e.g. anoxia[12], halothane anaesthesia[13], etc) which can precipitate the development of dysrhythmias. A decrease in conduction, even in a short segment of Purkinje fibres, can increase the probability of arrhythmias in many ways[14], and the shortening of the action potential duration, especially together with the slowing of conduction, might be expected to favour re-entry activity[15]. Another mechanism by which octanoate could promote the tendency towards arrhythmias is if it did not shorten the action potential duration of adjacent cardiac fibres or groups of fibres to the same extent. This might result in an increase in the normal asynchrony of recovery of excitability which has long been considered an important arrhythmogenic factor under various conditions, such as local myocardial ischemia, sympathetic activation, digitalis intoxication, etc[16,17]. Further studies to clear this point appear warranted.

An arrhythmia may also result from enhanced automaticity in ectopic pace-maker areas. A number of factors known to cause or potentiate development of ventricular arrhythmias can produce an increase in spontaneous firing of cells in the distal parts of atrioventricular node and in the His-Purkinje system[14,17]. It has been postulated that unbound FFAs might have detergent effect on cardiac cell membranes, thereby causing cation loss and resultant increase in ectopic impulse formation[18]. This assumption did not prove to be correct in our experiments in which after 60 min exposure to octanoate (2,4 mM), the spontaneous activity of ventricular strips completely (Figure 2). The initial, entirely regular spontaneous rate (about 70/min) was progressively reduced, and from time to time arrhythmias appeared. By the end of the test period, no activity was detected in 9 out 10 preparations. After 60 min washing with control solution, an almost complete recovery occurred. In this context, it is of interest to note that, in an unpublished series of experiments, octanoate (2,4 mM) did not alter substantially the intrinsic firing rate of the sinoatrial node of isolated rabbit atria[19].

In spite of the fact that in our experiments octanoate did not increase the automaticity in certain ectopic pacemaker areas of the normal cardiac tissue, it might still be shown to potentiate the effect of local myocardial ischaemia or catecholamines, both of which can cause spontaneous discharge of Purkinje fibres resulting in ventricular arrhythmias[14,17]. It is, of course, hardly to be expected that among the complex haemodynamic, autonomic, local and general metabolic changes occurring after acute coronary occlusion, the elevation of unbound FFA level would be alone responsible for the development of early post-infarction arrhythmias. In this respect, there is a real need for further studies of the cardiac electrophysiological effects of FFAs under in vivo conditions, with due regard also to the pathological processes characteristic of an acute myocardial infarction.

Whatever the role of a FFA may be as an additional factor in the genesis of arrhythmias due to a pathological situation, from our results with octanoate it seems reasonable to conclude that membrane function can be seriously altered by a FFA already in a normal Purkinje fibre. In favour of our suggestion, it has recently been shown that unbound FFAs (octanoate[10], oleate[20], and palmitate[20]) can produce severe arrhythmias, including ventricular fibrillation, even in normal isolated rat hearts.

Zusammenfassung. Am Beispiel von Octanoat (Kaprylsäure) wird nachgewiesen, dass Fettsäure das Aktionspotential von Purkinje-Fasern des Kalbes verändert. Dieselben Octanoatkonzentrationen von 2,4 mM unterdrückten auch die spontanen Aktivitäten isolierter Herzkammer-Streifenpräparate.

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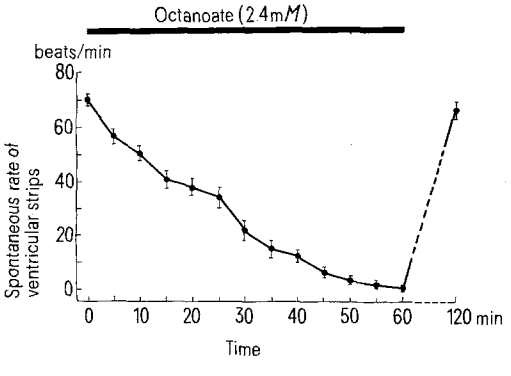

Fig. 2. Changes in the spontaneous rate of ventricular strips under the influence of octanoate. Exposure to octanoate started at 0 min right after the control measurements. Between 60 and 120 min, the preparations were washed with control solution. Vertical bars indicate ± S.E.; $n = 10$.

