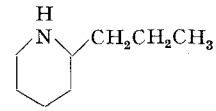
and stored at 0 °C. 45 kg of this plant material was chopped and steam distilled in batches in an all glass system for 3 h. The distillate was extracted with anhydrous ethyl ether which was removed in vacuo to give 8.0 g of are essential oil. The total distillate, which showed paralyzing activity, was placed on a jacketed Florisil column and eluted successively with 100% pentane, 5% ethyl ether in pentane, 15% ethyl ether in pentane, 50% ethyl ether in pentane, and 100% ethyl ether. Bioassay studies showed that physiological activity resided in the fractions eluted with 50% ethyl ether in pentane and 100% ethyl ether.

Separation of the basic components of these two fractions by standard techniques and further chromatography resulted in the isolation of a base (5 mg) with molecular formular $C_8H_{17}N$ (1.9%) from the 100% ethyl ether eluent. The GLC, TLC and mass spectrum of this base from *S. flava* leaves were in complete agreement with an authentic sample of coniine[8,9]. The 5 most intense fragment ion values of the mass spectrum of coniine in order of decreasing relative abundance with the proposed parent ion sixth are at *m/e* 59, 39, 41, 99, 126, 127.



Coniine (2-*n*-propylpiperdine)

Bioassay procedures. The bioassay apparatus consisted of a fibrous soxhlet thimble and a large test tube with a diameter just large enough to accomodate the thimble. The thimble was impregnated with a solution of the fraction to be tested and the solvent (ether or pentane) was allowed to completely evaporate. The thimble was placed in the bottom of the tube and 10 fire ants were placed in the bottom of the thimble. Paralytic activity was indicated when the ants failed to crawl out of the thimble. Thimbles to which only the solvent had been added were used as controls.

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Hepatic and Cerebral Coenzyme A Contents after Intravenous Injection of Coenzyme A in Rats

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Summary. Hepatic CoA concentrations and contents were significantly higher in rats having received i.v. CoA injections than in control rats. Maximum hepatic CoA concentrations were found 0.5–1 h after injection. In rat brain, no increase in CoA concentration was detected after i. v. injection of CoA.

In a previous paper[3] we have shown that addition of Coenzyme A (CoA) and some of its precursors led to an increase in the O_2-consumption of rat liver slices. The CoA concentration in the slices, however, only increased after addition of CoA itself or of dephospho-CoA (DPCoA). In this paper, the results of experiments on in vivo uptake of i.v. administered CoA by rat liver and brain are presented. In addition, we report our observations on the rise in CoA concentration in rat liver upon starvation.

Materials and methods. CoA (containing 80–85% CoASH), NAD, and the enzymes required for CoA determination were purchased from Boehringer, Mannheim.

Female white rats of 170–250 g body weight were used. Starved rats were fasted for 16 h with no restriction on water intake. The CoA injections were given into the tail vein. Each rat received 10 µmoles CoA dissolved in 0.25 ml 5% glucose in water and neutralized to a pH of 6–7 with $NaHCO_3$. Control animals received 0.05 ml isotonic saline +0.2 ml 5% glucose solution.

CoA determination were performed as previously[3], using the kinetic method with phosphotransacetylase described by MICHAL et al.[4]. Livers and brains were homogenized immediately after decapitation in 2 volumes of water at 0–4 °C. Dry weights were determined in the homogenates. Prior to measurement of CoA, the homogenates were deproteinized with perchloric acid at a final concentration of 0.4 *M*, and neutralized with K_2CO_3. In the resulting extracts the acid soluble fraction of CoA, i.e. the sum of CoASH, acetyl-CoA and CoA disulfide, was determined, the latter having been reduced by means of a preincubation with dithioerythritol.

To determine concentrations of total CoA, acid insoluble acyl-CoA was hydrolyzed prior to deproteinization by an incubation with 0.8 *M* NH_3 and 0.02 *M* mercaptoethanol at room temperature. Direct determinations of acyl-CoA in the washed precipitates from the perchloric acid extraction resulted in concentrations, which corresponded fairly well to the difference between total CoA and acid soluble CoA. The CoA tissue concentrations were not corrected for the CoA content in blood as CoA determinations revealed that the CoA blood concentrations $^1/_2$ h after injection were of the same magnitude as in untreated animals, namely 0.2–0.7 nmoles/ml.

Results. The effect of starvation on concentration and content of CoA in rat liver. Our experiments on uptake of CoA by rat liver slices[5] had revealed that CoA concentrations were sonsiderably higher in livers from starved than in livers from fed rats. A similar rise in heptatic CoA concentration following starvation has been observed by other authors[6–8].

