tionen kann dieser Effekt möglicherweise deshalb nicht sichergestellt werden, weil bei den gewählten Volumenrelationen zwischen Inkubationsmedium (1,5 ml) und RF-Substanz (6 µl) und einer einstündigen Inkubationszeit vermutlich nicht ausreichend Aminmoleküle in die Nähe von Ankergruppen der Fadenmoleküle gelangen. Auf Schüttelbewegungen während der Inkubation zur Beschleunigung der Diffusion musste weitgehend verzichtet werden, da die sehr geringe Masse des RF-Materials sonst an den Wänden der Untersuchungsgefässe haften und verkleben kann.

Gebundene Amine sind am RF auch nach mehrstündigen Waschungen mit den gewählten Medien nachweisbar. Damit dürfte der Bindungstyp im vorliegenden Falle nicht nur durch Ionenbeziehungen gegeben sein (vgl. [10, 12]). Möglicherweise bestehen ähnliche Verhältnisse, wie sie MOKRASCH und ANDELMAN[11] bei der Untersuchung eines Bindungstypes zwischen Lipoproteiden aus dem Hirn und Aminen beschreiben. Anzeichen dafür, dass an die RF-Substanz primär zusätzlich Aminmengen gebunden und während des Waschvorganges im Sinne eines Ionenaustausches freigesetzt werden, ergaben sich nicht.

Die Problematik einer möglichen physiologischen Bedeutung der Aminbindung an den RF ist sehr komplex. Grundsätzlich besteht die Möglichkeit, dass der RF an einer Regulation des Liquor-Amin-Pegels beteiligt ist, wenn die in vitro gefundene Bindung von Monoaminen auch in vivo stattfindet. Als Hinweise auf diese Möglichkeit ist zu werten, dass der RF von Katzen in vivo ^{3}H-Tyrosin binden kann[6].

Da im Bereich des Zentralkanales die RF-Masse (Frischgewicht) zum Teil mehrere Prozent der Liquormasse[3] beträgt und auffällige Unterschiede im Bindungsvermögen der RF-Substanz selbst gegenüber ähnlichen Molekülen (A und NA) gefunden werden, kann in den vorliegenden Ergebnissen ein erster Ansatzpunkt zur weiteren experimentellen Überprüfung der Bindungstheorie, die zum Beispiel auch unter Einsatz von bestimmten Metaboliten erfolgen sollte, gesehen werden.

Summary. The glycoprotein of Reissner's fibre is able to bind norepinephrine, epinephrine and serotonin. Bound amines can be demonstrated at the RF, even after washing with artificial cerebrospinal fluid for some hours. It is discussed whether it is a function of the RF to remove substances, for instance biogenic amines, from the cerebrospinal fluid.

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Further Studies on the Circadian Rhythm in the Proliferative Activity of Mouse Intestinal Epithelium

Circadian rhythm in cell proliferation of intestinal mucosal cells have been disputed. BULLOUGH[1] in 1948 and ALOV[2] in 1963 have reported slight and variable rhythms in proliferative activity while KLEIN and GEISEL[3], LEBLOND and STEVENS[4] and PILGRIM, ERB and MAUER[5] could not show similar results. SIGDESTAD et al.[6] using the crypt squash technique were able to demonstrate a distinct daily rhythm in the number of cells synthesizing DNA and the number of cells in mitosis. They found the peak and nadir of both parameters tested at 03.00 and 15.00 h respectively. It was the purpose of this investigation to further resolve the peaks in mitosis and DNA synthesis in mouse intestinal epithelium.

Male C57Bl (Schmidt, Madison, Wisc.), 140–150-day-old mice were used throughout this investigation. They were maintained on sterilized Purina Laboratory Chow and tap water ad libitum. The animal rooms were maintained at a constant temperature and humidity and on a strict 12 h light (06.00–18.00 h) and a 12 h dark (18.00 to 06.00 h) cycle. At hourly intervals between (24.00 and 05.00 h) 3 mice were injected i.p. with 50 µCi tritiated thymidine (^{3}HTdR, 0.36 Ci/m*M*) and sacrificed by cervical dislocation 30 min later. Cold Carnoy's solution was injected into the jejunum just below the ligament of Treitz. A segment was removed for the determination of the number of mitotic figures and labeled nuclei per crypt using crypt squash and autoradiographic techniques described by WIMBER et al.[7].

The amount of tritiated thymidine taken up by each crypt was determined by liquid scintillation counting. 50 crypts were pipetted into liquid scintillation vials containing 0.5 ml Soluene (Packard Instrument Co., Downers Grove, Ill.), allowed to stand at room temperature for 30 min then 10 ml scintillation solution (5.0 g PPO, 0.2 g M_2POPOP in 1000 ml toluene) was added. The vials were counted in a Packard Liquid Scintillation Spectrometer, Model 3380 operated at 5 °C; quench correction utilized the absolute activity Analyzer (model 544).

The results are presented in Figures 1–3. Utilizing closer test intervals in the present experiments it was possible to resolve the peak in the number of cells in mitosis and in the number of cells synthesizing DNA. The peak previously reported at 03.00 h was replaced by a peak at 02.00 h. Good correlation was seen in the data, at times duplicated in the previously reported results.

Unlike previous data there was no suggestion of a lag in the peak in mitosis relative to the DNA synthetic peak.

In a previous report[6] we reported a distinct diurnal rhythm in mitotic figures, S cells and dpm/crypt measurements in the mouse intestine. The peak was found to occur at 03.00 h while the nadir was seen at 15.00 h. The experiments reported here, have further resolved the peak in the 3 parameters tested to occur at 02.00 h. The significance of these results rests in the interpretation of the mechanisms involved in the diurnal control of cell proliferation in the gut.

