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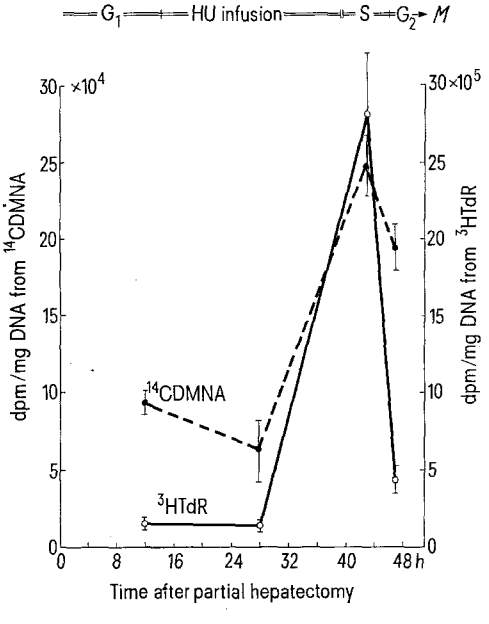
Synchronized Liver Cells: A New Tool for Analysis of Cell-Cycle Dependent Carcinogen-Binding to DNA in vivo

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

1 P. N. MAGEE and E. FARBER, Biochem. J. *83*, 114 (1962).
2 P. BROOKES and P. D. LAWLEY, J. Cell comp. Physiol. *64* Suppl. 1, 111 (1964).
3 V. M. CRADDOCK, Biochim. biophys. Acta *312*, 202 (1973).
4 R. GOTH and M. F. RAJEWSKY, Z. Krebsforschung *82*, 37 (1974).
5 H. M. RABES and H. V. TUCZEK, Virchows Arch. Abt. B Zellpath. *6*, 302 (1970).
6 H. S. SCHWARTZ, M. GAROFALO, S. S. STERNBERG and F. S. PHILIPS, Cancer Res. *25*, 1867 (1965).
7 M. F. RAJEWSKY, Expl. Cell Res. *60*, 269 (1970).
8 H. M. RABES, G. ISELER, H. V. TUCZEK and R. KERLER, Experientia *30*, 1116 (1974).
9 G. M. HIGGINS and R. M. ANDERSON, Arch. Path. *12*, 186 (1931).
10 H. N. MUNRO and A. FLECK, in *Methods of Biochemical Analysis* (Ed. D. GLICK; Interscience Publishers, New York, London, Sidney 1966), vol. 14, p. 113.
11 K. BURTON, Biochem. J. *62*, 315 (1956).

measured separately in a liquid scintillation counter (Packard 3214) and corrected for quench using an internal standard.

Results and discussion. Specific activities of DNA at different cell-cycle periods are summarized in the Figure. Specific activity of DNA from ^{3}H-TdR is low in presynthetic G_1 and during HU-induced inhibition of DNA synthesis at 28 h after PH. This is in agreement with previous results[8]. 4 h after release from the HU block DNA synthesis shows a high rate; it is reduced again after another 4 h, i.e. 8 h after termination of the HU block. At that time, hepatocytes have completed DNA replication and are accumulated in G_2.

Specific radioactivity as measured from ^{14}C in presynthetic G_1 and during HU-induced inhibition of DNA synthesis is low and without a significant difference (Figure). Coincidently with the upsurge of DNA synthesis as measured at 4 h after release from HU block, incorporation of ^{14}C radioactivity from ^{14}C-DMNA is increased significantly. Specific activity of DNA from ^{14}C-DMNA stays at an increased level even after decline of DNA synthesis at 8 h after HU block, i.e. in G_2.

