into the test chamber, and allowed to acclimate to the situation for 1.0 h. Then a predrug control EEG sample was recorded. Immediately thereafter the animal was dosed by oral intubation and 30 min later the first posttreatment EEG sample was recorded. Similar EEG samples were repeated every 30 min for a total of seven per 180-min test session.

Pramiracetam was placed into solution in deionized water, which also served as the placebo substance. Similar studies on piracetam served to provide a reference standard. All drug doses are expressed in terms of the active drug moiety.

Results and discussion. A representative result showing the difference in EEG spectral profiles between a young and an old rat is shown in figure 1. A logarithmic scale has been imposed on this display in order to expand the important delta and theta bands into the center portion of the graph, thereby facilitating viewing.

As can be seen from both the spectrum analysis and from the brief sample of actual EEG tracing, the cortical EEG of the aged rat is dominated by very slow wave (< 4 Hz), high voltage activity. The young rat's cortical EEG samples do not show this kind of slow-wave activity. Equally well, the aged rat's hippocampal EEG activity differs from that of the young rat's; that is, the theta rhythm which is so dominant in the young rat's hippocampus is largely absent in the old rat's hippocampus. Moreover, the spectrum analysis shows clearly that slow-wave activity (< 4 Hz) is predominant also in the hippocampus of the aged rat. These findings indicate that the aged rat's brain suffers from a deficit in vigilance, which is revealed clearly by testing under basal conditions. It should be added that these animal findings, plus the concept of a vigilance deficit, are consistent with work done in man[1,2]. Of course, the human studies could not investigate the electrical activity of the hippocampus for obvious reasons.

Figure 2 presents a representative result showing how pramiracetam affected an aged rat's cortical and hippocampal EEG. In these tests, 20 mg/kg of pramiracetam was administered orally once per day for seven consecutive days. The drug effects shown are for the EEG sample 90-min posttreatment, which is the time of peak action of pramiracetam following oral dosing.

As can be seen from figure 2, 20 mg/kg of pramiracetam produced cortical and hippocampal activation on the first day of dosing and also on the seventh day. These arousal effects can be seen in the shape of the spectral profile as well as in the millivolt score associated with the electronic window sampled in each spectral analysis. It is important to note that after seven days of treatment the arousal effect in cortex and hippocampus were stronger than on the first day. In fact, by the seventh day the spectral wave analyses appear indistinguishable from a young rat's. This finding shows that rapid tolerance does not develop to the central effect of pramiracetam.

The table shows the results of similar studies in a number of aged rats employing daily doses of vehicle (deionized water), the reference drug piracetam at two doses, and pramiracetam at two doses. The findings are presented as mean millivolts RMS and also as the mean percent change from the initial baseline level (all measured in the same manner as previously described for the 90-min posttreatment EEG sample).

The results for vehicle shown in the table indicate that the largest change associated with vehicle was +2.7%. The changes associated with pramiracetam treatment were very much larger than this, and all attained statistical significance. Generally, piracetam produced results similar in pattern to pramiracetam, although the effects were smaller in magnitude despite employing much greater doses. Moreover, the cortical arousal effect of piracetam was always rather weak compared to pramiracetam's, and by the seventh day of dosing piracetam's effects on hippocampus and cortex had largely disappeared (apparent tolerance development), while pramiracetam's seemed to be still increasing. Thus, the present method enables one to distinguish between drugs in terms of the arousal patterns they produce in the aged brain after acute doses and also after a period of daily doses.

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Increased formation of arginine deiminase by *Clostridium perfringens* FD-1 growing in the presence of caffeine

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Summary. Caffeine slowed growth and markedly increased the formation of arginine deiminase in growing *C. perfringens* FD-1 when dextrin, but not maltose or maltotriose, served as the energy source. It is postulated that the ability of caffeine to induce arginine deiminase is related to an inhibition of polysaccharide utilization, resulting in a shift-down condition known to induce arginine deiminase and other enzymes in bacteria.