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Table I. Concentration and content of CoA in livers from fed rats and rats starved for 16 h

	Weight of liver (g)	Dry weight (%)	CoA concentration		CoA content	
			Acid sol. CoA (nmoles/g dry wt.)	Total CoA (nmoles/g dry wt.)	Acid sol. CoA (μmoles)	Total CoA (μmoles)
Fed rats	8.6 ± 0.7	30.6 ± 0.8	430 ± 40	610 ± 60	1.13 ± 0.14	1.60 ± 0.25
Starved rats	5.7 ± 0.6	30.0 ± 1.3	800 ± 90	1 020 ± 120	1.37 ± 0.24	1.73 ± 0.28
p	< 0.01	> 0.5	< 0.005	< 0.01	> 0.2	> 0.5

The figures represent mean values from 3 animals ± SE. Probabilities, calculated by simple t-test, are for significances in the differences between the 2 groups.

In order to eliminate a possible influence of differences in food intake upon the effect of CoA injections on liver CoA, we found it necessary to examine the effect of starvation on CoA concentration in rat liver. A comparison of CoA concentrations in livers from as few as 3 starved and 3 fed rats confirmed that the concentrations of acid soluble and total CoA were signifcantly higher in livers from starved rats (see Table I). However, taking account of the liver weights, the difference in CoA concentration seems to find its explanation in a marked weight loss of rat livers during starvation for 16 h. As appears from the figures in Table I, no significant difference exists between the liver contents of total CoA in the 2 groups, whereas the fraction of acid insoluble CoA is clearly, though not significantly, smaller in livers from starved than in livers from fed rats.

The surprizingly large difference between the weights of livers from fed rats and from rats starved for a period of only 16 h, was further examined in a larger group of animals. In 14 fed rats of 210 g mean body weight (SE 30 g) the liver weight constituted 3.9% (SE 0.5%) of the body weight. In 9 starved rats of 200 g mean body weight (SE 40 g), the mean liver weight was only 2.9% (SE 0.3%) of the body weight. The difference is highly significant ($p < 0.001$).

Hepatic and cerebral CoA concentrations and contents in rats after i.v. administration of CoA.

Observation of a group of rats for 1 week after i.v. injection of 0.5–20 μmoles CoA revealed no adverse effects of the injections.

To find the time lapse between i.v. injection of CoA and maximum hepatic and cerebral CoA concentration, CoA was determined in livers and brains from fed rats killed at varying time intervals after i.v. injection of 10 μmoles CoA per rat. The CoA concentration in liver was elevated as compared to controls in all rats killed from 15 min to 7 h after injection of CoA. Maximum concentrations of 1.0 and 0.9 μmoles of total CoA per g dry liver were found after 30 and 60 min, respectively. These concentrations were higher than ever observed by us in livers from untreated fed rats. On the other hand, the elevation of hepatic CoA concentration after CoA injection was of the same magnitude as that found after starvation. In brains, no elevation of CoA concentration was observed on i.v. administration of CoA. Concentrations of total CoA were 0.17–0.22 μmoles/g dry weight.

A further investigation on the rise of hepatic CoA concentration after i.v. CoA administration was carried out by determining CoA in livers from fed rats killed 30 min after injection of 0.25 ml solution containing 10 or 0 μmoles CoA. Brain CoA concentrations were determined simultaneously. The results from this experiment are displayed in Table II. In livers from rats which received CoA injections, the concentration of acid soluble CoA was about 50% higher than in livers from control rats. The liver concentration of total CoA was increased by the same amount, showing that the concentration of acyl-CoA was unaffected 30 min after administration of CoA.

Contrary to our findings on untreated starved rats, also the contents of acid soluble and of total CoA were significantly higher in livers from rats having received CoA than in livers from control rats.

In brain, no increase in CoA content or concentration was observed after administration of CoA.

Discussion. From 1962 to 1969, 20 patients with endogenous hepatic coma of stage 2–4 have been treated with infusions of CoA, NAD, α-lipoic acid and thiamine pyrophosphate. 10 patients survived[9]. This survival rate is higher than that for patients not receiving specific treatment[10]. An effect of the cofactors is to be expected

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Table II. Concentration and content of CoA in rat liver and brain 30 min after i.v. injection of 55 and 0 μmoles CoA/kg, respectively

	Liver		p	Brain	
CoA injected (μmoles/rat)	10	0		10	0
Weight of organ (g)	6.7 ± 0.6	7.0 ± 0.7	> 0.4	1.6 ± 0.1	1.7 ± 0.2
Dry weight (%)	30.6 ± 0.5	30.0 ± 0.6	> 0.1	21.6 ± 0.6	21.3 ± 0.3
Total CoA concentration (nmoles/g dry wt.)	920 ± 130	650 ± 80	< 0.005	220 ± 10	220 ± 20
CoA content (μmoles)	1.90 ± 0.32	1.38 ± 0.21	< 0.01	0.076 ± 0.007	0.081 ± 0.007
Acid soluble CoA concentration (nmoles/g dry wt.)	810 ± 130	530 ± 70	< 0.001	180 ± 10	170 ± 20
Acid soluble CoA content (μmoles)	1.64 ± 0.25	1.12 ± 0.17	< 0.005	0.061 ± 0.004	0.063 ± 0.008

Values are means ± SE from determinations on 6 (liver) and 4 (brain) pairs of rats. Significances for differences between control and experimental animals are calculated by simple t-test.

only if one or more of them or their degradation products permeate into the cells of the target organs. As to CoA, its uptake by cells after in vivo administration has not been demonstrated until now. An in vitro permeation of CoA or DPCoA through biological membranes has been demonstrated by BREMER et al.[11] on isolated mitochondria and by us on liver slices[3,5].

