gy of the disease came in 1921 when a volunteer developed cutaneous leishmaniasis after being inoculated with a triturate of wild-caught *Phlebotomus* (*8*). In 1941 *Leishmania tropica* was transmitted to volunteers through the bite of experimentally infected *Phlebotomus papatasi* (*9*). Subsequent investigations have supported the hypothesis that *Phlebotomus* sand flies are the principal if not the sole vectors of human bartonellosis and leishmaniasis.

The first written account describing human bartonellosis appeared in 1630 (*10*) in Peru, only a century after the arrival of the first Spaniards. Carrion's disease, and a peculiar epidemiological form of cutaneous leishmaniasis called uta, are endemic in certain areas of the Peruvian highlands. Their antiquity has been substantiated mainly on the basis of linguistic considerations (*11*) and certain pathological representations in Peruvian anthropomorphic potteries from the Inca and pre-Inca times (*12*). The endemism of both diseases in small and isolated areas of the Peruvian Andes has also been pointed out as an indication of their great antiquity (*13*). In 1764 Cosme Bueno discussed in *El Conocimiento de los Tiempos* the folklore about the natural transmission of both diseases as follows (translated from the Spanish):

> The narrow valleys are very unhealthy where two kinds of maladies are noted; these diseases are also present in other cool provinces. One is verruga [Carrion's disease] which happens to be very troublesome and dangerous if not accompanied by cutaneous eruptions. The other results in corrosive ulcers, located on the face, is very difficult to cure, and causes the death of some people. It is said that both diseases originate from the bite of a small insect called uta [sand fly].

The term uta is still used synonymously for the vector and the disease of cutaneous leishmaniasis in certain areas of the Peruvian highlands.

Cosme Bueno was born in Spain and arrived in Peru in 1730 at the age of 19. He studied in Lima, where he became recognized as a distinguished physician, mathematician, and geographer. He traveled extensively throughout the Peruvian territory and other South American countries. Much of the information published in *El Conocimiento de los Tiempos* was gathered by Bueno himself. His report precedes other early records implicating sand flies as the vectors of leishmaniasis and Carrion's disease by about a century and a half.

An epidemiological peculiarity of uta and Carrion's disease is their persistence in the same areas for centuries. The diseases remain endemic today in the regions where Carrion's disease was reported by Vadillo in 1630 (*10*) and both diseases were reported by Bueno in 1764 (*14*). The antiquity of both diseases in Peru may explain the existence of the rich folklore about them.

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15. Supported in part by PHS grant AI-01251.

28 April 1975

Delivery of a Quaternary Pyridinium Salt Across the Blood-Brain Barrier by Its Dihydropyridine Derivative

Abstract. *A dihydropyridine-pyridine type redox system was successfully applied for delivering a quaternary pyridinium salt,* N*-methylpyridinium-2-aldoxime chloride (2-PAM), through the blood-brain barrier. The dihydropyridine derivative of 2-PAM was quickly oxidized to 2-PAM after crossing the blood-brain barrier. As a result of this approach, the brain cholinesterase blocked by organophosphates could be reactivated. The new method should be useful in delivering numerous drugs which are otherwise inaccessible to the brain because of their polar ionic character.*

An appropriately designed transient derivative of a drug enables the drug to be transported efficiently, without any unwanted metabolism prior to and during its delivery to the site or sites of action where the drug is released from its derivative by chemical or enzymatic (or both) cleavage. We now report successful application of the transient derivative (hereafter called pro-drug) concept for delivering a drug of the quaternary ammonium salt type across the blood-brain barrier (BBB).

