increasing age, a continuous decrease in per cent reticulocytes occurred with a final value of less than 2% reached in adult (220–240 g) rats. Corresponding with the decrease in reticulocyte counts, there was a pronounced decrease in adenyl cyclase activities; in 220–240 g animals, NaF- and isoproterenol-stimulated enzyme activities were only 10 to 15% of those found in the 30 to 40 g group.

The obvious correspondence between per cent reticulocytes and adenyl cyclase activities can be clearly seen in Figure 2 where the adenyl cyclase activities are plotted against the respective reticulocyte counts: enzyme activities and per cent reticulocytes were linearly correlated, the correlation coefficients amounting to 0.99 for the basal and the NaF-stimulated, and to 0.98 for the isoproterenol-stimulated activities ($n = 5$). Moreover, it can be concluded from Figure 2 that mature erythrocytes contain only negligible amounts of adenyl cyclase activity: the respective regression lines tend to cross the origin.

Discussion. The results presented lead to the conclusion that adenyl cyclase activity in the non-nucleated red blood cells from rats is localized in the reticulocytes. Support for this view comes also from preliminary experiments[16] with rats which were treated with 1-acetyl-2-phenyl hydrazine: these animals show marked enhancement of both reticulocyte counts and adenyl cyclase activities (Table).

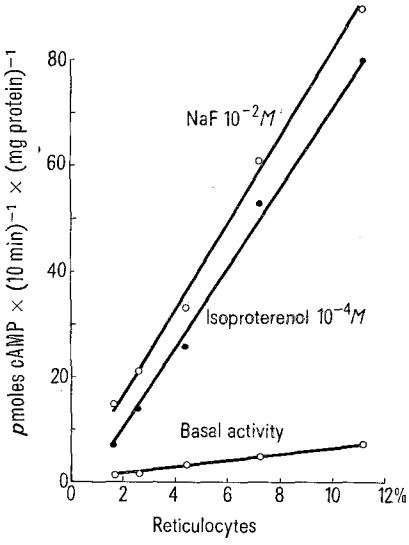

Fig. 2. Linear correlation between adenyl cyclase activities (basal, NaF- and D(-)-isoproterenol-stimulated) in erythrocyte ghost preparations, and reticulocyte counts in the respective blood samples (rat).

Comparison of adenyl cyclase activities in red blood cells from different species seems also to support the assumption that adenyl cyclase is localized in the reticulocytes. In our investigations (Table) adenyl cyclase activities in erythrocyte ghosts from 180–220 g rats were found to be several times higher than in ghost preparations from man. Likewise, the respective mean reticulocyte counts amounted to 2–3% in rat and about 1% in human blood samples. In addition, several cases of pernicious anaemia were investigated in which the reticulocyte crisis occurring after treatment with cyanocobalamine was accompanied by a marked increase in the adenyl cyclase activities measured in red cell ghost preparations (see Table).

If – according to our results – adenyl cyclase is confined to the reticulocytes, then this enzyme is not necessarily localized in the plasma membrane: it is known that reticulocytes contain – besides mitochondria – also ribosomes which cell constituents are, in part, firmly bound to the plasma membrane[17].

Concerning the physiological significance of adenyl cyclase in red blood cells, it seems that the enzyme has a functional role only in very young erythrocyte forms or, perhaps, in the erythrocyte precursors. This view is supported by recent experimental results[18,19] from which a functional role of cyclic AMP and of an adrenergic β-receptor system in the regulation of erythropoiesis may be assumed.

Zusammenfassung. Die durch Natriumfluorid und Isoproterenol stimulierbare Adenylcyclase-Aktivität in Erythrocyten-Schatten von 30–240 g schweren Ratten ist direkt proportional dem prozentualen Retikulocytengehalt des Blutes. Hinweise darauf, dass die Adenylcyclase-Aktivität kernloser Erythrocyten nahezu ausschliesslich in den Retikulocyten lokalisiert ist, konnten auch an menschlichem Blut erhalten werden.

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A New Peripheral Monoamine Oxidase Inhibitor: 2,9-Dimethyl-β-carbolinium Iodide

The origin of the hypotensive action of certain drugs and perhaps hypertension itself may be determined by utilizing a specific peripheral monoamine oxidase (MAO) inhibitor. Furthermore, the mechanism of reversal of reserpine-induced sedation by this type of compound and ultimately the site of reserpine action could also be ascertained. In addition, a specific peripheral MAO inhibitor would be valuable in the therapy of angina pectoris because of the absence of central behavioral effects.

We have achieved selective inhibition of peripheral MAO with the use of 2,9-dimethyl-β-carbolinium iodide (DMCI). In the in vitro studies using tryptamine as substrate, DMCI exerts greater inhibition of MAO from human liver mitochondria than that from rat and bovine liver[1]. This report describes the selective inhibition of peripheral MAO in vivo.

