As $[NTA]_t \gg [E]_t$, the problem can be divided into two parts: 1. an independent MgNTA/NTA system, which can be calculated according to

$$(1)\quad [Me] = \frac{[L]_t - [Me]_t + K_{MeL}}{2} + \sqrt{\frac{([L]_t - [Me]_t + K_{MeL})^2}{4} + \frac{}{K_{MeL}[Me]_t}}$$

$$(2)\quad [MeL] = [Me]_t - [Me]$$

$$(3)\quad [L] = [L]_t - [MeL]$$

and 2. a system EMg/E, which is a function of the first system. From

$$(4)\quad K_{EMg} = \frac{[E] \cdot [Mg^{2+}]}{[EMg]}$$

and

$$(5)\quad [E] = [E]_t - [EMg]$$

we get

$$(6)\quad \frac{[EMg]}{[E]_t} = \frac{[Mg]}{[Mg] + K_{EMg}}$$

As the substrate concentration is constant,

$$(7)\quad v = k_2\,[EMg]$$

Introducing (7) and $V = k_2\,[E]_t$ into (6) we get

$$(8)\quad \frac{v}{V} = \frac{[Mg]}{[Mg] + K_{EMg}}$$

From equations (1) and (8) the theoretical curves, e.g. Figure, have been calculated.

2. Calculation of $[Zn^{2+}]$ as a function of $[Zn^{2+}]_t$, $[Mg^{2+}]_t$ and $[L]_t$ in the system ZnL/MgL/Mg^{2+}. To calculate the dissociation constant of the Zn-enzyme complex, we had to obtain definite concentrations of Zn^{2+} in the region of 10^{-10} *M* to 10^{-14} *M* at 0.1 m*M* Mg^{2+}. Because of the extreme differences in concentrations, this problem could only be solved by combination of a Zn^{2+} buffer system with unbuffered Mg^{2+}: ZnEDTA/MgEDTA/Mg^{2+}.

Wolf[13] has evaluated the equations necessary to solve this problem. Ackermann[14] has calculated the concentrations of free metal ions in this system by means of a FORTRAN IV programm.

When the following conditions are fullfilled

$$(9)\quad [Zn]_t < [L]_t$$

and

$$(10)\quad K_{ZnL} \ll K_{MgL}$$

then

$$(11)\quad [ZnL] \approx [Zn]_t$$

and

$$(12)\quad [L]_t \approx [L]_t - [ZnL] \approx [L]_t - [Zn]_t$$

These approximations enable us to calculate the concentration of free Zn^{2+} ions more easily. From

$$(13)\quad K_{ZnL} = \frac{[Zn]\,[L]}{[ZnL]}$$

and

$$(14)\quad K_{MgL} = \frac{[Mg]\,[L]}{[MgL]}$$

we obtain

$$(15)\quad [Zn] = \frac{K_{ZnL} \cdot [ZnL]}{K_{MgL} \cdot [MgL]} \cdot [Mg].$$

Substituting equation 11 we get

$$(16)\quad [Zn] = \frac{K_{ZnL} \cdot [Zn]_t}{K_{MgL}} \cdot \frac{[Mg]}{[MgL]}.$$

$[Mg^{2+}]$ we calculate from equation (1) as a function of $[Mg]_t$ and $[L]_t$ and MgL from equation (2).

The results obtained by this approximation are in complete agreement with those calculated from the exact equation for all measurements taken.

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Acetylcholinesterase in Erythrocytes and Lymphocytes: its Contribution to Cell Membrane Structure and Function

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Summary. Based on biochemical, histochemical and immunofluorescent studies on erythrocytes and lymphocytes, we propose an active function for acetylcholinesterase in membrane structure.

There is considerable recent interest and research in the structure and function of plasma membranes in the dynamic phenomena involved in their participation in physiologic processes. There are several existing hypotheses of the physicochemical structure of membranes[2–4]. Acetylcholinesterase (AchE) is known to be a membrane component on the basis of biochemical[5] and histochemical data[6]. We present immunofluorescent localization of AchE in human lymphocytes, particularly the thymus-dependent (T) cells which form spontaneous rosettes with sheep red cells, and propose that AchE forms the bond between lecithin and protein in structural membranes and contributes to their stability and function.

