2 methylene groups at 8.14 and 7.22 τ (both triplets; $J = 7$ cps). Methylation with methyl iodide and sodium hydride in dry benzene gave N-methylkoenigicine (III), $C_{21}H_{23}NO_3$, mp 189°; $\lambda_{max.}^{EtOH}$ 241, 303, 346 and 361 nm (log ε 4.55, 4.56, 3.92 and 3.82 respectively), which on catalytic reduction (Pt-AcOH) yielded N-methyldihydrokoenigicine (IV), mp 193–194°, $C_{21}H_{25}NO_3$ (M^+ 339 by mass-spectrum); $\lambda_{max.}^{EtOH}$ 242, 273, and 318 nm (log ε 4.58, 4.28, and 4.22 respectively). The mass-spectrum of IV showed, apart from the M^+ peak, ions at m/e, 324, 283, 268, 240, 225, and 169.5 [m* 309.6 (339 → 324); 253.7 (283 → 268); and 236.2 (339 → 283)]. The combined data on II, III, and IV thus provide interlocking evidence for the structure of koenigicine as I.

The second base, $C_{19}H_{19}NO_2$, mp 194–195° (mol. wt. 293 by mass-spectrum) is again optically inactive and is shown to be identical with koenimbin recently isolated from the stembark of *Murraya koenigii*[3]. The following derivatives were prepared: dihydrokoenimbin, mp 249°; UV, $\lambda_{max.}^{EtOH}$ 243, 258, 312, 332, and 346 nm (log ε 4.21, 4.07, 4.03, 3.62 and 3.22), N-methylkoenimbin, mp 148°, $\lambda_{max.}^{EtOH}$ 242, 301, 339, 357 and 371 nm (log ε 4.55, 4.26, 3.83, 3.75 and 3.68 respectively), N-methyldihydrokoenimbin, mp 180°, $\lambda_{max.}^{EtOH}$ 247, 266, 314, 337 and 352 nm (log ε 4.49, 4.33, 4.29, 3.73 and 3.51 respectively). Our data is in agreement with the findings of NARASIMHAN et al.[3]. We think, however, that although the structure (V) proposed by the latter for koenimbin is highly likely to be correct, the alternative structure (VI) cannot yet be ruled out. This point can be settled only by synthesis, which is in hand.

These alkaloids are of considerable biogenetic interest. As an alternative to the suggestion of CHAKRABORTY and DAS[2] that the formation of the carbazole ring in plants precedes C-methylation of the aromatic ring by electrophilic attack, we propose that the biosynthesis of these alkaloids (and, in particular, the origin of the 3 or 6 C-methyl) involves the intermediacy of MVA. Appropriate tracer experiments on this aspect of the problem are in hand.

Zusammenfassung. Zwei neue Alkaloide, Koenigicine und Koenimbin, wurden aus Blättern von *Murraya koenigii* isoliert und als (I) und (V oder VI) charakterisiert. Es wird vermutet, dass bei der Biosynthese dieser Alkaloide das 3-C-Methyl aus Mevalonsäure stammt.

S. P. KUREEL, R. S. KAPIL and S. P. POPLI

Central Drug Research Institute, Lucknow (India), 24 March 1969.

Hepatic DNA Synthesis After Partial Hepatectomy in Rats Treated with Protamin-Zn-Insulin Under Different Nutritional Conditions

In the liver tissue remaining after 65–70% hepatectomy (PH) the changes suggesting the shift in the metabolic and endocrine balance develop regularly. Among these changes especially the rise of triglycerides content[1], the increase of fatty acids oxydation[2], the decrease of fatty acids synthesis[3] and the decrease of glycogen content[4] could be taken into account. The development of these changes is, as a rule, dependent on the stimulation of sympatho-adrenal system[5] and on the activation of the axis hypophysis-adrenal cortex[6]. The relationship of the changes in the metabolic and endocrine balance after PH to the liver regenerating process is not yet clear. In our experiments devoted to the study of this relationship, we also intended to determine the development of liver regeneration after PH in rats that received an s.c. injection of protamin-Zn-insulin (PZI) (Spofa, Czechoslovakia), the hormone influencing the metabolic and endocrine balance in the opposite way as compared with glucocorticoids and catecholamines.

Methods. For the experiments male rats (230–280 g), fed the standard laboratory diet[7] containing 25% of proteins, 53% of carbohydrates and 22% of lipids, were used. At partial hepatectomy 65–70% of liver tissue was removed[8]. The operations were performed at 10.00 h. One hour before death, all rats received an injection of thymidine-C^{14} (2.5 μc/100 g body wt.; specific activity, 44 mc/mM) into the femoral vein. The rats were killed by decapitation. The nuclei of the liver cells were isolated[9], then washed on filters with trichloroacetic acid (5%), alcohol and ether. The dry sample had been dissolved by hyamin before the scintillation fluid was added. The radioactivity of samples was measured in liquid scintillation counter Mark I (Nuclear, Chicago). The content of DNA in the liver tissue was estimated according to DISCHE[10]. The results were evaluated statistically using Student's t-test.