Materials and methods. Male Sprague-Dawley rats (200–250 g) were injected i.v. via the tail vein with

15 mg/kg of DMCI-9-^{14}C (79 nCi/mg) in saline, and sacrificed at designated time intervals. The tissues were homogenized in 2 volumes of 0.01 *N* HCl and the radioactivity was assayed using liquid scintillation spectrometry.

Tissues from animals receiving the same dose of non-labeled DMCI were assayed in vitro for MAO activity using tryptamine-2-^{14}C as substrate as previously described[2]. The in vivo inhibition of the enzyme was also evaluated by injecting intravenously serotonin-^{14}C (5-HT-2-^{14}C) bioxalate (10 μCi/ml/kg) in saline into DMCI-or saline-treated rats. The concentration of 5-HT-2-^{14}C in several tissues was then assayed using a modification of the method by WIEGAND and PERRY[3].

For the metabolic study, rats were injected i.v. with 15 mg/kg of tritiated DMCI (270 nCi/mg). At 8 h, the heart tissue was homogenized in 5 volumes of 95% ethanol and the compound in the ethanol extract was identified by TLC in solvent systems mentioned in Table II. Before measurement of radioactivity, the extract was evaporated to dryness to eliminate any contamination by tritiated water.

Results and discussion. Thirty min following an i.v. injection of DMCI9-^{14}C into rats, the heart accumulated 2.4% per g tissue of the administered radioactivity, nearly twice that found in liver (1.3% per g tissue). The initial level of radioactivity in the liver declined to half after 1 h, followed by a slight decrease at 2 h, while that in the heart remained nearly constant throughout 2 h. In the brain, radioactivity was barely detectable at all time intervals studied. To eliminate the possibility that the 9-$^{14}CH_3$ group of DMCI was being demethylated, an equivalent dose of ring-labeled tritiated DMCI (359 nCi/mg) was administered to rats; at 2 h, the distribution of radioactivity showed no variation from that found using the ^{14}C compound.

When the tissues from animals receiving DMCI were assayed in vitro for MAO activity, that from the liver showed greatest inhibition at 30 min (29.8 ± 1.9%), which then declined to 15.8 ± 2.0% at 1 h, and was maintained at this level at 2 h. When inhibition was measured at 4 h, a slight increase to 20.2 ± 1.8% was observed; this decreased at 8 h to 10.8 ± 1.8% and remained at nearly the same level at 16 h. In the heart, 51.5 ± 3.3% inhibition was found at 30 min, which elevated to 66.8 ± 2.9% at 1 h; at 2 h this level declined to 57.0 ± 1.2%, remained constant throughout 8 h, then decreased to 28.8 ± 5.5% at 16 h. At all time intervals the inhibition of heart MAO surpassed that of the liver. This was in agreement with the results from the distribution study described above. DMCI had no effect on MAO activity in brain at any time interval studied, which further supports the insignificant uptake of DMCI by this tissue.

Further substantiation of the inhibitory activity of DMCI on MAO was made by injecting a substrate of the enzyme, 5-HT-2-^{14}C, into the DMCI-treated animals, followed by measuring the radioactive amine in tissues. The results in Table I indicate an increase of 5-HT-^{14}C in the liver at all time intervals studied. In the heart, a considerable elevation of 5-HT-^{14}C was observed which surpassed that found in the liver at the same intervals. The elevation of 5-HT-^{14}C levels in both tissues corresponded well with the degree of MAO inhibition at these intervals, and also the relative distribution of DMCI in the two tissues. There was no significant elevation of 5-HT-^{14}C in the brain after the organ had been perfused free from blood with saline (%increase of 5-HT-^{14}C per g tissue from controls, before perfusion: 32.2 ± 9.4, 0.5 h; 63.9 ± 13.6, 1 h; 44.0 ± 6.9, 2 h. After perfusion: −3.4 ±

Table I. Effects of DMCI on the levels of 5-HT-^{14}C in rat tissues

Time after DMCI injection (h)[a] ≦	Increase of 5-HT-^{14}C per g tissue from controls[b] [%] Liver	Heart
0.5	138.8 ± 14.5 (7)	182.3 ± 34.4 (3)
1	97.5 ± 11.3 (4)	252.4 ± 21.4 (5)
2	97.1 ± 13.0 (4)	200.0 ± 20.5 (3)
2	104.0 ± 7.2[c] (4)	369.1 ± 24.5[c] (3)

[a] The radioactive amine was injected i.v. to DMCI-treated animals 10 min prior to sacrifice. [b] To achieve more consistency in the results, the animals were paired; i.e., for each rat receiving DMCI, a control rat of corresponding weight was used in the event that serotonin would not be distributed proportionately on a weight basis. 5-HT-^{14}C was extracted with *n*-butanol at pH 2 as described by WIEGLAND and PERRY[3]. The final aqueous phase was assayed for ^{14}C after the addition of 20% BBS-3 in liquifluor, and the percent increase of 5-HT-2-^{14}C per g of tissue from controls were determined. Each value represents the mean ± S.E. Numbers of animals are given in the parentheses. [c] Results obtained from perfused tissues.

