ported that the problem of high-background have minimized this difficulty by extensively diluting the reaction mixtures before precipitation. However, Stevens et al. used the sediment by Ba^{2+} in which most ^{35}S-compounds were present, while in our method, phosphotungstic acid was used, and phosphotungstic acid did not precipitate the AAS of substrate. Therefore, AA in the supernatant, after deproteinization by phosphotungstic acid, was used for HPLC, and the procedure was found to be more sensitive than that reported by Stevens et al. In the present study, with HPLC-amperometric detection, such co-precipitation and high-background problems could be excluded: that is, disturbing protein was removed by precipitation after the enzymatic reaction, and only the product of enzyme activity (AA) was detected electrochemically.

5) Application of the method for the detection of arylsulfatase A activity in biological materials. The method was used for the measurement of AS-A activity in human leukocytes. Using the natural substrate for AS-A activity, AS-A activity could be specifically determined, as shown in the figure. In preliminary trials, attempts were made to determine AS-A activity in rat brain, and it was found that even when a different tissue and animal species were used, the present method could be satisfactorily applied for the determination of AS-A activity (table). The present method may be applicable for the discriminative determination of AS-A and AS-B in connection with diseases, particularly related to allergy. Such investigations are presently being undertaken and the results will be reported in future.

Conclusion. It was possible to apply the present method of AS-A activity determination to crude biological materials containing low enzyme activity, because of 1) the specific nature of the substrate and 2) the method of detection of the enzymatic reaction product. In addition, the time of analysis was shortened considerably because only the product, AA, is determined by HPLC-amperometric detection. In addition, the substrate, AAS, did not disturb the chromatographic process and detection. That is, the retention time of AAS was long (approx. 40–50 min). Thus, high sensitivity could be achieved with a much shorter time of analysis.

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Failure of coffee to inhibit the pharmacodynamic activity of morphine in vivo

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Summary. High doses of caffeine-containing as well as decaffeinated instant coffee neither inhibited morphine-induced analgesia in mice nor the morphine-induced fall of blood pressure, heart rate and respiratory rate in rats. On the contrary, caffeine-containing coffee even enhanced the analgesic effects of morphine in mice. Coffee thus does not exhibit opiate-antagonizing activity in the whole organism in vivo. The very weak morphine-antagonistic efficacy of coffee powder in the myenteric plexus-longitudinal muscle preparation from the guinea pig ileum is of no practical importance.
Key words. Coffee; caffeine; morphine; naloxone; analgesia.

Boublik and coworkers[1] recently reported the startling discovery that solutions of instant coffee powder, both regular and decaffeinated, are able to inhibit the in vitro binding of ^3H-labeled naloxone and other ligands to opiate receptor binding sites of rat brain membranes. Furthermore, a partially purified material from instant coffee powder antagonized the effects of morphine on the guinea pig ileum in vitro. From their results they estimated that one cup of coffee contains the equivalent of one-third of an ampoule of naloxone, and raised the possibility that drinking coffee might be followed by opiate receptor blockade not only in the gastrointestinal tract but also in the central and peripheral nervous system. We report here that instant coffee even in very high doses does not inhibit the pharmacodynamic activities of morphine in mice and rats in vivo. On the contrary, caffeine-containing coffee powder even enhanced the analgesic effects of morphine in mice.

The analgesic activity of morphine was assessed in male NMRI mice weighing 18–20 g by the well-known tail flick response test to radiant heat using the analgesia-meter of Joh. Friedrich Bundschuh (Griesheim, FRG). The reaction time was measured once before treatment with coffee and morphine and thereafter at 15-min intervals for the 1st, and at 30-min intervals for the 2nd hour. For each animal, the differences between the reaction times measured after morphine and the initial value were calculated. A commercially available instant coffee powder, both regular and decaffeinated (Jacobs Cronat Gold and Jacobs Nacht & Tag, caffeine content as measured gaschromatographically amounting to 20.2 mg/g and 2.6 mg/g, respectively), was dissolved in distilled water for oral administration and in saline for i.v. administration. The volume administered of these solutions was kept constant at 10 ml/kg b.wt. Morphine was dissolved in saline (10 ml/kg b.wt) and injected i.p. 15 min after coffee administration. Control animals treated with vehicle instead of coffee were studied simultaneously with the coffee-treated mice.

