started at the same time and were maintained for 30 days. Animals were then killed and their livers were analyzed for hydroxyproline and free proline. Proline was determined by the method of Troll and Lindsley[6] as modified by Rojkind and Gonzalez[7]. Hydroxyproline was determined as described by Prokop and Udenfriend[8].

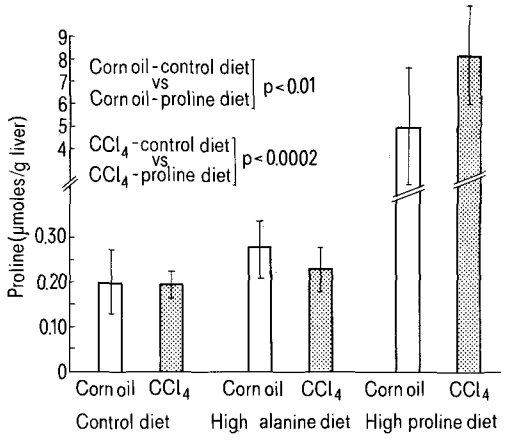

Fig. 1. Proline content of the liver in animals fed either a normal chow diet or chow diets enriched with proline or alanine. Animals were given 1 ml/kg CCl_4 3 times weekly for 30 days, or an equivalent volume of corn oil (controls).

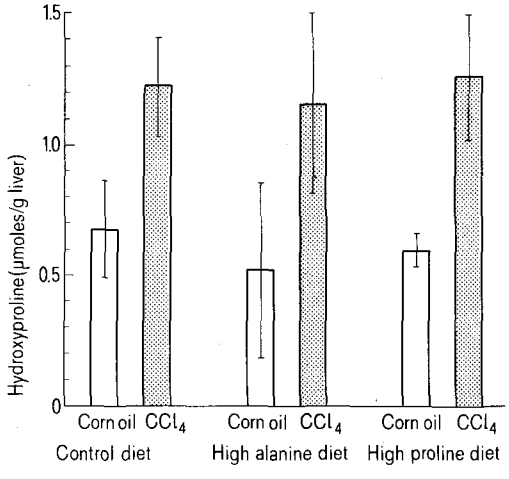

Fig. 2. Hydroxyproline content of the liver in animals fed either a normal chow diet or diets enriched with proline or alanine. Carbon tetrachloride and corn oil were administered as described in figure 1.

Results. Figure 1 shows that supplementation of chow diet with proline, as used in our experiments, resulted in a 20-fold increase in free proline in the liver. Concomitant administration of CCl_4 for 30 days did not result in an increase in free proline expressed per g of liver, as compared to that in the corn oil control group. However, when data are expressed per total liver, rats receiving the high proline diet and CCl_4 showed a significant increase in free liver proline, as compared to corn oil controls (CCl_4: 106 ± 22; Corn oil: 60 ± 26 μmoles/liver, $p < 0.03$). Figure 2 shows that hydroxyproline accumulation in the liver following CCl_4 is not influenced by the amount of proline in the diet. The high proline diet which resulted in a 20-fold increase in the levels of proline in the liver did not induce an increase in hydroxyproline over the levels in controls fed normal chow diet. Similarly, when expressed as hydroxyproline per total liver, CCl_4-treated animals fed the high-proline diet did not show higher hydroxyproline levels when compared to animals fed normal chow diet. Actually, a small but significant reduction was observed: (CCl_4-high-proline diet, 16.6 ± 2.3 μmoles/liver; CCl_4-chow diet, 20.9 ± 1.5 μmoles/liver; $p < 0.02$). For all the parameters studied in this work, animals fed the high-alanine diet did not differ from chow-fed rats.

Discussion. While a number of studies have shown a correlation between liver contents of proline and collagen, measured as hydroxyproline, in several conditions leading to cirrhosis[2-4] these do not necessarily imply a cause-effect relationship in which the regulatory mechanism of collagen synthesis is the availability of proline.