Key words. Clostridium perfringens; caffeine; arginine deiminase; shift-down; dextrin.

Several isolated reports have implicated caffeine in the induction of gene-expression of luciferase[1], adenylosuccinate lyase[2], and λ-prophage[3] in prokaryotes, ornithine decarboxylase[4] and tobacco mosaic virus[5] in eukaryotes. In no case was the mechanism elucidated. Caffeine and related methylxanthines have been shown to stimulate sporulation in certain strains of *C. perfringens*[6–11]; sporulation is considered to be a primitive form of differentiation[12]. This caffeine effect does not appear to be related to increased levels of cyclic AMP[13] resulting from inhibition of cAMP phosphodiesterase by the methylxanthine. We report here that induction of arginine deiminase in *C. perfringens* FD-1 is greatly increased by caffeine, an effect pronounced when dextrin serves as the energy source but slight in the presence of maltose or maltotriose.

Materials and methods. Strain; spore stocks. *C. perfringens* strain FD-1 was obtained from S.M. Harmon, Food and Drug Ad-

Influence of carbohydrate energy source on growth rate and level of arginine deiminase in *C. perfringens* cells

Carbohydrate	Control cells		Cells grown with 4 mM caffeine	
	τ	Arginine deiminase $\mu M \cdot 10^6$ cells $^{-1}$ h^{-1}	τ	Arginine deiminase $\mu M \cdot 10^6$ cells^{-1} h^{-1}
Dextrin	54	23	95	57
Maltose	36	25	37	33
Maltotriose	39	18	40	20

τ = estimated doubling time, min. KU = Klett units.

ministration, Washington, D.C. Spore stocks were prepared on CPS medium as previously described[7] and maintained in serum bottles at 5 °C.

Medium. The medium (CY) used for all experiments reported here contained: Casitone (Difco), 6,0%; yeast extract (Difco), 0.5%; K_2HPO_4, 1.0%, and was supplemented with carbohydrates, as indicated in the text, at a level of 1%. Caffeine (4 mM) was incorporated as indicated in the text. Sodium thioglycollate (0.02%) was added immediately prior to autoclaving.

Preparation of cells. Inocula were prepared from spore stocks by growing overnight in Fluid Thioglycolate Broth (Difco) at 37 °C[6]. Culture tubes (16 mm) containing 13 ml of freshly autoclaved CY medium were inoculated with 0.5 ml of the overnight culture in Fluid Thioglycolate Broth, and placed in a water bath at 37 °C. Turbidity was measured immediately with a Klett-Summerson colorimeter (Nr. 66 filter) and monitored periodically thereafter; doubling time (τ) was estimated from the turbidity readings[9]. The young cultures were harvested by centrifugation at 19 °C at 2.5 h, or at times indicated and washed once in 0.5 M Na/K phosphate buffer, pH 6.5, supplemented with 0.1% glucose to protect viability[8]. Since a linear relation between Klett readings and the log of the direct cell count (as determined with a Petroff-Hausser chamber) was observed in the turbidimetric range encountered in these experiments, specific activity of arginine deiminase was based upon cell number, as determined from Klett units (K.U.).

Arginine deiminase. Washed cells prepared as above were resuspended to ¼ the original culture volume in the same 0.5 M phosphate buffer, containing 10 mM arginine, and 2.5 ml transferred to thermal death time (TDT) tubes (8 × 150 mm). After 40 min, the cells were removed by spinning in a microcentrifuge, and the supernate filtered through a 0.2-μm membrane filter. Residual arginine was then determined.

