and the number of spermatozoa diminished. The tissue consisted almost entirely of spermatogonia. In 4 males intramuscularly pretreated with Methallibure, the i.m. injection of 0.06 mg/g body wt. 17-α-methyl-testosterone stimulated the intensity of aggressive behaviour significantly to 55% above the value before this injection, and the frequency of opercular spreads (+ 40%, difference significant) within 2–3 days p.i. In another 4 males pretreated with Methallibure, the i.m. injection of mammalian luteinizing hormone (NIH; 0.05 mg/g body wt.) increased the frequency of sexual (+222%) and aggressive (+283%) motor patterns as well as the frequency of opercular spreads (all at least $p < 0.05$). The i.m. injection of reserpine (0.0005 mg/g body wt.), which depletes the intraneuronal storage granules of catecholamines (especially norepinephrine), stimulated the aggressivity of 10 males significantly within 6 h. p.i. for a period of several days, whereas leading, a motor pattern which is performed by sexually highly excited animals, significantly diminished. Even a 2-, a 4- and an 8-fold dose of reserpine resulted in elevated aggressivity already 1 h p.i. and did not produce sedation. Chlorpromazine (0.125 mg/l dissolved in the aquarium water), which inhibits the action of released norepinephrine, depressed the aggressivity of 5 males significantly, as well as the nest building behaviour and the opercular spreads ($p < 0.05$). The sexual tendency remained high. The last mentioned observations support the hypothesis that the actual control of the aggressive and the nest-building tendencies may be mediated by norepinephrine. It is suggested that there is a long term (LH, testosterone) and an additional short term (catecholamines) control of reproductive behaviour in *L. gibbosus*.

Zusammenfassung. Ein synthetisches Antigonadotropin (Methallibur, I.C.I) hemmt das Sexual- und Nestbauverhalten von Sonnenbarsch-Männchen (*Lepomis gibbosus*). Testosteron hingegen steigert die Aggressivität, und Säuger-LH zudem noch die Sexualtendenz. Reserpin erhöht die Kampfstimmung; Chlorpromazin hemmt dagegen die Kampf- und Nestbaustimmung, nicht aber die Sexualtendenz.

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Effect of Phenobarbital on Hepatocyte Proliferation in Rats Following Partial Hepatectomy

Administration of phenobarbital to experimental animals produces enlargement of the liver[1]. Hepatomegaly in mice following phenobarbital and other drugs has been ascribed to hepatocyte hypertrophy[2–4] or chiefly hyperplasia[5–8]. PAULINI et al.[9,10] showed that phenobarbital in young rats produced both hepatocyte hypertrophy and hyperplasia. The present study examines the effect of phenobarbital on hepatocyte proliferation in partially hepatectomized rats, measuring thymidine incorporation and the mitotic index.

Method. 36 male 10-week-old OFA (Lyon) rats, weight 260 to 377 g, were used. They were caged singly, allowed free access to food (Altromin®) and water, and randomly allocated to 4 treatment groups: Group 1: Controls, 2 rats/time interval, no treatment. Group 2: Phenobarbital alone, 2 rats/time interval; phenobarbital 80 mg/kg i.m. daily for 2 days prior to time zero (time of hepatectomy), 40 mg/kg i.m. at time zero. Group 3: Hepatectomy alone, 4 rats/time interval; 2/3 hepatectomy (HIGGINS[11]) under ether narcosis at time zero. Group 4: Hepatectomy plus phenobarbital, 4 rats/time interval; phenobarbital 80 mg/kg i.m. daily for 2 days prior to time zero, 40 mg/kg i.m. at time zero.

Sacrifice of rats was done at 16, 24 and 39 h after time zero. The time of hepatectomy was arranged so that all rats were sacrificed between 09.00 and 10.00 h. ^{3}H-thymidine, 3 mC/kg, was given i.p. 60 min before sacrifice. Liver tissue following sacrifice was prepared histologically for mitotic counts. Paraffin sections were coated with Ilford-G-5 emulsion, exposed for 14 days, and then stained with hematoxylin and eosin. Thymidine-labelled hepatocyte nuclei and hepatocyte mitoses (metaphase, anaphase and telophase) were counted in 200 consecutive microscopic fields for each rat. In every 10th field (i.e. 20 fields per rat)

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Table I. ^{3}H-thymidine index of rat hepatocyte nuclei after partial hepatectomy and/or phenobarbital pre-treatment

Time after hepatectomy (h)	^{3}H-thymidine index ± S.D. (%)			
	Controls (no treatment) ($n = 2$)	Phenobarbital ($n = 2$)	Partial hepatectomy alone ($n = 4$)	Phenobarbital + partial hepatectomy ($n = 4$)
16	0.042 ± 0.059	2.059 ± 2.718	0.268 ± 0.344	12.365[a] ± 7.572
24	0.254 ± 0.308	1.631 ± 2.032	19.615 ± 9.274	30.291 ± 15.466
39	0.144 ± 0.108	0.293 ± 0.175	16.884 ± 8.466	22.980 ± 8.282

[a] $p < 0.01$ (vs. hepatectomy alone). Student t-test.

