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

means of solving some of the problems[4,5] indicated. In this respect it seemed of interest to follow up the finding, first reported by NACHMANSON in 1939[6], that strychnine was in vitro a rather potent inhbitor of acetylcholinesterase (AChE, acetylcholine acetyl-hydrolase, E.C. 3.1.1.7), particularly since that study was carried out before the differences existing between AChE and cholinesterase (ChE, acylcholine acyl-hydrolase, E.C. 3.1.1.8), especially with respect to their substrate specifities, were recognized[7,8]. Later studies have shown that also the plasma acetylcholine hydrolyzing activity is inhibited by this drug[9,10], however no studies have been performed using specific substrates for AChE and ChE, and the type of inhibition exerted by strychnine on these enzymes is unknown, thus rendering the bearing that those findings have on the in vivo pharmacological effects of the drug only a matter of conjecture.

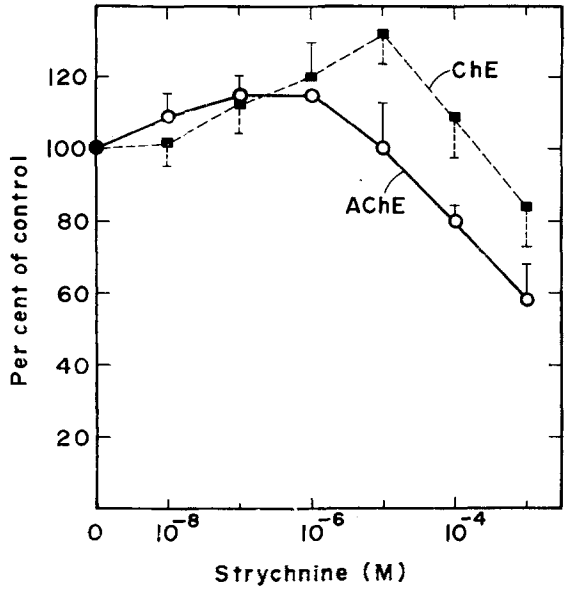

Fig. 1. Inhibition of AChE and ChE by Strychnine. ○, AChE assayed with 3 mM acetylbetamethylcholine; ■, ChE assayed with 3 mM butyrylcholine. Each point represents 4 to 6 assays ± 1 probable error.

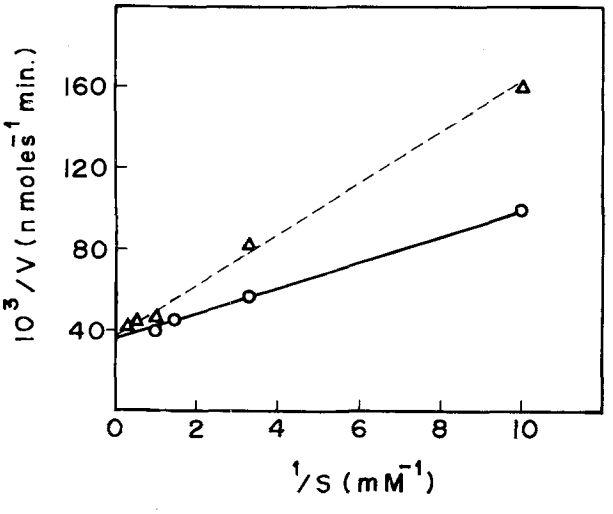

Fig. 2. Lineweaver-Burk Plot of AChE. ○, enzyme alone; △, enzyme plus 5×10^{-5} M strychnine.

Materials and methods. Acetylbetamethylcholine and butyrylcholine were used as specific substrates for AChE and ChE, respectively, and the acid liberated was recorded continuously by means of an automatic recording pH stat (Radiometer SBR2 Titrigraph), exactly as previously described[11]. Under these conditions, reaction rates are linear for at least 40 min. The partially purified rat brain AChE and ChE utilized were also obtained as indicated in our previous report[11].

Strychnine sulfate was from Sigma Chemical Co. and was homogeneous in 2 chromatographic systems: Thin layer on silical gel G with xylene, butanone, methanol, diethylamine (20:20:3:1) as solvents, or ascending on Whatman No. 1 paper using n-butanol, concentrated HCl and water (100:15:34) as solvent system.

Results. When AChE was incubated with 3 mM acetylbetamethylcholine (the K_m of the enzyme for this substrate being 2.3×10^{4} M), strychnine had an inhibitory effect at concentrations higher than 10^{-5} M (Figure 1). On the other hand, ChE incubated with 3 mM substrate (K_m for butyrylcholine: 6.6×10^{-4} M) was markedly less sensitive to strychnine, and only at 10^{-3} M drug was inhibition detectable. With both enzymes, low strychnine concentrations consistently enhanced enzyme activity.

The inhibition of AChE by strychnine was next studied at different substrate concentrations, and the results expressed in a double reciprocal plot (Figure 2). Strychnine behaves as a competitive inhibitor and a K_i of 7×10^{-5} M was estimated from the Lineweaver-Burk plot. A Dixon plot[12] (not shown), using 0.5 and 2.5 mM substrate concentrations gave a similar K_i value (4×10^{-5} M). ChE was also inhibited by strychnine in a competitive manner (Figure 3), and a K_i of 1.7×10^{-4} M was estimated in this case.

Discussion. While confirming previous reports that strychnine is an anticholinesterase[6,9,10,13], the main finding of this study is that strychnine inhibits competitively both AChE and ChE, showing a somewhat higher affinity for the former. This should allow a more definite assessment of the bearing that this activity of strychnine has on its in vivo mechanism of action.