Serious poisoning with anticholinesterase agents such as organophosphates is still a big and possibly increasing problem (*1*). It is known that pyridine aldoxime type quaternary ammonium salts are the best cholinesterase reactivating agents and among them, *N*-methylpyridinium-2-aldoxime chloride (2-PAM chloride) is the preferred drug, although as an ionic compound, 2-PAM would not be expected to cross the BBB. Consequently, it could not reactivate the blocked cholinesterase in the brain. It is generally accepted that one of the crucial points in overcoming organophosphate poisoning is the extent of reactivation of the blocked cholinesterase in the brain, since it was concluded (*2*) that a certain minimum cholinesterase activity level is necessary for the survival of an organism poisoned by an organophosphate compound. Although this minimum level appears to be different for different parts of the brain as well as different for various species, it appears that access by relatively small quantities of 2-PAM to some parts of the central nervous system (CNS) might be enough to elicit pharmacologic effects and reactivate a small percentage of the blocked cholinesterase, and thus save the life of the intoxicated patient. However, no experiments have been reported on the influence of unreactivated acetylcholinesterase (AChE) on the brain functions of the

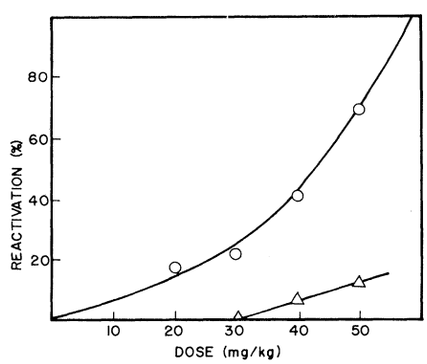


Fig. 1. In vivo reactivation of AChE in mice brains inhibited by subcutaneous administration of DFP (2 mg/kg) as a function of the dose of 2-PAM (△) or Pro-2-PAM (○). The percent of reactivation is equal to $(A_r - A_i)/(A_n - A_i) \times 100$, where A_n is the normal activity of AChE in brain; A_i is the activity after intoxication; and A_r is the activity after intoxication followed by treatment with a reactivator.

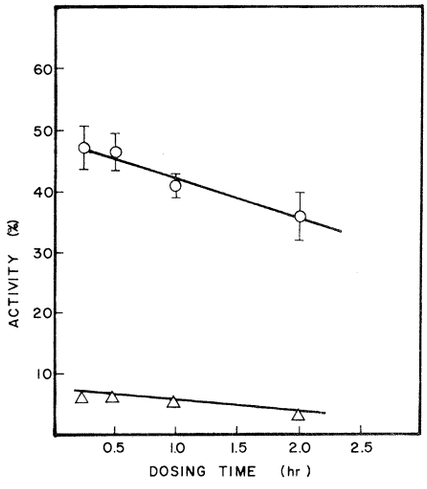
Fig. 2. Activity of AChE in mice brains inhibited by DFP (2 mg/kg) given subcutaneously and reactivated with either 2-PAM (△) or its derivative Pro-2-PAM (○) (40 mg/kg, each), given intravenously as a function of the time elapsed between the administration of the poison and of the antidote.

surviving patient. In addition, a process of "aging" of the deactivated AChE takes place, which results in the irreversible inhibition of the enzyme in most parts of the brain where 2-PAM does not gain access. Such inhibition has two major consequences: (i) some brain functions are disturbed during the time that the "aged" deactivated AChE is being replaced. A recent study suggests that the replacement process is slow, as indicated by evidence that after accidental intoxication with cholinesterase inhibitors, patients had psychiatric sequelae persisting for many weeks (*3*); and (ii) the patient with low brain AChE activity is much more susceptible to a possible new intoxication; much lower doses of poison could deactivate the remaining active AChE in the brain and result in death due to failure of the respiratory center of the brain.

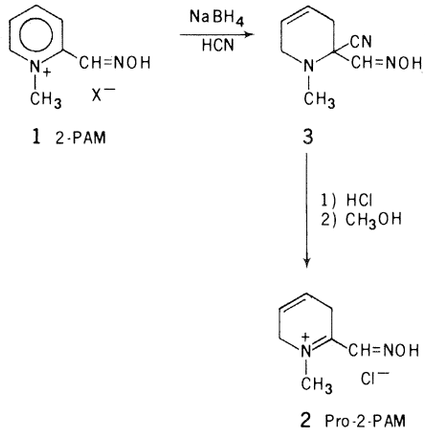
It would consequently be desirable to reactivate the blocked cholinesterase in the brain concurrently with that in other parts of the body. It would also be desirable to extend the biological half-life of 2-PAM [$t_{1/2}$ < 1 hour in humans (*4*)] in order to assure further prophylactic usefulness. Although the distribution characteristics of 2-PAM could be somewhat improved by the use of *N*-dodecyl derivative (*5*) instead of the *N*-methyl, or with a tertiary amine type oxime (tetrahydropyridines) (*6*), the activity of these compounds was far too low.