Results and discussion. In the first experiment, rats fed ad libitum before the operation and after it were used. PZI (3 IU/100 g body wt.) was injected 1 h before PH. In the same interval before the operation, the control rats received an injection of saline (0.1 ml/100 g body wt.). In rats that received insulin, the hepatic DNA synthesis 20 ($p < 0.01$) and 44 h after the operation ($p > 0.05$) was

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Table I. Specific activity of hepatic DNA after partial hepatectomy in rats fed ad libitum before and after this operation

Groups	DNA specific activity (cpm/mg DNA) Before the operation	After partial hepatectomy (h) 20	44
Partial hepatectomy + saline (0.1 ml/100 g body wt.)	321 ± 68	6006 ± 1170	3070 ± 389
Partial hepatectomy + PZI (3 IU/100 g body wt.)	284 ± 75	11,990 ± 3430	4436 ± 1465

One hour before partial hepatectomy the rats were injected protamin-Zn-insuline (3 IU/100 g body wt.). The control rats received at the same time before the operation an injection of a corresponding amount of saline 0.1 ml/100 g body wt.). Means and confidence limits for $p = 0.05$ are given.

Table II. Specific activity of hepatic DNA after partial hepatectomy in rats that fasted 4 days before this operation

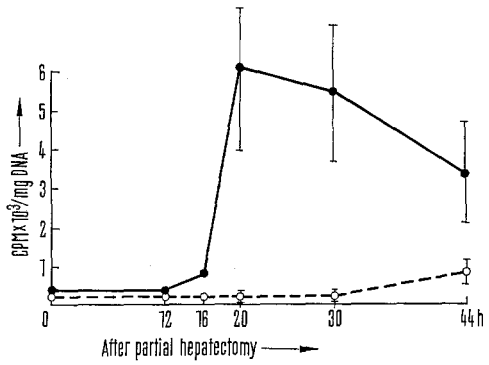
Groups	DNA specific activity (cpm/mg DNA) Before the operation	After partial hepatectomy (h) 20	44
Partial hepatectomy + saline (0.1 ml/100 g body wt.)	340 ± 49	2650 ± 940	2143 ± 634
Partial hepatectomy + PZI (3 IU/100 g body wt.)	318 ± 64	862 ± 368	3586 ± 480

After partial hepatectomy the rats were given the food. 1 h before the operation the rats were injected protamin-Zn-insuline (3 IU/100 g body wt.). At the same time before the operation, the control rats received an injection of a corresponding amount of saline (0.1 ml/100 g body wt.). Means and confidence limits for $p = 0.05$ are given.

higher than in control rats (Table I). The interpretation of these results was however rather complicated. It was not certain if the compensation reactions evoked by hypoglycemia, regularly developing after PH and further stressed by the application of insulin, for instance the activation of the axix hypophysis-adrenal cortex had taken part in these experiments[11]. The development of these consequences in rats fed ad libitum is made possible by the fact that these rats do not receive the food spontaneously for 8 or even more hours after the operation. To achieve the typical effect of insulin on intermediary metabolism, it was therefore necessary to create conditions under which the rats would receive the food even in the early postoperative period. The stimulation of postoperative eating was achieved by exposing rats to 4 days fasting before PH. During this time interval, the weight of rats decreased from 260 ± 23 g to 239 ± 28 g, i.e. 8% only. The fasted rats received 3 IU of PZI/100 g body wt. 1 h before PH. These rats and the control rats were given the food immediately after the operation. In rats that received insulin, the hepatic DNA synthesis 20 h after PH was significantly lower ($p < 0.001$) and 44 h after the operation significantly higher ($p < 0.001$) as compared with the control values (Table II).

In the following group, the rats at the end of the 4-day-fasting period received the food for 3 h only. At the end of this time, when the food was removed again, the rats received an injection of PZI 3 IU/100 g of body wt. One hour later PH was performed. In the rats that received insulin, the increase of hepatic DNA synthesis after PH was at first completely suppressed (Figure). In these rats the small increase of hepatic DNA synthesis was observed 44 h after PH at least.

The results presented above show that insulin temporarily inhibits the increase of hepatic DNA synthesis in rats receiving the food for several hours before the actual PH or immediatelly after the operation. Insulin is not considered to be a mitogenic hormone. Its effect on hepatic DNA synthesis after PH was probably mediated by influencing the intermediary metabolic reactions[12]. Nevertheless the details of the relationship between the changes of intermediary metabolism and liver regeneration process remain unclear. We suppose that our results support the theory about the main role of the humoral mechanism in the stimulation of liver regeneration[13].



Specific activity of hepatic DNA after partial hepatectomy in rats fasted 4 days, then given food for 3 h. When the food was removed again the rats received an injection of protamin Zn-insulin (3 IU/100 g body wt.) (○---○). 1 h later partial hepatectomy was performed. The control rats had the same nutritional regime but instead of insulin they were injected a corresponding amount of saline (0.1 ml/100 g body wt.) (●—●). Means and confidence limits for $p = 0.05$ are given.

Zusammenfassung. Bei Ratten mit dauerndem Zutritt zur Nahrung wurde die Zunahme der Desoxyribonukleinsäure-Synthese in der Leber (60–70% Hepatektomie) durch Applikation von Protamin-Zn-Insulin (3 IE/100 g Körpergewicht) s.c. 1 h vor Hepatektomie stimuliert. Im Gegensatz dazu wurde bei Ratten mit einem stimulierten postoperativen Zutritt zur Nahrung die Synthese der Desoxyribonukleinsäure im Lebergewebe nach partieller Hepatektomie mit Protamin-Zn-Insulin gehemmt.

J. Šimek, A. Husáková,
F. Deml and I. Dvořáčková

Department of Physiology, Radioisotope Laboratories and Department of Pathology, Faculty of Medicine, Charles University, Hradec Králové (Czechoslovakia),
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