PILGRIM, ERB and MAUER[5] and more recently BROWN and BERRY[8] suggested that diurnal rhythm may be due to partial synchronization of the cells in the DNA syn-

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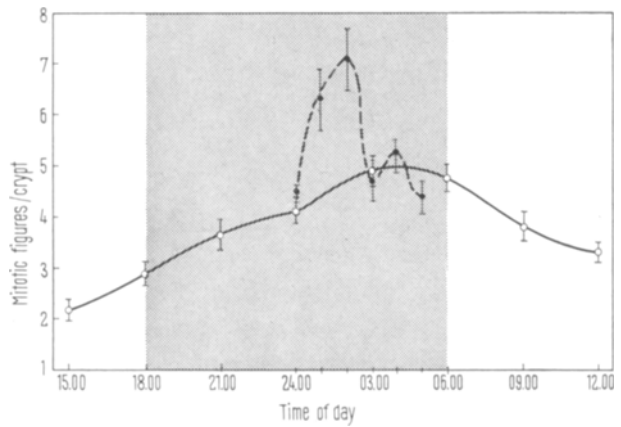

Fig. 1. Daily variation in the number of mitotic figures per crypt. Open circles are previously reported results. Shaded area represents the dark cycle in the animal rooms.

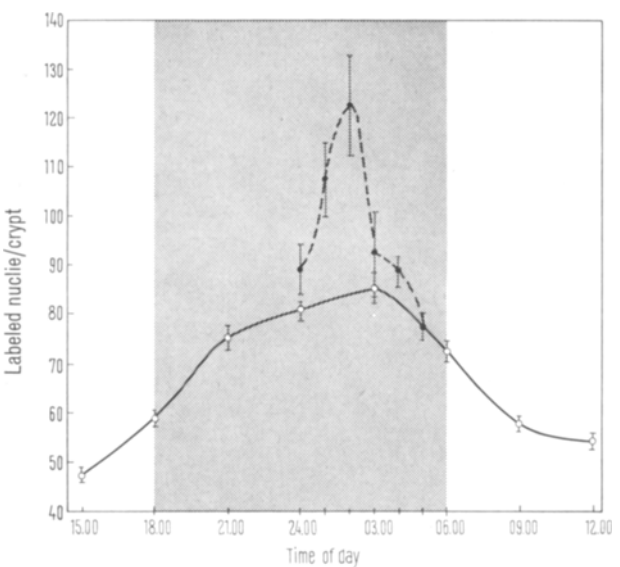

Fig. 2. Variation in the number of cells synthesizing DNA (S-cells) as a function of time of day. Open circles are previously reported results. Shaded area represents the dark cycle in the animal rooms.

thetic phase of the cell cycle. This cohort would then be expected to peak in mitosis after $0.5\ T_S + T_{G_2} + 0.5\ T_M$ has elapsed. Suggestive evidence for this hypothesis was obtained in our previous study. However, when hourly intervals are tested no difference was noted between the peak in mitosis and the peak in DNA synthesis. This argues against the proposed mechanism occurring in the gut.

Gelfant[9] has demonstrated in the mouse epidermis 2 distinct populations of cells which are blocked in G_1 and G_2, and which can be triggered into S or M respectively by appropriate stimuli. This theory fits our results; but studies of Lesher[10] would seem to argue against its occurrence in the intestine because: 1. The percent labeled mitosis curve reaches 100% in 1–2 h after ^{3}HTdR injection, and 2. after 4 injections of ^{3}HTdR each separated by 2 h, the entire proliferative compartment is labeled.

A more recent study of Pederson and Gelfant[11] had demonstrated a population of G_1 and G_2 cells in the intestine and kidney which are non-cycling. Release of the block could cause a wave of cells into S and M resulting in the simultaneous peaks noted, however, it is extremely doubtful that the small number (4%) of these non-cycling cells could account for the large daily variation noted.

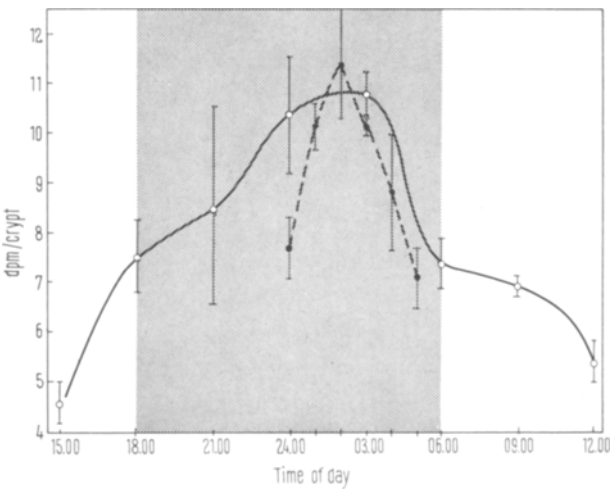

Fig. 3. Diurnal flucuation in dpm per crypt. Open circles are previously reported results. Shaded area represents the dark cycle in the animal rooms.

Zusammenfassung. Im Tagesrhythmus der Proliferationstätigkeit von Darmepithelien wurde in drei verschiedenen Parametern um 02.00 Uhr ein Maximum gefunden. Mögliche Mechanismen werden erörtert.

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Sex and Age Differences in Hyperthermia Response to Ether Anesthesia in Weanling Rats with Ventromedial Hypothalamic Lesions[1]

The role of the hypothalamus in temperature regulation has been known since the early work of Isenschmid and Krehl[2], who demonstrated that lesions in the posterior hypothalamus prevented appropriate homeostatic re-

[1] This investigation was supported by U.S.P.H.S. Grants No. HE 06 975 and No. HD 03331, N.I.H.

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