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Table II. Excretion and metabolism of DMCI in rat bile following i.v. injection of the tritiated DMCI

Interval after injection [h]	Administered radioactivity[a] [%]	Cumulative percentage	Unchanged DMCI[b] [%]	Glucuronide of 9-hydroxymethyl metabolite[b, c] [%]
0 –0.5	4.0	4.0	22.6	77.4
0.5–1	5.9	9.9	7.5	92.5
1 –2	8.1	18.0	5.6	94.6
2 –4	5.5	23.5	—[d]	—[d]
4 –8	8.4	31.9	—[d]	—[d]

Each value represents the mean obtained with 3 animals. [a] The radioactivity in bile samples was measured by liquid scintillation spectrometry following the addition of BBS-3 in liquifluor. [b] DMCI and the metabolite were identified by TLC in the solvent systems of *n*-butanol-acetic acid-water (4:1:1) and 2-propanol-ammonium hydroxide (4:1) against reference standards. [c] Hydrolysis of the conjugate was carired out by incubation of the bile samples in acetate buffer, pH 5, with Glusulase (1,000 U/ml, Endo Laboratories) and Ketodase (500 U/ml, Warner-Childott) at 37 °C for 2 days. [d] Not determined.

2.6, 2 h). In heart and liver, increased 5-HT-^{14}C levels were still observed after perfusion (Table I).

Eight h after administering tritiated DMCI, the radioactivity in rat heart was due essentially to the unchanged compound. Thus, the inhibition of rat heart MAO was most likely produced by DMCI and not by a metabolite. In the bile collected from cannulated rats, a total of 32% of administered radioactivity was recovered throughout 8 h (Table II). The major metabolite in bile was identified as the glucuronide of 2-methyl-9-hydroxymethyl-β-carbolinium iodide, a product resulting from the oxidation of the 9-methyl group of DMCI. About 30% of the administered radioactivity was excreted in the urine of cannulated rats during the 8-h period; in the urine the glucuronide of the 9-hydroxymethyl metabolite accounted for 13% and the unchanged DMCI, 87% of the radioactivity.

In view of the results of this study, DMCI exhibits properties required of a peripheral MAO inhibitor. It is significant that this compound demonstrates considerable inhibition of heart MAO which is thought to be representative of the enzyme located in the peripheral nervous system.

Resumen. Yoduro de 2,9-dimetilo-carbolinio (DMCI) y no sus metabolitos inhibió la enzyma monoamino oxidasa y elevó ^{14}C-serotonia en el corazón e higado, pero no en el cerebro de ratas.

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The Effect of the Carbohydrate Moiety on Biological Activities of Synthetic Glycosides of Medicagenic Acid

The various biological activities exerted by saponins are generally attributed to their aglycone moieties, whereas to the composition and constitution of their carbohydrate moieties only small, if any, significance is assigned[1,2].

With regard to lucerne saponins, it was found[3,4] that their fungistatic and haemolytic activities are dependent solely on the presence of one aglycone, medicagenic acid, which is a dicarboxylic triterpene acid. The structure of a highly active lucerne saponin was recently established as 0-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosylmedicagenic acid[5]. In continuation of this work, the synthesis of medicagenic acid containing glycosides, the carbohydrate moieties of which are composed of 1 or 2 sugars, either α- or β-linked, was undertaken, and the influence of the sugar moiety on the extent of the fungistatic and haemolytic activities was examined. Medicagenic acid was prepared as described[3] and its carboxyl groups converted to the benzohydryl ester[6]. The following sugars were used: glucose, galactose, maltose, cellobiose and gentiobiose. They were converted to their acetobromo derivatives as described by JEANLOZ and STOFFYN[7]. The synthesis of the glycosides was carried out by the modified Koenigs-Knorr method[8], as described also by MORRIS and TANKERSLEY[6] for the synthesis of a β-D-glucoside of medicagenic acid. After removal of the benzohydryl groups by catalytic hydrogenolysis, the synthetic saponins were purified by thin layer chromatography on Kieselgel HR plates, using the mixture of ethylacetate-acetic acid-water (7:2:2) as solvent. The saponins containing only 1 sugar residue had an Rf value of 0.85, and those which contained one of the disaccharides had Rf values of 0.80–

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Haemolytic and fungistatic activities of native and synthetic glycosides and of medicagenic acid assayed with ram red blood cells[a] and *Sclerotium rolfsii* Sacc.[a]

Compound assayed	Mode of linkage in carbohydrate moiety	Haemolytic index	Mycelial growth inhibition by $1 \times 10^{-4}M$ saponin in the culture medium (% of control)
Medicagenic acid	—	6600	92.5
Native lucerne saponin	β 1→6, β 1→3	15000	88.0
Glucose-medicagenic acid	—	13500	83.7
Galactose-medicagenic acid	—	14000	88.3
Maltose-medicagenic acid	α 1→4	14600	88.6
Cellobiose-medicagenic acid	β 1→4	14200	86.2
Gentiobiose-medicagenic acid	β 1→6	14000	86.5

[a] For methods, see GESTETNER et al.[3].