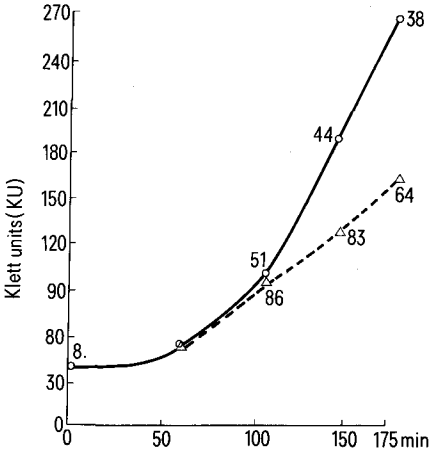
Amylase. For amylase determinations, cells were sedimented and resuspended in 0.1 M phosphate, pH 6.0[14]. The procedure of Priest[14] was followed, except that incubation temperature was 37 °C, and cell suspensions were incubated in TDT tubes.

Analytical procedures: Arginine was determined by the method of Van Pilsum et al.[15]; ornithine by the method of Chinard[16]; and citrulline by the method of Prescott and Jones[17] as modified by Shindler and Prescott[18]. These compounds, and ammonia, were also determined chromatographically with an amino acid analyzer, using a Durrum resin[19,20].

The iodine test for starch, was performed by the method of Smith and Roe[21] as modified by Priest[14], using soluble starch as a substrate.

Chemicals. Dextrin was obtained from Difco Laboratories. Soluble starch was obtained from Merck & Co. Maltotriose and maltose were obtained from Sigma Chemical Co.; other maltose samples were obtained from Fisher Scientific and from Difco.

Results. When *C. perfringens* FD-1 was grown on CY medium with dextrin, induction of the arginine dihydrolase system[22] was evident within 3 h, as determined by the ability of the washed cells to convert arginine to citrulline, ornithine and ammonia (data not shown). When caffeine (4 mM) was added to the culture medium, growth slowed markedly after 100 min (fig.); arginine deiminase (EC 3.5.3.G) activity was consistently higher in the caffeine-grown cells sampled after 100 min. The addition of caffeine to washed cells did not affect activity; evidently caffeine was enhancing the formation of arginine deiminase, not its activity. If maltose or maltotriose replaced dextrin in CY medium, caffeine (4 mM) did not significantly alter the growth rate, and only relatively minor effects on arginine deiminase levels were observed (table). Other experiments (not shown) demonstrated that caffeine (4 mM) did not affect growth rates or arginine deiminase levels when glucose or sucrose served as the energy source. The results suggested that caffeine exerted its primary effects on an early stage of dextrin metabolism. Preliminary experiments suggested that caffeine-grown cells, resuspended in minimal medium-casein hydrolysate[16] formed very little extracellular amylase, whereas control cells formed amylase normally, in the two-phase fashion demonstrated previously for *Bacillus subtilis*[14].



Growth of *C. perfringens* FD-1 in CY medium ± caffeine (4 mM). Control (○——○); 4 mM caffeine added (△——△). Specific activity of arginine deiminase was significantly higher in caffeine-containing cultures than in control at all sampling times; maximal difference was observed at 147 min, at which time arginine deiminase specific activity was 120% higher in the caffeine containing culture. Numbers refer to arginine deiminase activity of cells at times indicated in $\mu M \cdot KU^{-1}$ h^{-1}.

Discussion. The results presented here show that when *C. perfringens* FD-1 is grown with a dextrin energy source, the addition of caffeine slows growth and markedly enhances the induction of arginine deiminase. These effects were not observed to any marked degree when a number of simple sugars served as the energy source. In particular, no large increase in arginine deiminase activity was observed when caffeine was present in cultures in which maltose or maltotriose served as the energy source, as contrasted with a striking increase in dextrin-containing cultures. Apparently caffeine in some way adversely affects polysaccharide utilization; the increased synthesis of arginine deiminase might be an indirect result of an inhibitory effect of caffeine on the formation of extracellular amylase, resulting in nutrient depletion and a slowing of growth. Mercenier et al.[23] found that the arginine deiminase pathway in *Pseudomonas aeruginosa* was used to obtain energy under conditions of nutrient depletion; in *Bacillus*, arginase, as well as sporulation, is known to be induced under 'shift-down' conditions[12].