Our experiments show that vastly different proline levels in the liver do not influence the accumulation of collagen in the liver following the administration of a cirrhogenic agent such as CCl_4. We therefore suggest that the correlation that exists between proline levels and hydroxyproline accumulation in the liver in several conditions is an epiphenomenon and not the mechanism for increase in collagen formation.

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Effect of centrally active drugs on dopamine oxidation by rat brain catecholamine oxidase[1]

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Summary. Centrally active drugs of the phenothiazine-, butyrophenone- and iminodibenzyl class markedly decreased the rate of dopamine oxidation in the presence of rat brain catecholamine oxidase.

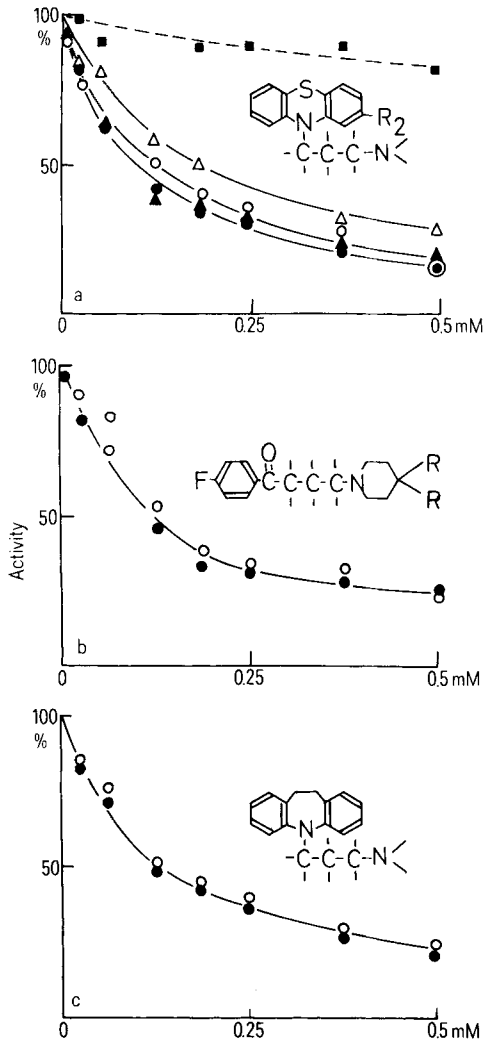
Vander Wende and Spoerlein[2] reported the presence of an enzyme in rat brain, which catalyzes the oxidation of dopamine, noradrenaline and adrenaline in vitro. Although the enzyme appears to be widely distributed in the central nervous tissue, the role of the enzyme is unknown. It has been suggested that catecholamine oxidase activity may play a role in the maintenance of normal mental function by controlling the relative levels of catecholamines[3,4], or by

generating oxidation products of catecholamines, which may serve as neurohumours or neuroregulators[2,5]. For instance, Hoffer and Osmond[6] have suggested that adrenochrome has an important role in nervous function.

It was, therefore, of interest to investigate if centrally active drugs could modify the catecholamine oxidase activity of the enzyme described by Vander Wende and Spoerlein[2]. The present investigation shows that the enzyme activity is markedly decreased in the presence of several antipsychotic- and antidepressive drugs.

Materials and methods. Promazine was obtained from AB Ferrosan, chlorpromazine and imipramine from A/S Dumex, trifluprómazine from Squibb & Sons, desipramine from Ciba Geigy, haloperidol from AB Mekos, trifluperidol from Janssen Pharm., chlorpromazine sulfoxide from Rhone-Poulenc and dopamine from Sigma Chem. All aqueous solutions were prepared in deionized, glass-distilled water.

The enzyme was prepared from rat brain (female Wistar, 200–250 g) according to the method of Vander Wende and Spoerlein[2]. The final residue, containing the enzyme, was washed and resuspended in 0.5 M sodium phosphate buffer, pH 6.8. The enzyme activity was associated with the mitochondrial membrane fraction (R.A. Løvstad, unpublished data).



