkylation, and in particular, if replicating DNA provides the most favourable target for specific carcinogen-binding. Using regenerating rat liver as a model system, no clear-cut evidence for dependence on cell-cycle phase of the degree of alkylation of liver DNA by alkylating carcinogens has been obtained[3,4]. Experiments with regenerating liver are hindered by the fact that, even after two-thirds hepatectomy, the degree of synchrony of cell-cycle phases of hepatocytes is rather low[5]. However, synchrony of DNA synthesis in regenerating rat liver is enhanced dramatically after an accumulation of hepatocytes at the G_1-S boundary by means of a continuous infusion of hydroxyurea (HU) following partial hepatectomy. HU is given at a dose level known to inhibit the start of DNA synthesis in vivo[6,7]. Following this treatment up to 90% of hepatocytes starts DNA synthesis simultaneously[8]. This model system seems to be adequate for quantitative comparison of the degree of DNA alkylation prior to, during, and after DNA replication in vivo, i.e. in G_1-, S- and G_2-phase of the cell cycle.



Specific activity of DNA after simultaneous i.p. injection of a carcinogen, N,N-di (^{14}C) methylnitrosamine, and a DNA precursor, ^{3}H-thymidine, at different intervals after partial hepatectomy (abscissa) prior to, during ,or at different intervals after an inhibition of DNA synthesis induced by a continuous infusion of hydroxyurea from 14 to 39 h after partial hepatectomy. Open circles, specific activity from 3H-TdR (right ordinate); closed circles, specific activity from ^{14}C-DMNA (left ordinate). Rats sacrificed 120 min after injection of label. Mean and S.E. (vertical bars) of 6 rats in each group.

Materials and methods. Male Wistar rats (220–240 g, AF/Han) were two-thirds hepatectomized[9] and received, in groups of 6 rats each, an i.p. injection of both a labelled carcinogen, N,N-di(^{14}C)methyl-nitrosamine (0.18 μCi/g body weight, spec. act. 9.3 mCi/mmol, ^{14}C-DMNA, Radiochemical Centre Amersham), and a tritiated DNA precursor, ^{3}H-thymidine (0.8 μCi/g body weight, spec. act. 5 Ci/mmol, ^{3}H-TdR, Radiochemical Centre Amersham) following different pretreatments: Group a) injection of ^{14}C-DMNA and ^{3}H-TdR 12 h after partial hepatectomy (PH); group b) injection of ^{14}C-DMNA and ^{3}H-TdR 28 h after PH during a continuous infusion of HU (1.25×10^{-3} *M*/kg/h, starting 14 h after PH); group c) injection of ^{14}C-DMNA and ^{3}H-TdR 43 h after PH, 4 h after stopping a continuous infusion of HU from 14 to 39 h after PH; group d) injection of ^{14}C-DMNA and ^{3}H-TdR 47 h after PH, 8 h after stopping the continuous infusion of HU. All rats were sacrificed 120 min after injection of the labelled material and liver DNA was extracted[10,11]. Radioactivity from ^{14}C and ^{3}H was

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