Effect of Methylcobalamin on Protein Content in Liver and Serum of Partially Hepatectomized Rat

A decrease of the hepatic vitamin B_{12} content and a concomitant increase of the serum vitamin B_{12} level were observed in rats with experimental hepatic damage[1,2]. The possibility was suggested by several investigators that vitamin B_{12} prevents the decrease of ribonucleic acids and proteins in damaged liver[3,4]. Furthermore, this vitamin was suggested to have a facilitating effect on liver regeneration of healthy and damaged livers after hepatectomy[5,6]. GUEST et al.[7] have shown that methylcobalamin, a derivative of vitamin B_{12}, had a more marked effect than any other derivatives of vitamin B_{12} on the formation of B_{12}-enzyme in vitro from a crude source of *E. coli*. Recently, MISRA et al.[8] reported that a marked amount of CH_3 group of methylcobalamin was incorporated into deoxyribonucleic acids and acid-soluble fraction of regenerating rat liver.

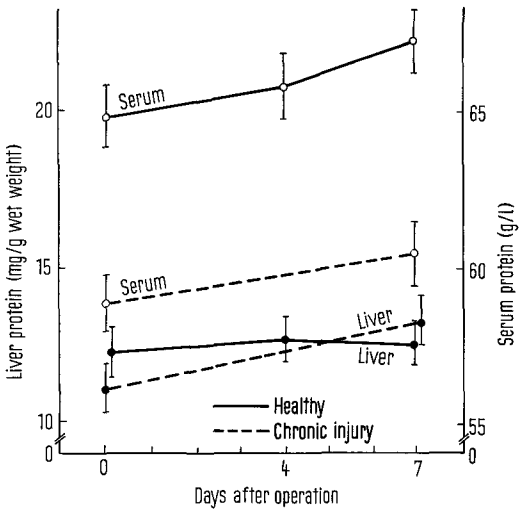

Fig. 1. Effect of methylcobalamin administration on liver and serum protein of healthy rats and those with chronic injury. Rats were administered i.m. 20 μg of methylcobalamin/100 g body wt./day. The vertical bars show ± S.D.

In the present study, we investigated the effect of methylcobalamin on the protein concentration in the regenerating liver and serum of rats with chronic hepatic damage after partial hepatectomy.

Material and method. Male albino rats of Sprague-Dawley strain, weighing 170 g at the beginning of the experiment, were used. 4–8 rats of each group were fed ad libitum on a standard stock laboratory diet. Chronic hepatic damage was induced by carbon tetrachloride inhalation twice a week for 5 months. Partial hepatectomy was performed under ether anesthesia, and right and middle lobes of the liver were removed by the method of HIGGINS and ANDERSON[9]. Both the hepatectomized rats and non-operated were given i.m. 20 μg of methylcobalamin/100 g body weight/day, beginning 3 days before the operation and continuing at daily intervals during the pre- and post-operative period until the day before sacrifice. Rats of the control groups received physiological saline. The rats were bled under anesthesia on 4 and 7 days after the operation, and the serum was prepared by centrifugation immediately. The livers were perfused in situ by the portal vein with an ice-cold 0.25*M* sucrose solution and removed. The liver homogenates (10%, w/v) were prepared in an ice-cold 0.25*M* sucrose solution in a Potter-Elvehjem glass homogenizer for 2 min at about 1,000 rev/min and followed by cen-

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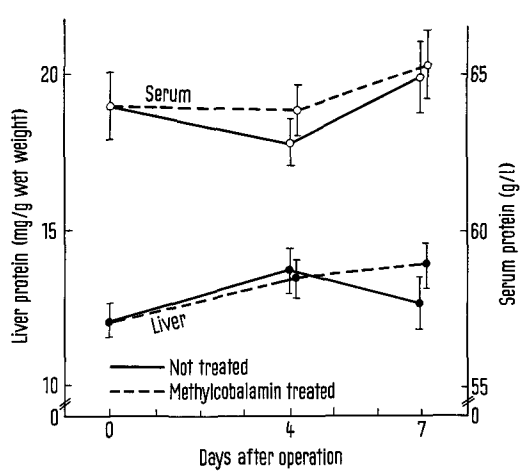

Fig. 2. Effect of methylcobalamin on regenerating livers of healthy rats after partial hepatectomy. Hepatectomized rats were administered i.m. 20 μg of methylcobalamin/100 g body wt./day. The injection began 3 days before the operation and continued until they were sacrificed.

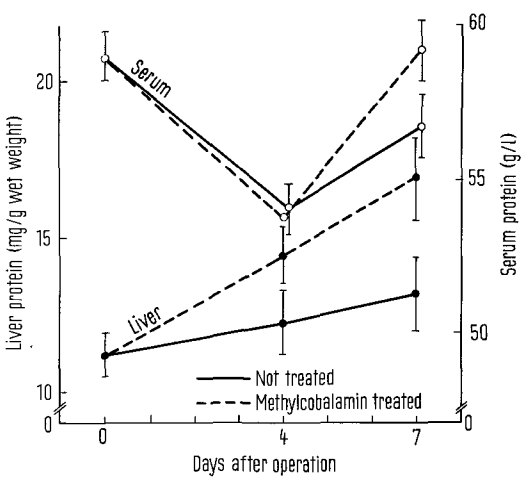

Fig. 3. Effect of methylcobalamin on regenerating liver after partial hepatectomy of rats with chronic hepatic injury. Hepatectomized rats were administered i.m. 20 μg of methylcobalamin/100 g body wt./day. The injection began 3 days before the operation and continued until they were sacrificed. Chronic hepatic damage was induced by carbon tetrachloride inhalation twice a week for 5 months.

trifugation at 13,000 *g* for 20 min. Protein in the supernatant fraction of the liver and serum was determined colorimetrically by the method of LOWRY et al.[10] with crystalline bovine serum albumin as a standard.