The mean reaction time of mice to radiant heat initially ranged between 5.6 and 7.7 s and did not change consistently upon treatment with saline or coffee (table 1). Morphine (10 mg/kg i.p.) doubled the reaction time in most experiments, with the analgesic effect reaching its maximum 45 min after the treatment

Table 1. Effect of coffee or naloxone on morphine-induced analgesia in mice

Pretreatment	Morphine (i.p.)	n	Reaction time to tail flick response Before morphine (s)	45 min after morphine (△s)
Water, 10 ml/kg p.o.	–	6	5.8 ± 0.3	± 0 ± 0.5
Instant coffee, 150 mg/kg i.p.	–	6	7.7 ± 0.4	− 0.5 ± 0.6
Water, 10 ml/kg p.o.	10 mg/kg	24	6.1 ± 0.4	+ 5.8 ± 1.3
Instant coffee, 150 mg/kg p.o.	10 mg/kg	14	6.1 ± 0.4	+ 10.1 ± 2.7*
Decaffeinated instant coffee, 150 mg/kg p.o.	10 mg/kg	10	5.6 ± 0.3	+ 4.5 ± 1.6
Water, 10 ml/kg p.o.	10 mg/kg	20	6.1 ± 0.2	+ 5.4 ± 1.3
Instant coffee, 2 g/kg p.o.	10 mg/kg	10	6.7 ± 0.6	+ 10.2 ± 2.6
Decaffeinated instant coffee, 2 g/kg p.o.	10 mg/kg	10	5.6 ± 0.3	+ 7.0 ± 1.4
Saline, 10 ml/kg i.v.	10 mg/kg	30	6.8 ± 0.3	+ 7.0 ± 1.4
Instant coffee, 60 mg/kg i.v.	10 mg/kg	10	7.3 ± 0.3	+ 10.8 ± 2.6
Decaffeinated instant coffee, 60 mg/kg i.v.	10 mg/kg	10	7.0 ± 0.3	+ 5.8 ± 2.8
Saline, 10 ml/kg i.v.	10 mg/kg	10	6.5 ± 0.5	+ 6.1 ± 2.1
Naloxone, 0.1 mg/kg i.v.	10 mg/kg	10	6.7 ± 0.3	+ 2.0 ± 0.4*
Naloxone, 0.5 mg/kg i.v.	10 mg/kg	10	7.3 ± 0.3	+ 0.6 ± 0.5*

Pretreatment was performed 15 min before administration of morphine. *Statistically significant difference from the simultaneously investigated control group not pretreated with coffee or naxolone, respectively ($p < 0.05$; range test of Wilcoxon, Mann and Whitney[8]).

(fig.). Pretreatment with 60 mg/kg coffee powder i.v. – a dose equivalent to that ingested per kg b.wt by an adult with two cups of coffee – did not inhibit but rather enhanced the analgesic activity of morphine (fig.). This effect may be related to the caffeine content of the coffee powder, because the same amount of decaffeinated instant coffee did not influence morphine-induced analgesia (fig.).

The ability of caffeine to enhance the efficacy of non-opioid analgesics has been proved experimentally[2-4] and was recently confirmed by an analysis of 30 clinical studies involving more than 10,000 patients[5]. According to the recent investigations of Misra et al.[6] and to our results the efficacy of caffeine as an analgesic adjuvant also proves true for opioid-induced analgesia.

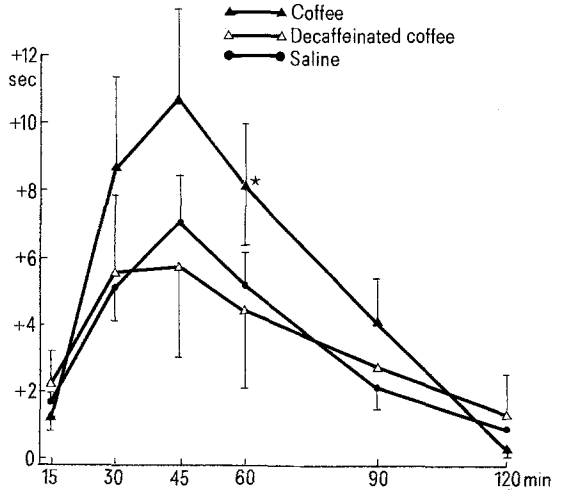
The presentation of the other experiments in mice will be limited to the results measured 45 min after treatment with morphine (table 1). Coffee powder administered orally in doses which correspond to the ingestion by an adult of about five or six cups of coffee, respectively, also enhanced the analgesic effects of morphine though the limit of statistical significance was reached only with the lower dose. As in the experiments with intravenous injection, also orally administered decaffeinated coffee did not influence the analgesic effects of morphine (table 1). Naloxone, on the other hand, reduced the analgesic activity of morphine by two-thirds in a dose of 0.1 mg/kg i.v. and inhibited it completely in a dose of 0.5 mg/kg i.v.