Even 0.2 mg/kg of i.v. injected strychnine is able considerably to reduce the IPSPs of spinal motoneurons[2]. If the unwarranted assumption is made that strychnine is only distributed in the extracellular fluid space, the maximal concentration that it could reach is 3×10^{-6} M, that is more than one order of magnitude lower than the K_i of the AChE-strychnine complex. It does not therefore appear feasible to attribute to this anticholinesterase activity of the drug its mechanism of action when acting in low doses. A similar conclusion had already been reached by LAPICQUE[14] on other grounds. On the other

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hand, a large variety of other actions of strychnine have been demonstrated with higher doses, namely an antagonism to the inhibitory effects of GABA[15,16], and a curare-like action in sympathetic ganglia and in neuromuscular synapses[17,18], D-tubocurarine being itself an excitatory agent when applied to the cerebral cortex[19], possibly also acting there as anti-GABA compond[20].

Strychnine at concentrations higher than 10^{-3} M apparently also expands plasma membranes[21]. This latter effect, because of the very large concentrations needed, does not appear to be related to the anticholinesterase nor to the in vivo effects of strychnine. On the other hand, a discrimination between the anticholinesterase, the anti-GABA and the curare-like effects cannot be made on the basis of concentration, since all occur at similar ones. However, the relative inefficacy as neuronal excitors of anticholinesterases more potent than strychnine[22,23], make it unlikely that the anti-AChE action of this alkaloid is the main mechanism for its excitatory effects when applied topically to the brain cortex, although it could contribute in some degree to this highly complex effect[24], since it is known that acetylcholine, which is a minor cortical transmitter, has predominantly excitatory effects on this structure[25,26]. Since nicotinic inhibitory receptors in the cerebral cortex are yet to be found[27], and D-tubocurarine itself has some anti-GABA effects[20], it seems probable that the primordial mechanism for the excitatory action of strychnine in the cerebral cortex is its anti-GABA activity.

Resumen. La estricnina inhibe competitivamente a la acetilcolinesterasa (AChE, acetilcolina acetil-hidrolasa, E.C. 3.1.1.7) y a la colinesterasa (ChE, acilcolina acil-hidrolasa, E.C. 3.1.1.8) de cerebro de rata. Se calculó un K_i de 7×10^{-5} *M* para AChE y de 1.7×10^{-4} *M* para ChE cuando sus actividades enzimáticas se midieron con acetilbetametilcolina y butirilcolina, respectivamente. Se analizó la relevancia de estos hallazgos para el mecanismo de acción de dosis altas de estricnina in vivo, concluyéndose que esta acción anticolinesterásica es de escasa importancia.

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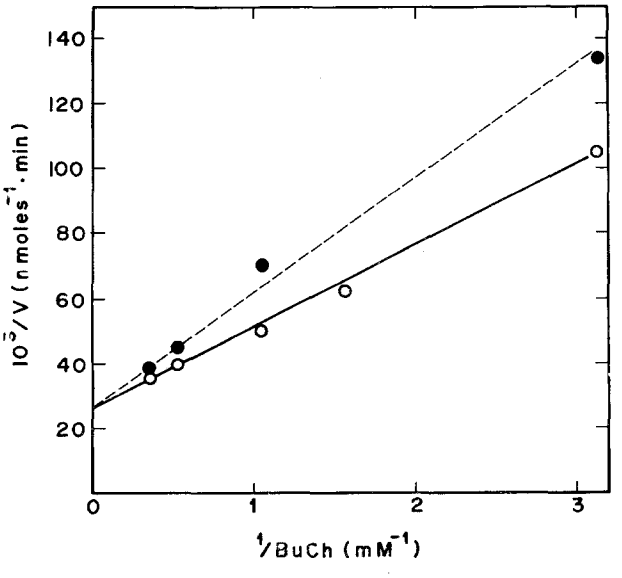

Fig. 3. Lineweaver-Burk Plot of ChE. ○, enzyme alone; ●, enzyme plus 5×10^{-5} *M* strychnine.

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Inhibition of Vasopressin-Induced Morbid Effects on the Rat Kidney by Pindolol and Propranolol

Studies on renal blood flow have shown that low amounts of vasopressin diminish the circulation in the renal medulla of the rat[1] and hamster[2]. From this finding a selective sensitivity to ADH of arteries of this area has been suggested[1].

In recent experiments in our laboratories, it has been observed that vasopressin, in doses nearly proportional to those which have been occasionally used in man[3], induces rat renal ischemic lesions localized in the region between medulla and cortex[4], supporting also the selective arterial sensitivity.

Since some reports indicate that in the ADH effect in animals[5,6] and in man[7] adrenergic mechanism is involved the present experiments were designed to examine whether the vasopressin-induced rat kidney damage is influenced by β-adrenoceptive receptor blocking drugs.

Material and method. The experiments to be described were conducted on young adult albino rats (Wistar origin) of both sexes, averaging 220–240 g, housed 4 in each cage, at 21–22 °C and air humidity 48–51%, 10–12½ h light/day, on pellets diet (Zootrofiki Athens-Greece) and tap water ad libitum; food was only removed 12 h before the experiment.

The antidiuretic hormone (Vasopressin-Sandoz) was injected i.p. once daily at a dose of 100 mU/100 g body wt. for 3 successive days. As β-adrenergic receptor blocking