The exact manner in which caffeine adversely affects dextrin metabolism is uncertain. Coleman et al.[24] postulated that synthesis of extracellular enzyme was limited by the nucleic acid precursor pool; caffeine affects nucleotide pools in bacteria[25]. Another possibility is inhibition of export of amylase[26]. The amylase(s) of *C. perfringens* have been given only cursory attention[27] and further studies are required to establish the effects of caffeine on this enzyme(s).

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Organophosphate-mediated inhibition of choline acetyltransferase activity in rat brain tissue[1]

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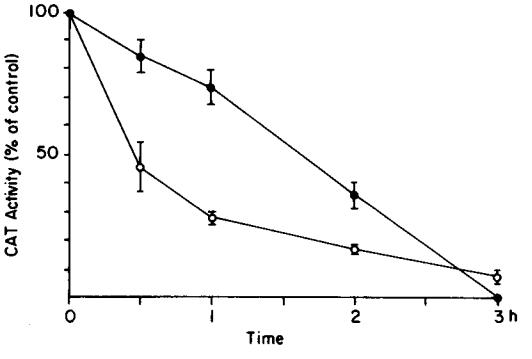
Summary. Administration of the organophosphate compound soman in rats resulted in an inhibition of choline acetyltransferase activity in almost all brain regions examined. Enzyme activity was inhibited by 20–50% in various brain regions 30 min after soman injection (94–120 µg/kg). Enzyme activity in two regions decreased with time to a near zero level by 3 h after injection.
Key words. Choline acetyltransferase; organophosphate; soman; acetylcholinesterase; enzyme inhibition.

The organophosphate compounds appear to exert their acute toxic effects by inhibiting cholinesterase enzymes throughout the body[3]. These enzymes include the cholinesterases of plasma (acylcholine acyl-hydrolase, ChE, E.C. 3.1.1.8) and various tissues, particularly the acetylcholine acetyl-hydrolase (AChE, E.C. 3.1.1.7) in nervous tissue and at the neuromuscular junction. Lethality associated with organophosphate intoxication usually results from accumulation of acetylcholine (ACh). The accumulation of ACh can cause respiratory failure as a consequence of synaptic blockade at the diaphragm neuromuscular junction or in CNS respiratory centers. While the mechanism underlying the acute effects of organophosphates is known, intoxication with these compounds can also produce long-lasting psychiatric effects[4–6]. The mechanism by which these persistent psychiatric changes are produced is uncertain.

Soman (methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester) is a highly toxic organophosphate compound[7–10]. Soman exhibits most of the general features of the organophosphate group, it is a potent inhibitor of AChE and has an LD_{50} in rats in the range of 80–100 µg/kg[8]. The work described in this report represents an investigation of one particular type of potential organophosphate-induced secondary effect. This secondary effect involves presynaptic alterations in the activity of the synthetic enzyme of ACh, choline O-acetyltransferase (CAT, E.C. 2.3.1.6), after soman administration. Such an effect may reveal the basis for long term psychiatric changes caused by organophosphates. For this reason, mechanisms of action of the organophosphates other than AChE inhibition are of interest.

Methods. Sprague-Dawley rats (200–300 g) were used in all experiments. Rats were administered soman (94–120 µg/kg) by s.c. injection. The soman was administered in physiological saline with an injection volume of 0.2 ml. Control animals were given saline injections. Animals which did not survive were not used. All animals surviving were sacrificed by decapitation at a given time interval after injection. Whole brains were removed and the indicated areas of tissue were manually dissected. Tissue was homogenized in buffered saline and CAT activity was assayed according to the radiometric procedure of Fonnum[11]. The activity of AChE was also determined in the different brain areas from the same animals. The assay used to determine AChE was that described by Reed et al.[12]. In this way, CAT activity could



Time-dependent inhibition of CAT activity by soman in two different areas of brain tissue. All animals were injected with 94 µg/kg of soman and sacrificed at the indicated time intervals thereafter. Enzyme activity in the basal forebrain (○) and cerebellum (●) was then determined. The 100% value at time 0 corresponds to the control level of CAT activity in each tissue.