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Absence of Evidence of Biotransformation of Morphine to Codeine in Man

In studies of the human metabolism of morphine, it was found that in addition to morphine glucuronide, morphine ethereal sulfate, normorphine and normorphine conjugate were metabolites of morphine[1]. A recent paper[2] reporting the formation of codeine from morphine in man prompts the publication of the present paper.

Material and methods. Urine was collected for consecutive 24-hour periods from post-addict prisoner volunteers receiving chronic administration of morphine sulfate, 60 mg q.i.d., s.c., and was analysed for morphine and its metabolites with thin-layer chromatography (TLC)[3] and gas-liquid chromatography (GLC)[4]. Spots of morphine and

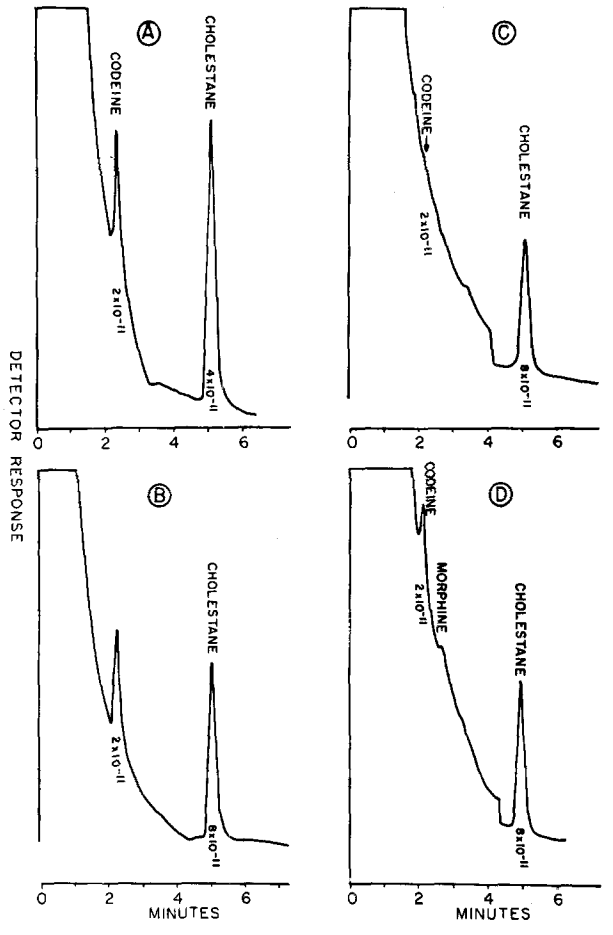

Fig. 1. Gas-liquid chromatograms of extracts from aqueous solutions containing 10 µg of codeine phosphate (A) and 10 mg of stock morphine sulfate (B), and from acid hydrolyzed urine of a subject receiving chronic administration of 240 mg morphine sulfate per day, 5 ml (C) and 20 ml (D) of pooled urine collected during a 24-hour period. The number under the curves represent the attenuation × range (Amps/mV).

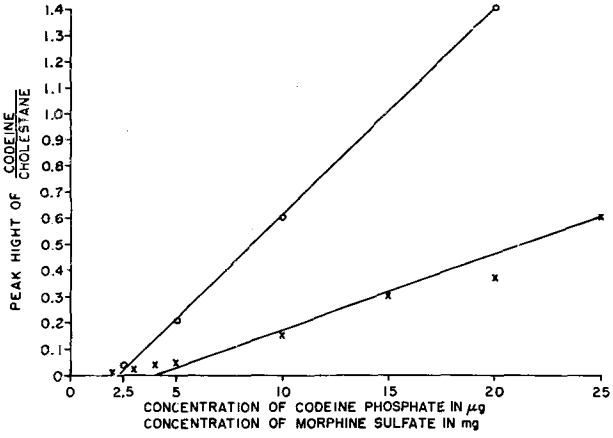

Fig. 2. Standard curve of codeine phosphate (○—○), and codeine found in morphine sulfate (×—×), U.S.P.

normorphine, but not of codeine were observed on TLC of the free drug fraction of the XAD-2 resin extract of the urine[1] and of the organic solvent extract of the acid hydrolyzed urine[5]. A small shoulder corresponding to the peak of authentic acetylcodeine was observed on the GLC of the urine extract derivatized with acetic anhydride[6] (Figur 1C); whereas, extracts derivatized with Tri-Sil-Z (a silanizing agent), trifluoroacetic anhydride and pentofluoropropionic anhydride did not show this peak. TLC of the extract obtained from 75 ml acid hydrolyzed urine, theoretically containing 24 mg of administered morphine sulfate, showed a faint spot corresponding to codeine. GLC of the acetyl derivative of the extract obtained from 20 ml hydrolyzed urine showed a small peak corresponding to acetylcodeine (Figure 1 D).