Effect of different concentrations of centrally active drugs on the dopamine oxidase activity of the enzyme. *a* ○, Thioridazine; ●, chlorpromazine; △, promazine; ▲ triflupromazine; ■, chlorpromazine sulfoxide. *b* ○, Haloperidol; ●, trifluperidol. *c* ○, Imipramine; ●, desipramine.

The enzyme activity was determined according to the method of Vander Wende and Spoerlein[2], by measuring the amount of melanin produced from dopamine (10 mM) in 0.5 M sodium phosphate buffer, pH 6.8. Melanin was dissolved in a mixture of deoxycholate and NaOH[2], and the absorption of melanin was measured spectrophotometrically at 450 nm, using a Beckman DB 26 instrument. It was corrected for a slight nonenzymic formation of melanin.

The rate of melanin formation increased initially, and then remained constant[2]. The enzyme activity was estimated after the initial lag period. The activity obtained in the absence of drug was set to 100% (figure 1), corresponding to 0.32 OD units/mg protein/h. Protein was determined according to the method of Lowry et al.[7].

Results and discussion. The figure shows the effect of different concentrations of various centrally active drugs on the enzyme-catalyzed oxidation of dopamine. The phenothiazine derivatives (promazine, chlorpromazine, triflupromazine and thioridazine), the butyrophenone derivatives (haloperidol and trifluperidol) and the iminodibenzyl derivatives (imipramine and desipramine) were found markedly to inhibit the dopamine oxidase activity of the enzyme, while chlorpromazine sulfoxide was less effective (figure 1a). The inhibitory effect of the phenothiazines was not much affected by the nature of the substituent in 2-position. It was not possible to obtain a correlation between the inhibitory effect and the antipsychotic activity ($CF_3 > Cl > H$), the latter increasing with decreasing electron donor ability of the drug[8]. The inhibition by the powerful antipsychotic butyrophenones (figure 1b) was almost the same as the inhibition observed with the phenothiazines (figure 1a). This was also the case with the antidepressive drugs, imipramine and desipramine (figure 1c). Judging from the comparative study of imipramine and desipramine, desmethylation of the nitrogen atom in the basic side chain does not significantly affect the degree of inhibition. The phenothiazine derivatives are closely related to the iminodibenzyl derivatives, and conformational studies suggest that there are also certain similarities between butyrophenone- and phenothiazine derivatives[9,10].

Although the different drugs studied markedly inhibited the enzyme activity, they did it in a nonspecific manner. The frequently observed nonspecific effects of centrally active drugs are often attributed to adsorption of drugs to membranes, altering the membrane structure[11–15], as in the case of chlorpromazine and imipramine influence on various mitochondrial activities[11]. Examination of several enzymes affected by phenothiazine derivatives indicates that they are associated with membranes[13]. The catecholamine oxidase investigated is located in the mitochondrial membrane fraction, and the nonspecific inhibitory effect observed could be due to adsorption of drugs to the membrane.

The enzyme reacts with several catecholamines in vitro, and different intermediates of melanogenesis accumulate, depending on the substrate used[2]. However, the function of the enzyme within the cell remains obscure. The nonspecific effect obtained with different centrally active drugs (figure 1), a common observation in biological studies[11–13], seems to imply that the enzyme is not intimately involved in the maintenance of normal mental function, as has been suggested.

1 The financial support from Gotfred Lie and Marie Lie's Fond is gratefully acknowledged.

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An insect acetylcholinesterase inhibitor from compound eyes of *Triatoma infestans* (Hemiptera)[1]

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Summary. The presence of an acetylcholinesterase inhibitor in the compound eyes of adult *Triatoma infestans* was demonstrated. The inhibitory activity was localized in the ocular pigments separated by disc gel electrophoresis. The inhibitor was selective against insect acetylcholinesterase, reversible, noncompetitive and heat stable.

