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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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].

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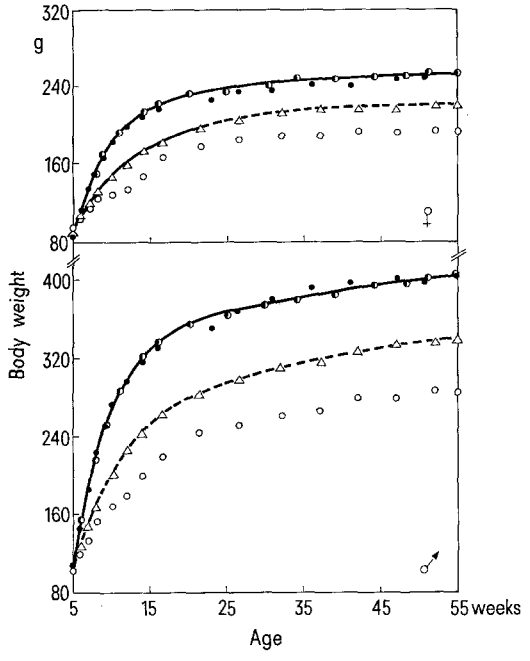
Lethality and tumor induction in rats with X-radiation and IUdR

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.

Results. The IUdR treatment alone did not appear to interfere with the growth of the animals (Figure) or to induce tumor development (Table). However, the injections of IUdR did increase markedly the short-term lethal effects of X-radiation (Table); there was also a marked interference with the growth of the surviving animals after the exposure to both agents together (Figure). The radiosensitization of the whole animal by IUdR is thus in agreement with the effects of IUdR on isolated mammalian cells[1-3]. However, IUdR failed to increase the incidence of tumors elicited by X-radiation (Table).



Effect of X-radiation and of IUdR on the mean body weight of female (top graph) and male (bottom graph) rats. Treatments were A) control (◐–◐); B) IUdR alone (●); C) X-radiation alone (△ – △); D) IUdR plus X-radiation (○).

It is possible that different schedules of IUdR treatment might stimulate skin tumor production by X-radiation, in the same manner as demonstrated in mice when using local applications of methylcholanthrene[5]. However, the present data show that incorporation of sufficient IUdR into the DNA of the animal to increase appreciably the lethal effects of X-irradiation did not augment the total carcinogenic effects of whole-body radiation among the surviving animals. These results differ from those obtained when ionizing radiation was combined with other carcinogenic agents such as urethane or fluorenylenebisacetamide, in which case the pattern of tumor development was altered markedly[7,8]. The difference may reflect the fact that IUdR is selectively incorporated into the DNA of only those cells which are actively synthesizing DNA at the time of injection.

Zusammenfassung. Nach Injektion von Jod-Desoxyuridin bei Ratten hat die letale Wirkung von Ganzkörper-Röntgenbestrahlungen zugenommen ohne die Anzahl der entstandenen Tumoren zu vermehren.

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Effects of Ether Anesthesia on Plasma Prolactin Sampling

There is considerable evidence to indicate that the stress of etherization in rats causes a rapid increase in serum prolactin[1-6]. GROSVENOR et al.[1] showed that laparotomy under ether depleted pituitary prolactin as effectively as nursing. NEILL[2] has reported increases in serum prolactin following the 'stress' of ether anesthesia. More recently, TERKEL et al.[3] and AJIKA et al.[4,5] have demonstrated elevated serum prolactin levels following 2 min of etherization. NEILL[2,6] has warned that considerable care should be taken in animal handling and in selecting a method of blood collection when assaying blood for prolactin.

In this study an intra-atrial cannula was used to withdraw blood which was subsequently assayed for prolactin by radioimmunoassay. This method of blood collection markedly facilitated the evaluation of the relationship of ether 'stress' and blood prolactin levels. It has also provided insight into the optimal time period from which a 'normal' sample can be safely withdrawn for prolactin analysis in rats under ether anesthesia.

Materials and methods. 17 60-day-old male Sprague-Dawley rats from Spartan Animal Farms, Inc., (Haslett, MI) weighing 275–325 g were used. The rats were housed in a room maintained at 24 °C which was illuminated 14 h/day. They were fed a standard laboratory chow (Allied Mills, Chicago, Ill.) and water ad libitum. After installation of cannulas, the rats were housed in separate cages for the remainder of the experiment.

In rats anesthetized with Nembutal (35 mg/kg body weight), a silicone rubber (Silastic) cannula was passed down the right external jugular vein into the right side of the heart as described by TERKEL[7] and WEEKS[8]. The intravascular portion of the cannula consisted of 0.012 inch (i.d.), 0.025 inch (o.d.) Medical Silastic (Dow-Corning, Midland, MI) tubing connected to PE-60 polyethylene tubing (Clay-Adams, Inc., N.Y., NY) joined with a silicone seal (General Electric Co., Silicone Products Dept., Waterford, N.Y.). The cannulas were exteriorized and fastened at the back of the neck, filled with heparinized saline (300 U/ml), and sealed by inserting a straight pin into the end of the cannula.

The rats were placed in a container saturated with ether (ether for anesthesia, Mallinckrodt, St. Louis, MO) at

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