Table II. Mitotic index of rat hepatocytes after partial hepatectomy and/or phenobarbital pre-treatment

Time after hepatectomy (h)	Mitotic index ± S.D. (%)			
	Controls (no treatment) ($n = 2$)	Phenobarbital ($n = 2$)	Partial hepatectomy alone ($n = 4$)	Phenobarbital + partial hepatectomy ($n = 4$)
16	0.017 ± 0.024	0.032 ± 0.018	0.051 ± 0.044	0.118 ± 0.083
24	0	0.018 ± 0.025	0.014 ± 0.017	1.081[a] ± 0.497
39	0	0	4.702 ± 1.127	1.749[a] ± 0.349

[a] $P < 0.01$ (vs. hepatectomy alone). Student t-test.

the total number of hepatocyte nuclei was counted. Percentage values, giving the ^{3}H-thymidine index and the mitotic index, were calculated.

The data were subjected to statistical analysis following transformation (Student's t-test and analysis of variance).

Results. The controls showed the expected minimal thymidine labelling in the hepatocyte nuclei (Table I). Phenobarbital alone produced no significant increase in labelling. Hepatectomy alone produced no obvious increase after 16 h. After 24 h there was a marked increase in thymidine incorporation, which was maintained at 39 h. Hepatectomy together with phenobarbital produced a greater increase in thymidine incorporation. At 16 h, phenobarbital pre-treatment in the hepatectomized rats was associated with an obvious and significant ($p < 0.01$) increase in the mean thymidine index. At 24 h the increased labelling in phenobarbital-hepatectomized rats was still greater than in only hepatectomized rats, although not significantly so, probably due to the large individual variation. Again at 39 h the phenobarbital pretreated rats had increased thymidine labelling compared with the hepatectomy-alone rats. The overall increase in thymidine incorporation in phenobarbital-hepatectomized rats was supported statistically using analysis of variance ($p < 0.01$).

Hepatectomy caused no increase in the mitotic index after 16 and 24 h (Table II). However, at 39 h hepatectomy produced a marked rise. Pre-treatment with phenobarbital induced a slight increase compared with hepatectomy-alone results at 16 h and a significant increase at 24 h. At 39 h, the mitotic index was significantly less in phenobarbital-treated hepatectomized rats, compared to non-treated hepatectomized animals. Analysis of variance revealed no overall significant difference between these two groups.

Comment. Our findings show that pre-operative treatment with phenobarbital of partially hepatectomized rats leads to an earlier onset of hepatocyte proliferation; in such animals, thymidine incorporation was pronounced after 16 h and mitotic activity was distinct after 24 h. In the untreated hepatectomized animals, neither was so advanced at these times. In addition, phenobarbital-pretreated rats had slightly increased thymidine incorporation after 24 and 39 h, and the analysis of variance showed a significant overall increase of labelling. There was no difference between mitotic indices in phenobarbital-pretreated and non-treated hepatectomized rats. It can be seen from Table I that maximum thymidine labelling was found 24 h after hepatectomy. The somewhat smaller values in both treated and untreated rats after 39 h indicate that thymidine uptake was on the decline at that time. Peak thymidine incorporation probably occurs between 24 and 39 h. BÜRKI et al.[12] gave rats phenobarbital immediately after partial hepatectomy. The increase of 131-iododesoxyuridine labelling and mitotic activity which they found set in a few hours later than in our study. We selected our examination times to coincide with the probable start of DNA synthesis and mitotic activity and to be near the postulated maximum DNA activity, and did not consider the time of maximum mitotic activity. Consequently we cannot be certain of the effects of phenobarbital treatment on this latter parameter. It seems probable, however, that mitotic activity is also advanced (and increased) in analogue to the advance and increase in thymidine incorporation found.

Zusammenfassung. Mit Phenobarbital behandelte Ratten zeigen nach partieller Hepatektomie in der Leber ein beschleunigtes Einsetzen der DNS-Synthese und der Mitose-Aktivität sowie eine Erhöhung der DNS-Synthese.

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In vivo Generation of Hydrogen Peroxide from 6-Hydroxydopamine

We suggested previously[1,2] that hydrogen peroxide (H_2O_2) was responsible for the degeneration of adrenergic nerve terminals caused by the injection of 6-hydroxydopamine (6-OHDA) into experimental animals[3,4]. When 6-OHDA is added to aqueous solutions at neutral pH, H_2O_2 is generated[2]. H_2O_2 is a cytotoxic agent that can oxidize sulfhydryl groups of enzymes and peroxidize structural lipids. One form of cytotoxicity that we studied was the irreversible inhibition of uptake of biogenic amines into either rat brain homogenates[1] or tissue slices[2]. The inhibition of uptake by H_2O_2 was prevented by catalase[1].

Catalase can serve as a convenient intracellular marker for H_2O_2. H_2O_2 forms a complex with catalase (complex I), which on further reaction with 3-amino-1, 2, 4-triazole leads to irreversible inhibition of catalase activity[5-7]. The reaction scheme is as follows:

$$H_2O_2 + \text{catalase} \longrightarrow (\text{Catalase}\text{-}H_2O_2)\ \text{complex I}$$

$$\text{Complex I} + \text{aminotriazole} \longrightarrow \text{irreversibly inhibited catalase.}$$