To examine the possibility that the small amount of codeine found in the urine might have originated as a contaminant of the injected morphine and not a biotransformation product of morphine, 0.5 to 25.0 mg of stock morphine sulfate was extracted, acetylated and chromatographed. The GLC showed that in the morphine samples larger than 2.0 mg there were peaks corresponding to acetylcodeine (Figure 1 B). The concentration of codeine in the morphine sample calculated from a standard curve prepared from 2.5 to 20 µg of codeine phosphate was found to be 0.04% (Figure 2). As can be seen from Figure 1 D the amount of codeine present in the 20 ml hydrolyzed urine is less than $^1/_2$ the amount found in a 10 mg sample of morphine sulfate. Based on the assumption of the daily urine containing all daily administered drug, the sample theoretically should contain about 7 mg of morphine sulfate and it was found to contain 5.2 mg with GLC analysis. The amount of codeine found was about 31.1% of the codeine present as impurity in 7 mg of administered morphine sulfate. The observation of codeine in the urine of morphine addicts during the 24-hour period of urine collection, therefore, could be accounted for by contamination of the injected morphine sulfate.

Discussion. Codeine in small amounts was detected in the human urine after chronic administration of 240 mg morphine sulfate daily and in this regard the present data partially confirms the observation of BÖNER and ABBOTT[2]. However, their finding of a large amount of codeine (0.7 to 6% relative to morphine) in urine samples cannot be confirmed. The source of codeine observed in the

[1] S. Y. YEH, Fedn. Proc. *32*, 763 (1973).

[2] U. BÖRNER and S. ABBOTT, Experientia *29*, 180 (1973).

[3] TLC was performed on Quantum Silica Gel precoated plates, developed with ethyl acetate:methanol:ammonium hydroxide (28%) (17:2:1) and sprayed with pot. iodoplatinate reagent.

[4] A Varian Aerograph, Series 2700, equipped with a 5 foot × 2 mm stainless steel column packed with 3% SE-30 coated on Varaport 100/120 and a 3 foot × 2 mm glass column packed with 3% OV-17 coated on gas-chrom Q, 60/80 mesh and flame ionization detectors. Temperatures for injector, column and detector, were 255°, 250° and 295 °C, respectively.

[5] S. Y. YEH, Report of the 36th Annual Meeting of NAS-NRC Committee on Problems of Drug Dependence (1973), p. 215.

[6] Five ml urine was hydrolyzed with 20% by volume of concentration HCl in a steam jacketed autoclave at 15 lb pressure for 30 min, adjusted to pH 14 with NaOH, salted with NaCl and extracted with 15 ml of $CHCl_3$ by shaking for 15 min and centrifuged for 15 min. The aqueous phase was discarded and 13 ml of the organic phase was extracted with 2.5 ml of 2*N* HCl. After removal of the organic phase the acidic aqueous phase was again adjusted to pH 14 and extracted with $CHCl_3$. 13 ml of the organic phase and 0.05 ml of cholestane (0.1 mg/ml) solution were evaporated to dryness and the residue acetylated with 0.2 ml of acetic anhydride and 0.1 ml of pyridine in a sealed tube at 60–70 °C for 1 h. The excess acetic anhydride was removed and the residue dissolved in 0.05 ml ethyl acetate and 1 µl of the solution injected into the GC.

urine in the present studies was attributed to the codeine that was present as an impurity in the morphine injected, and not to a biotransformation product of morphine.

The finding of a large amount of codeine in urine samples by BÖRNER and ABBOTT[2] might be due to on column acetylation of the urine extract with both monoacetylmorphine and acetylcodeine in the same peak.