Results and discussion. As shown in Figure 1, the protein concentration of the serum of rats with chronic hepatic damage was lower than that of healthy rats, and no significant difference of the protein concentration in the liver was seen between healthy and hepatic damaged rats. By the administration of methylcobalamin for 7 days, a slight elevation, but not statistically significant, was observed in the protein concentrations of the liver and serum both of the healthy and chronic hepatic damaged rats, as shown in Figure 1. In healthy rats with partial hepatectomy, methylcobalamin administration showed no influence on the content of protein in the liver and serum as given in Figure 2. On the contrary, as shown in Figure 3, after the hepatectomy of rats with chronic hepatic damage, the protein concentrations in the liver and serum of the group administered methylcobalamin were significantly higher than those of the control group on the 7th postoperative day. The administration of cobamide (5,6-dimethyl-benzimidazolyl-5′-deoxyadenosyl cobamide) showed, in this condition, no effect such as seen by the methylcobalamin administration. This effect of methylcobalamin apparently seems to enhance protein synthesis in the process of regeneration of the liver with chronic hepatic damage, and it is assumed from these results that methylcobalamin accelerates the liver regeneration.

Zusammenfassung. Die Zunahme der Leber- und Serumeiweissmenge bei chronischer Leberstörung von Ratten mit partiel extirpierter Leber wird nach Applikation von Methylcobalamin gefördert. Eine Methylcobalamin induzierte Förderung der Eiweißsynthese in der Leber wird angenommen.

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Strychnine Antagonistic Potency of Pyrone Compounds of the Kavaroot (*Piper methysticum* Forst.)

Up to now several aromatically substituted δ-lactones (formulas see [1]) have been isolated from the root-stock of the kavaplant (*Piper methysticum* Forst.), indigenous to many islands of the South Pacific. These compounds, the so-called kavapyrones, mainly act in pharmacological experiments as central depressant drugs. In detail we have found: depression of spontaneous motor activity, potentiation of anaesthesia[2], anticonvulsive effects against electroshock and pentylenetetrazol induced seizures[3], and, in particular, a centrally caused muscle relaxation as well as a blocking effect of polysynaptic reflexes[4]. On account of these properties, an antistrychnine action can be expected for the kavapyrones. For one of them, dihydromethysticin (DHM), such an effect was described earlier[5,6].

Methods. In mice, poisoned with various doses of strychnine, the following 5,6-hydrogenated pyrones, kavain (K), dihydrokavain (DHK), methysticin (M), and again dihydromethysticin, were tested in comparison with mephenesin and phenobarbital. The anticonvulsive and the antilethal effect were evaluated. For each of these effects, the $ED_{50} \pm s_{\bar{x}}$ was calculated by the method of MILLER and TAINTER using 10–30 animals for each dose. Male albino mice (weight 20–27 g) were poisoned with strychnine doses from 2 mg/kg (the minimum certainly effective lethal dose) up to 60 mg/kg injected s.c. After the injection of strychnine, the animals were set in separate cages. The compounds tested were injected i.p., the pyrones and phenobarbital 30 min before, mephenesin only 1 min before strychnine. The pyrones were dissolved in peanut oil because of low solubility in water.

Results. All the kavapyrones showed a marked antagonistic effect upon the convulsant and lethal action of strychnine. The mean dose of the compounds tested which antagonize the lethal effect of 2, 3 and 5 mg/kg strychnine are summarized in the Table. M proved to be the most potent compound of all pyrones tested. The ED_{50} of M against 2 mg/kg strychnine amounts to approximately $^1/_{33}$ of the LD_{50} of this drug, against 5 mg/kg strychnine to $^1/_7$ of the LD_{50}. Pretreatment of the animals with the maximum effective dose of M (300 mg/kg) increases the LD_{50} of strychnine 5–6 times. M even protects some animals against the lethal effect of 10 mg/kg strychnine, a dose 5 times higher than the minimum certainly effective lethal dose of the alkaloid. Except for DHM, the other pyrones act in the same range as M, as far as the maximum effective doses and the absolute limits of activity against strychnine are concerned. The protective effect of K and DHK becomes apparent only with higher doses, close to the intrinsic toxic activity of these drugs. DHM acts against strychnine poisoning of 2 mg/kg in the same range as M, but this drug has the lowest absolute limit of activity. Except for DHM, 2–5 mg/kg of strychnine are antagonized by pyrone doses which otherwise influence only very little the behaviour of the animals. The ED_{50} against these strychnine doses are lower than those producing loss of righting reflex or paralysis.

The pyrones have a higher protective activity against strychnine poisoning than mephenesin, which acts only against 2 mg/kg strychnine. The ED_{50} amounts to 40% of the LD_{50} of the latter compound. This dose of mephenesin otherwise provokes complete paralysis of the animals.

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