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Methods and observations. Lymphocytes from a healthy adult donor were obtained by Hypaque-Ficoll density gradient centrifugation and washed with Hanks balanced salt solution. To a suspension containing 25×10^6 lymphocytes per ml, 50 µl of monospecific anti-human cholinesterase raised in rabbits (Behringwerke) was added and the mixture incubated at 37 °C for 20 min. The cells were washed once and treated with 50 µl of fluorescein-conjugated anti-rabbit IgG raised in goat (Behringwerke). After 2 washings, rosette-forming T-lymphocytes were identified by the method of JONDAL et al.[7]. T-lymphocytes showed an area of bright aggregate fluorescence close to the cell membrane (Figure 1). Bone marrow derived (B) lymphocytes exhibited a thin rim of fluorescence.

Fresh human erythrocytes were washed and a 50% suspension made in plasma. 4 ml aliquots were incubated with 5 mg per ml of phospholipase D (Sigma) or with 2.5 mg per ml of eserine (B.D.H.) at 37 °C for 2 h. Plasma potassium was measured by flame photometry, erythrocyte osmotic fragility by estimation of percentage hemolysis using 0.5% sodium chloride[8] and AchE by a test kit[9]. Drug treatment resulted in a release of potassium into the supernatant associated with increased fragility of red cells (Table). Phospholipase D treated cells showed a small but statistically significant increase in AchE activity.

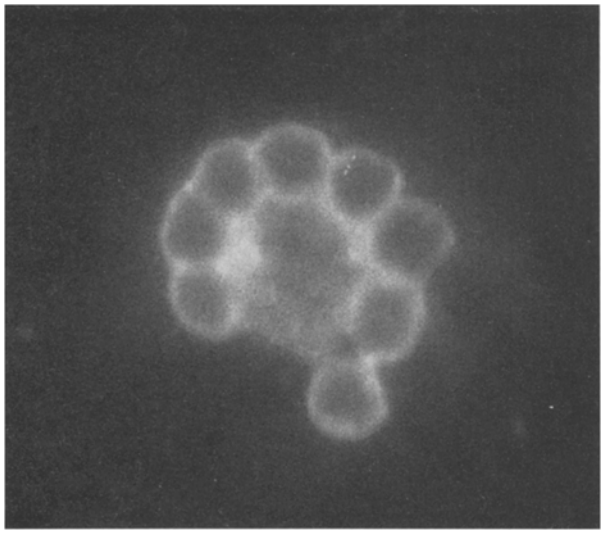

Fig. 1. Immunofluorescent localization of acetylcholinesterase in the cell membrane.

Effect of drug treatment (in vitro) on human erythrocyte fragility and acetylcholinesterase activity

Groups	Potassium (mEq/l)	Osmotic fragility (% of hemolysis)	AchE (units)
Control	4.58 ± 0.25	3.72 ± 0.042	134 ± 5
Eserine	8.26 ± 0.61	5.93 ± 0.21	Nil
Phospholipase D	10.90 ± 0.63	14.56 ± 1.93	152 ± 2

The results are expressed as the mean ± SEM. 6 experiments were done. The values for the study groups are significantly different from the controls ($p < 0.05$) on all 3 parameters. AchE unit is defined as µmoles of acetylthiocholine hydrolyzed/min/ml of packed red cells.

0.025 mg of phospholine iodide, an inhibitor of AchE, was administered to a batch of 5 guinea-pigs. Their erythrocytes showed an increased uptake of H^3-cholesterol (Mean 999 ± SEM 430 cpm per ml of packed red cells) compared with cells from 4 control animals (46 ± 3).

Liposomes prepared with egg lecithin were incubated with 2.5 mg per ml of bovine erythrocyte AchE[10] for 6 h at 37 °C. This resulted in a significant ($p < 0.05$) drop of enzyme activity (Mean 0.093 AchE units ± SEM 0.04) when compared to the controls without the liposome (0.178 ± 0.05).

Discussion. The heterogeneity of lymphocytes, based on function and cell surface receptors, is established[11]. Electron microscopy of replicas of membranes of mouse lymphocytes prepared by freezefracture technique showed marked differences between T and B cells[12]. Biochemical and biophysical studies on lymphocyte subpopulations separated by free-flow electrophoresis and characterized immunologically, showed significant differences in the chemical composition of the surface membranes of T and B cells[13]. T-lymphocytes which carry a higher net negative charge than B cells, are relatively rich in neuraminidase-susceptible α-carboxyl groups but poor in sulphydryl and ribonuclease-sensitive phosphate groups. Preliminary studies had shown that mitogen-stimulated lymphocytes contain esterases closely linked with the cell membrane[6]. The demonstration of acid α-naphthyl acetate esterase activity resulted in the appearance of dot like reaction products in lymphocytes present in the thymus-dependent paracortical areas of murine lymph nodes[14]. In the germinal centres populated mainly by B lymphocytes, very few cells gave a positive result.