It has been pointed out that on column acetylation of morphine shows 2 peaks, the first corresponding to monoacetylmorphine and the second to diacetylmorphine[7]. GLC results obtained in the present studies indicate monoacetylmorphine and acetylcodeine have similar retention times, and cannot be resolved in either 3% OV-17 or 3% SE-30 columns at various temperatures. Therefore, there is the possibility of measuring the first peak of acetylmorphine as acetylcodeine when on column acetylation is used for samples containing both codeine and morphine.

The findings of codeine (8 to 15% relative to morphine) in the urine of heroin addicts[2] might be due to the contamination of heroin with acetylcodeine and column acetylation. It is well known that acetylcodeine is a byproduct of heroin, especially when the heroin is obtained from an illicit source[8]. The presence of acetylcodeine and codeine in the illicit heroin has been detected with paper chromatography and TLC. The mean ratio of codeine to morphine in the 20 hydrolyzed heroin samples has been quantitatively determined and found to be 0.11 (0.10–0.14)[8]. BÖRNER and ABBOTT[2] stated that the heroin sample obtained from the street was analysed and no codeine or acetylcodeine were found. However, the size of sample used for analysis of codeine and acetylcodeine could affect the detectability of a small amount of contaminants in the drug.

In studies of the metabolism on morphine in vivo. ELISON and ELLIOTT[9] observed a radioactive spot corresponding to codeine in the extract of a pooled 24-hour urine sample of Long-Evans rats pretreated with morphine-N-$^{14}CH_3$ (the dose given was not stated, it was presumably 20 mg/kg) and a Gunn rat s.c. given 20 mg/kg of morphine-N-$^{14}CH_3$. The authors pointed out that "a 24-hour urine sample from a single jaundiced rat was sufficient to give detectable metabolite spots in duplicate, whereas 4 pooled 24-hour urine samples from normal rats were necessary to give one single detectable spot". The radioactive spot corresponding to codeine in normal rats observed by ELLISON and ELLIOTT was interpreted by me to possibly come from the original source as a contaminant in the morphine injected. The data of ELISON and ELLIOTT were therefore interpreted that *O*-methylation of morphine does not take place in normal rats[9] but does in Gunn rats which possess hereditary defects in glucuronide formation[10]. Thus, only when the major metabolic pathway, glucuronidation at the 3-position, is blocked then *O*-methylation of morphine takes place.

Codeine has been qualitatively identified in the urine of dogs after s.c. injection of 350 mg/kg of morphine sulfate[9]. The codeine might also come from the original source; presumably all or at least a large quantity of the urine had been used for those studies.

Using highly sensitive techniques the metabolism of N-^{14}C-methylcodeine in man[11], monkeys[12], dogs[13,14], rabbits[14], cats[14] and rats[15] has been studied and it was found that about 90% of the administered codeine was biotransformed. *O*-demethylation (except in dogs) and glucuronidation were the major metabolic pathways for codeine in man, monkeys, dogs, rabbits and rats, and N-demethylation was the major metabolic pathway for cats. The apparent morphine, a biotransformation product of codeine, was further conjugated. In other words, if biotransformation of morphine to codeine does take place, then the apparent codeine will be metabolized. Nevertheless, no evidence was observed in the present studies for the formation of codeine from morphine in man.

Zusammenfassung. Nachweis von Codein im Harn von chronisch mit Morphin-Injektionen behandelten Personen. Diese Codein-Spuren können jedoch aus Verunreinigungen des benützten Morphins stammen und bilden daher keinen Beweis dafür, dass Codein beim Menschen durch Biotransformation als Metabolit von Morphin entsteht.

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Strychnine as an Anticholinesterase: in vitro Studies with Rat Brain Enzymes

The convulsive effects of relatively low doses of intravenously injected strychnine have been largely explained by the finding of BRADLEY, EASTON and ECCLES[1], who showed that this alkaloid reduces or abolishes inhibition in the central nervous system, especially in the spinal cord, medulla and pons. The mechanism by which it so acts appears to be the binding of the drug to post-synaptic receptors for a 'glycine-like' inhibitory transmitter, probably in a competitive manner[2,3]. However, neither the molecular mechanism by which strychnine acts at low concentrations, nor the mechanisms by which higher doses of strychnine are excitatory, especially when topically applied to brain cortex, a structure largely insensitive to intravenous strychnine, and which is the basis for the classical neuronography, are sufficiently understood at present.

Our laboratory has been concerned with the in vitro effects of strychnine on different neural components, as a