In our search for a better cholinesterase reactivator, we did not look for a new drug, but considered the problem as a specific delivery problem of 2-PAM itself. Thus, we attempted to synthesize a novel derivative form of 2-PAM, that is, the corresponding dihydropyridine derivative, which would not possess the polar quaternary ammonium structure. This transient derivative was expected to pass through various biological membranes such as the BBB and the gastrointestinal mucosa, after which it would be oxidized to 2-PAM for performing its pharmacological effects. In other words, we intended to deliver 2-PAM as the active species. The in vivo oxidation of the dihydro derivative was expected to be a very fast process, probably mediated by one of the hydrogen transferase enzymes.

Direct reduction of 2-PAM (**1**) with various reaction conditions and reagents (*7*) always led to the tetrahydro derivative.



The dihydro derivative (**2**) of 2-PAM was finally synthesized by a modification of the method described by Fry (*8*). We used a combined reduction-addition first step, followed by elimination of the nucleophile added in the first step:



The intermediates and the final dihydro derivative gave satisfactory analysis data and their structures were demonstrated by nuclear magnetic resonance, infrared, and ultraviolet spectra as well as by chemical transformations. The pK_a of **2** was determined to be 6.32 ± 0.06, while the pK_a of the oxime function was estimated to be around 10.5. In solution, **2** is easily and quickly oxidized into 2-PAM by various oxidizing agents.

The ability of **2** (Pro-2-PAM) to protect against an organophosphate poison and to pass the BBB and, subsequently, to reactivate the phosphorylated cholinesterase in the brain was compared to that of 2-PAM with white mice. Distribution and metabolism studies were also done with beagle dogs. The results have shown that the in vivo oxidation of Pro-2-PAM into 2-PAM in both species is a very fast process and no new metabolite was formed after intravenous administration [in which ^{14}C-labeled 2-PAM and Pro-2-PAM and a modification of the analytical methods of May (*9*) were used] of Pro-2-PAM. Pro-2-PAM gave as good a protection against phospholine iodide poisoning as 2-PAM (**1**).

The penetration into the CNS of Pro-2-PAM as compared to **1** was studied by analysis of the distribution of the corresponding ^{14}C-labeled material as well as by determination of the cholinesterase activity (*10*) and relative reactivation of the mice brain homogenates after administration of 0.5 LD_{50} (lethal dose, 50 percent effective) of diisopropylfluorophosphate (DFP), followed by various doses of 2-PAM and Pro-2-PAM, respectively.

The radioactive distribution studies have shown that Pro-2-PAM resulted in an average of 13-fold higher 2-PAM concentration in the brain than when 2-PAM was administered. (Radiochromatography has demonstrated that the source of radioactivity found in the brain after administration of the ^{14}C-labeled Pro-2-PAM is indeed 2-PAM.) The results of the relative reactivation studies are shown in Fig. 1. It can be seen that there is a dramatic difference between the ability of 2-PAM itself and that of Pro-2-PAM to reactivate inhibited cholinesterase in mice brains.

The ability of Pro-2-PAM to penetrate the BBB was also used for studying in vivo the "aging" process. For this, the reactivation of brain cholinesterase blocked by DFP was determined after various time intervals. As shown in Fig. 2, a significant decrease in the ability of the antidote to reactivate blocked AChE in the brain was observed as a function of time. Our oxidoreduction approach can certainly be extended to provide transportation through biological membranes for many other poorly permeable drugs containing an *N*-heteroaromatic ring as a structural part or as a carrier system.

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12 May 1975