These results indicate that the uptake of ^{14}C radioactivity derived from ^{14}C-labelled methyl groups of DMNA is different in various cell-cycle phases, low in G_1-, enhanced in G_2-, and highest in S-phase. In accordance with the observation of an increased susceptibility to a carcinogen of epidermal cells during stimulated DNA synthesis[12], different sensitivity of liver DNA to carcinogen-induced base alkylation could be assumed. However, the specificity of carcinogen-binding to DNA remains to be evaluated, as regards site, degree and persistence of a possible alkylation. Non-specific incorporation of label from the 1-carbon pool into purine bases and different levels of enzymatic activation of the alkylating carcinogen during the various phases of the hepatic cell cycle have to be taken into account, too. For attacking these questions, the model of synchronized rat liver cells in vivo might provide an appropriate tool.

Zusammenfassung. Die Bindung von Radioaktivität aus dem Carcinogen N,N-Di (^{14}C)methylnitrosamin an DNA ist in der Hydroxyharnstoffsynchronisierten, regenerierenden Rattenleber in der G_1-Phase und während der Hemmung der DNA-Synthese gering, erreicht während der synchronisiert ablaufenden DNA-Synthese-Phase maximale Werte und ist auch in der G_2-Phase noch erhöht.

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[12] J. V. Frei and T. Harsono, Cancer Res. *27*, 1482 (1967).

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Effect of Iododeoxyuridine on Tumor Induction in X-Irradiated Rats

The thymidine analogue, 5-iododeoxyuridine (IUdR), is incorporated specifically into the DNA of growing cells and increases the sensitivity of these cells to the lethal effects of X-irradiation[1–3]. This compound does not appear to be appreciably carcinogenic in rats[4] or mice[5]; however, it does stimulate the production of tumor-associated viruses by tissue culture cells[6]. Further, injection of IUdR in mice produced a small but significant increase in the number of skin papillomas caused by a subsequent application of 3-methylcholanthrene[5]. We have therefore examined the combined effects of IUdR and X-radiation in order to determine whether this compound would increase the carcinogenic effects of X-radiation in rats.

Methods. Each group of animals consisted of approximately equal numbers of male and female rats of a blackhooded Collip strain. The experimental treatments, which were commenced at 5 weeks of age, were as follows: A) controls, no treatment; B) 5 injections of 500 mg IUdR/kg i.p. over a total period of 5 weeks; C) 5 whole body exposures of 165 R X-radiation (300 kVcp) over a period of 5 weeks: D) 5 injections of IUdR as in B) plus 5 exposures of 165 R as in C), the X-radiation being given in each case 24 h after the IUdR injection. The surviving animals were held until they were 16 months of age and examined for tumors as in the previous experiments[7].

[1] B. Djordjevic and W. Szybalski, J. exp. Med. *112*, 509 (1960).
[2] R. J. Berry and J. R. Andrews, Nature, Lond. *196*, 185 (1962).
[3] R. L. Erikson and W. Szybalski, Cancer Res. *23*, 122 (1963).
[4] Z. Hadidian, T. N. Frederickson, E. K. Weisburger, J. H. Weisburger, R. M. Glass and N. Mantel, J. natn. Cancer Inst. *41*, 985 (1968).
[5] T. Arata, S. Tanaka and C. M. Southam, J. natn. Cancer Inst. *40*, 623 (1968).
[6] N. Teich, D. R. Lowy, J. W. Hartley and W. P. Rowe, Virology *51*, 163 (1973).
[7] D. K. Myers, Experientia *29*, 859 (1973).

Lethality and tumor induction in rats with X-radiation and IUdR

treatment	Lethality in 60 days (number dead/number treated)	Number of rats alive at 16 months of age	Cumulative % incidence of tumors by 16 months of age		
			Mammary tumors	Leukemia + lymphomas	Other tumors[a]
A) control	0/115	105	2	3	0
B) IUdR	0/30	26	0	3	0
C) X-ray	1/66	42	17	21	12
D) IUdR + X-ray	34/65	18	16	26	3

[a] The miscellaneous tumors included 1 osteosarcoma, 1 adenoma, 4 hemangiomas, 2 carcinomas and 1 sarcoma; small keratinized skin growths were not included.