The influence of morphine (5 mg/kg i.v.) on the mean arterial blood pressure, the heart rate and the respiratory rate was investigated in male Wistar rats (340–400 g) anesthetized with pentobarbital (60 mg/kg i.p.). Blood pressure was recorded from the left carotid artery with a Statham transducer and heart rate taken from the ECG using a ratemeter whereas respiratory rate was measured by personal observation. Morphine produced an immediate and transient fall of blood pressure and heart rate peaking already during the first minute after injection. Maximal respiratory depression, on the other hand, did not occur until 10 min after the administration of morphine, and in one animal was evident only 30 min afterwards when the experiments were stopped. Means of the maximal deviations from the initial values produced by morphine in rats pretreated with saline, instant coffee, decaffeinated instant coffee (60 mg/kg each) or naloxone (1 mg/kg), respectively, are compiled in table 2. There was no influence at all of normal and decaffeinated coffee on morphine-induced hypotension and bradycardia. Respiratory arrest occurred in one animal of the control group, in two animals of the coffee group and three animals of the decaffeinated coffee group (out of six each). Naloxone, on the other hand, inhibited the cardiovascular and respiratory effects of morphine nearly completely.

Table 2. Influence of coffee or naloxone on the morphine-induced changes in mean arterial blood pressure, heart rate and respiratory rate in anesthetized rats

Pretreatment	Saline	Coffee	Decaffeinated coffee	Naloxone
Blood pressure (mm Hg)				
Before morphine	131 ± 9	135 ± 7	120 ± 8	130 ± 8
After morphine (△)	−58 ± 10	−45 ± 10	−50 ± 7	−7 ± 3*
Heart rate (min^{-1})				
Before morphine	342 ± 18	365 ± 13	348 ± 19	355 ± 18
After morphine (△)	−73 ± 16	−80 ± 9	−92 ± 14	−15 ± 2*
Respiratory rate (min^{-1})				
Before morphine	69 ± 5.5	68 ± 9.4	66 ± 7.9	54 ± 6.8
After morphine (△)	−27 ± 5.0	−40 ± 9.0	−49 ± 11	−6 ± 2.0*

Saline (10ml/kg), instant coffee (60 mg/kg), decaffeinated instant coffee (60 mg/kg) or naloxone (1 mg/kg) were administered i.v. 5 min before the i.v. injection of morphine (5 mg/kg). The maximal deviations from the values measured before morphine were calculated for each animal. Means ± SEM of six animals each. *Statistically significant difference from the corresponding value in saline-pretrated rats ($p < 0.05$; range test of Wilcoxon, Mann and Whitney[8]).



The influence of a pretreatment with instant coffee powder (normal or decaffeinated, 60 mg/kg i.v. 15 min before morphine) on the increase in the reaction time to radiant heat induced by morphine (10 mg/kg i.p.) in mice. ▲, Normal coffee powder (n = 10); △, decaffeinated coffee powder (n = 10); ○, saline (controls, n = 30). Means ± SEM.
*Statistically significant difference from the value simultaneously measured in the control group ($p < 0.05$).

In order to elucidate whether coffee brand differences account for the discrepancy between our results and those of Boublik et al.[1] we also investigated the capability of our instant coffee in antagonizing morphine in vitro. As in the paper of Boublik et al.[1] this was tested on the myenteric plexus-longitudinal muscle preparation from guinea pig ileum[7]. The muscle was set up isometrically in 36 °C tyrode and field-stimulated with rectangular pulses (0.1 Hz; 1 ms) of supramaximal voltage. After an equilibration period of 2–3 h, a dose-response relation was established by cumulative addition of morphine hydrochloride. The mean EC_{50} of morphine (n = 3) was 65 nM. Decaffeinated instant coffee (dissolved in bidestilled water) at a final concentration of 1 mg/ml shifted the concentration-response curve of morphine to the right, which resulted in a mean EC_{50} for morphine of 127 nM (n = 3). Additional experiments showed that this effect corresponded to that of 5 nM naloxone (= 2 ng/ml). Our instant coffee, therefore, also possesses the morphine-antagonistic in vitro activity demonstrated by Boublik et al.[1]. However, the efficacy of coffee in antagonizing morphine pharmacodynamically is very low indeed: One cup of coffee prepared according to manufacturer's recommendation from 2 g of instant coffee contains only the equivalent of one hundredth of an ampoule of naloxone. It must be stressed in this respect that the much higher estimation of Boublik et al.[1] according to which one cup of coffee corresponds to one-third of an ampoule of naloxone is based on binding studies and not on pharmacodynamic investigations.