The presence of naturally occurring inhibitors of enzymes in insects has been demonstrated in various species. Chymotrypsin[2], mixed function oxidase[3], glutathion S-transferase[4] and cholinesterase[5] are enzymes for which inhibition caused by endogenous insect materials has been reported. In the particular case of acetylcholinesterase (AChE) inhibitors, there are few data available about their characteristics and localization. This fact makes it difficult to study the possible physiological role of these compounds and their eventual use in the development of new structures useful as insecticides.

In previous studies on the AChE of *T. infestans* head we failed to determine any acetylcholine(ACh)-hydrolysing activity by the titrimetric[6] or the Hestrin method[7]. Later experience with the more sensitive Ellman's procedure[8] allowed us to measure the cholinesterase activity and purify the AChE present in the head of *T. infestans*[9].

During the development of the purification scheme we observed anomalies in the behaviour of the total activity, whose value increased in each step. This fact could be interpreted as being due to the presence of an endogenous inhibitor of AChE in the *T. infestans* head. The localization and characteristics of that inhibitor are the subject of this report.

Materials and methods. Adults and eggs of *T. infestans* were obtained from the colony reared in our Institute for 5 years. Specimens of other insect species were obtained from the Malbran Institute and Insher (Argentina). Commercial enzymes (erythrocytes bovine AChE and horse plasma ChE), 5,5′-dithiobis-(2-nitrobenzoate) (DTNB) and acetylthiocholine iodide (ATC) were obtained from Sigma (USA).

The enzymatic activity was determined by Ellman's colorimetric method[8], measuring changes in absorbance at 412 nm with a Varian model 634 spectrophotometer.

Polyacrylamide disc electrophoresis in tris glycine buffer (pH 8.3) was performed using a Bio Rad apparatus according to reported procedures[9]. Gel concentration was 7% and the current applied was 5 mA per tube for 1 h.

Results and discussion. The 1st step in the purification procedure of *T. infestans* head AChE developed in our laboratory[10] was to centrifuge the crude homogenate at 18,800 × g in 0.5 M NaCl-0.02 M phosphate buffer pH 7.2. After centrifugation almost all the ChE activity was recovered in the pellet. When we rehomogenized it in 15 mM sodium deoxycholate (DOC) in 0.02 M buffer phosphate pH 7.2 and centrifuged at 10,000 × g, the enzymatic activity remained in the supernatant, and a 50% increase with respect to the total activity before centrifugation was observed. This fact suggests to us the presence of a reversible ChE inhibitor present in the colored 10,000 × g pellet.

The inhibition of the AChE present in the *T. infestans* head appeared to be related to the color of the homogenate.

In fact, the rehomogenized and boiled 10,000 pellet were shown to be inhibitor of the AChE present in the supernatant and was active against the AChE of housefly head too.

As most of the color in the *T. infestans* homogenates was due to the ocular pigment, the compound eyes of the insect were excised and the AChE activity in homogenates of heads without eyes was compared with the activity in homogenate of whole heads. The hydrolysis rate of ATC determined in extract of heads with eyes in 0.5 M NaCl-0.02 M phosphate buffer pH 7.2 was 20% less. In both cases

Inhibitory action of *Triatoma infestans* eyes homogenate against cholinesterases from different sources

Source of cholinesterase	Specific activity (units*/mg of protein)	ATC hydrolysis rate (Δ abs.$_{412}$/min)	Inhibition final concentration (%) 3 eyes/ml	6 eyes/ml
T. infestans head (eyes free)	23.4	0.038	25	40
R. prolixus head	9.1	0.028	36	50
M. domestica head	71.5	0.054	17	26
P. americana head	21.5	0.051	17	31
Bovine erythrocyte	711.8	0.044	0	0
Horse serum	1352.0	0.056	0	0

* A unit of acetylcholinesterase is the amount catalyzing the hydrolysis of 1 nmole of ATC per min at 22 °C and pH 7.2.