The experiments reported here demonstrate a significant elevation of hepatic CoA in rats after i.v. administration of CoA. Both concentration and content of CoA in liver were increased by about 40%, corresponding to 5% of the amount injected. Since no rise in CoA blood concentration could be demonstrated after CoA injection, the gradual increase in hepatic CoA concentration during the first 15–60 min after injection seems not to be caused by a permeation into liver cells of intact CoA, but rather by some degradation product, possibly DPCoA.

In rat brain, no increase in content or concentration of CoA is observed after i.v. injection. The improvement in the stage of consciousness often observed in patients with endogenous liver coma shortly after initiation of treatment with cofactor, may therefore, be a result of an improved liver function rather than of an improvement in brain function due to an increase in cerebral CoA concentration.

Even though a marked increase in hepatic CoA content occurs in rats after injection of high doses of CoA, this permits no definite conclusion on what happens in human patients with severely decreased liver function. Regarding, however, the disturbances in cell wall function, which are generally connected with liver diseases[9,12,13], a permeation of nucleotidic cofactors into cells of liver and even of brain would more likely take place in patients with liver coma than in healthy individuals.

The results of the experiments reported here, together with the clinical improvements observed after infusions of cofactors to our patients, suggest that administration of CoA could be responsible for the amelioration of consciousness in endogenous liver coma.

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Isoenzyme der alkalischen Phosphatase - Referenzwerte im jugendlichen Alter und Einfluss der Eiweissernährung
Isoenzymes of Alkaline Phosphatase - Reference Values in Young People and Effects of Protein Diet

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Summary. In 260 normal students, 20–25 years old, the variation in the activities of serum alkaline phosphatase and its isoenzymes with sex, ABO blood groups, and protein intake were studied. The values are on the whole higher in males than in females. The activity of the intestinal isoenzyme was higher in subjects taking protein-rich diet than in those taking protein poor diet.

Die Bestimmung der Isoenzyme der alkalischen Phosphatase (EC 3.1.3.1) kann für die Früherkennung, differentialdiagnostische Abgrenzung und Verlaufskontrolle – vor allem bei Leber- und Knochen-Erkrankungen – von Bedeutung sein[1–3]. Die Abhängigkeit der Gesamtaktivität als auch der Isoenzyme der alkalischen Phosphatase von Alter, Geschlecht, Blutgruppenzugehörigkeit und fettreicher Ernährung ist in der Literatur beschrieben worden[4–6]. In dieser Arbeit wird das Verhalten der Isoenzyme bei unterschiedlicher Eiweissernährung unter Prüfung der Geschlechts- und Blutgruppenabhängigkeit berichtet.

Material und Methoden. 260 Medizinstudenten (93 Männer, 167 Frauen, Alter 20–25 Jahre, im Durchschnitt 22 Jahre) ernährten sich über zwei Wochen je eine Woche eiweissreich und eiweissarm. Als Sollwerte für eiweissreiche Ernährung waren über 20% Eiweiss-kcal, für eiweissarme unter 10% Eiweiss-kcal unabhängig vom Körpergewicht der Probanden vorgegeben. Richtlinie für tägliche Kalorienzufuhr: Männer 2600–3000, Frauen 2000–2500 kcal. Nach jeder Woche und vor der Versuchsdurchführung erfolgte je eine Blutentnahme.

Die Aktivitäten der Isoenzyme der alkalischen Phosphatase wurden bei 37 °C in einem kombinierten Testeinsatz mittels L-Phenylalanin-Hemmung und Hitzeinaktivierung bestimmt[3]. Die Präzision in der Serie wurde anhand von 84 Doppelwertbestimmungen ermittelt. Die Streuung beträgt für die Gesamtaktivität 0.6, Darmphosphatase 0.2, Leberphosphatase 0.4 und Knochenphosphatase 0.5 U/l. Die Daten wurden vor der statistischen Auswertung 3 Gruppen zugeordnet: Normalernährte, eiweissreichernährte und eiweissarmernährte Probanden. Die Verteilungsprüfung erfolgte mit dem $\varkappa^2$-Anpassungstest. Mittelwert und Streuung wurden anhand der transformierten Werte berechnet. Prüfungen auf Signifikanz wurden mit der Varianzanalyse und Duncan-Test sowie dem *t*-Test durchgeführt.

Ergebnisse. Die Verteilungsprüfung, getrennt nach Geschlecht und Ernährungsform, ergab für die Gesamt- und Isoenzymaktivitäten eine log-normale Verteilung.

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