In conclusion, coffee even in very high doses does not inhibit the pharmacodynamic activities of morphine in vivo. The in vitro results reported by Boublik et al.[1] and partially reproduced by us thus cannot be extrapolated to the whole organism in vivo. This is presumably due to the fact that the morphine-antagonistic activity of coffee is much too low as to be of any practical importance. There is no necessity, therefore, for patients needing the pharmacodynamic actions of morphine to avoid coffee consumption.

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Myelin changes in the rats CNS following intraventricular injection of serum[1]

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Summary. Normal human or rat serum administered by intraventricular injection induced demonstrable changes in the rat CNS myelin as seen from an increased recovery of dissociated myelin (DM), i.e. a myelin-related low density membrane fragments, from the tissue homogenates. The yield of DM reached a maximum on the third postinjection day and returned to the control level by day 5. In spite of the increased recovery of DM, no physico-chemical alternations in myelin isolates and no histological abnormalities in the tissue could be detected. The production of DM seems to be a sensitive index of serum-induced alteration of the myelin sheath.
Key words. Serum; intrathecal injection; CNS; dissociated myelin; demyelination; multiple sclerosis.

Abnormalities in the CNS blood vessels[3] followed by increased vascular permeability[4,5] may be among the earliest changes that procede demyelination in multiple sclerosis (MS). Some of the extravassating blood components may have a detrimental effect on the CNS tissue and contribute to the process of demyelination. We have recently demonstrated that the exposure of isolated myelin to serum results in gross chemical and physical changes in the membrane[6]. Similar myelin alterations can also be detected in cerebral slices following their incubation with serum[7]. The present experiments are a continuation of these studies aimed at establishing whether the myelin deteriorations can be induced in the CNS tissue exposed in vivo to intrathecally injected serum.

Material and methods. Adult male Wistar rats weighing about 300 g were anesthetized with Na-pentobarbital, 50 mg/kg. After fixation of the head in a stereotaxic device, a burr hole was made over the left parietal region and 100 µl (human or rat) serum or Krebs-Ringer bicarbonate buffer was injected intraventricularly using a Hamilton syringe with a 30G steel cannula. The coordinates from bregma were: 0 mm A/P, 2 mm L, 3.5 V[8].

Different anatomical parts of the CNS were dissected out and weighed. The tissue from two animals was combined and homogenized in 0.32 M sucrose. The material floating on 0.32 M sucrose (DM) and the myelin subfractions were isolated as previously described[7]. The electrophoretic analysis of membrane proteins was performed by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulphate[9]. The immunoblot conditions were as described by Greenfield et al.[10].

Animals for histological examination were anesthetized with 1.5% Halothane and perfusion fixed with 300 ml Lillie's phosphate buffered formaldehyde through a cannula in the ascending aorta. The paraffin embedded brains were serially sectioned at 7 µm and stained with cresyl violet and luxol fast blue.

Results and discussion. No mortality or changes in the normal behavior of rats were observed following the intraventricular injection of human (or rat) serum or Krebs-Ringer bicarbonate buffer. The examination of the brain tissue at the light microscopic level revealed no large hemorrhages, cellular infiltrations or infarcts. Around the cannula track in the cortex a few macrophages with hemosiderin granules and some necrotic nerve cells were seen. Systematic examination of myelin stained specimens showed some myelin loss around the cannula track in corpus callosum with a few luxol fast blue positive swollen macrophages. There was also vacuolization of myelin sheaths in the surroundings of the tract. These changes seemed to be more pronounced in the serum injected animals. No remote signs of focal or diffuse demyelination were found. Our findings corroborate those from other laboratories reporting no pathological changes in the tissue due to intrathecal injection of control serum[11,12].

The physico-chemical changes in myelin observed in the system of tissue slices following incubation with serum, i.e. the increase