Our study employing a specific immunofluorescence method localized AchE to the cell membrane of lymphocytes. There was a quantitative and morphologic difference in the pattern of fluorescence seen in T and B lymphocytes. T cells showed a thick aggregate fluorescence whereas B cells had a thin rim of fluorescence. We have reported previously[15] that pretreatment of sheep erythrocytes or human lymphocytes with eserine or phospholipase D impairs rosetting, suggesting the significant contribution of AchE in modulating this phenomenon. It is possible that the enzyme acts as a key transducer at the cell membrane level. An alteration of the level of its functional activity would result in microdeformation or configurational change, which may underlie many cell-cell or cell-antigen interactions. In addition, AchE may have a role in the other functional characteristics of lymphocytes, viz. antigen binding, endocytosis and degradation of antigenic material, cytotoxicity, etc.

Eserine (physiostigmine), a specific competitive inhibitor of AchE, affects both the stability and permeability of the cell membrane. This is explainable on the basis of Ehrenpreis'[16] interpretation of the action of acetyl-

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choline on cholinergic systems. In this formulation, the active membrane site which controls ionic movements consists of AchE-phosphorylcholine complex located within the membrane. Acetylcholine dissociates it to form AchE-acetylcholine complex. This results in the opening of pores and increased permeability of the membrane.

Phospholipase D splits the link between phosphoric acid and choline in the lecithin molecule. Phosphorylcholine is a possible site for the location of AchE due to the former's close structural similarity to acetylcholine, a natural substrate for cholinesterase. Our observation of increased uptake of H^3-cholesterol by red cells of phospholine iodide treated animals suggests an increased turnover as a result of lipoprotein disorganization. The reduction of AchE activity by lecithin vesicles also shows the intimate relationship between the two molecules.

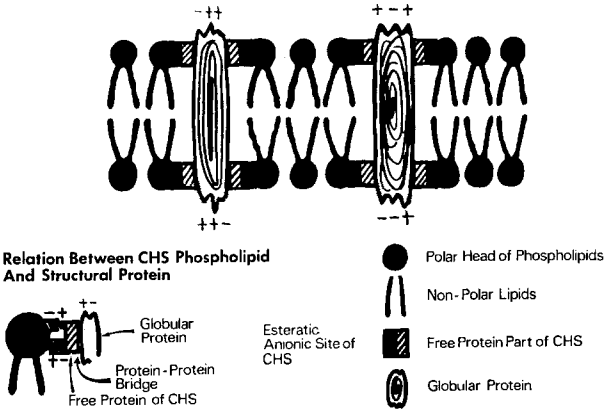

Fig. 2. Hypothetical structure of cell membrane incorporating acetylcholinesterase(CHS) (see text).

Moreover, both AchE and lipoprotein are extracted together when erythrocyte ghosts are treated with hypertonic saline[17]. These results suggest that AchE is associated with the phosphorylcholine site.

In the light of these observations, we propose a hypothetical model of membrane structure (Figure 2). This is based on the original proposal of GORTER and GRENDEL[18] that a lipid bilayer provides the structural framework of the membrane. We suggest an extension of the fluid mosaic concept[2], with lipoprotein-protein interaction and active participation of AchE. The polar head groups of lecithin form ionic bonds with the esteratic and anionic sites of the enzyme and the free protein or the non-active site of the enzyme forms a protein-protein bond with the structural proteins.

It is likely that membrane-bound AchE plays an important functional role in health and disease. In many pathological states, e.g. experimental muscular dystrophy[19], organophosphorus-induced demyelination[20], leukemia[21], duodenal ulcer[22], and some forms of hemolytic anemia[23], a membrane defect associated with reduced AchE activity has been described. If there is a causal relationship between the two, attempts at restoration of enzyme activity may possibly improve membrane stability and cellular function in these syndromes.

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A New Prostaglandin Metabolite of Arachidonic Acid. Formation of 6-keto-$PGF_{1\alpha}$ by the Rat Stomach

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Summary. Arachidonic acid was transformed by rat stomach homogenates into a new prostaglandin viz. 6-Keto-$PGF_{1\alpha}$. Its structure was confirmed by mass spectrometry.

Over a decade ago, arachidonic acid was shown to be the direct precursor of prostaglandins (PG) E_2 and $F_{2\alpha}$[2,3]. Studies on the mechanism of this transformation have led towards postulating an endoperoxide intermediate common to both PGE_2 and $PGF_{2\alpha}$[4] which has recently been isolated and resolved into two labile intermediates having a half life of approximately 5 min[5-9].

In our investigations of the prostaglandin synthetase system using the rat stomach tissue as source, we previously reported the isolation of two cyclic ether derivatives of prostaglandin $F_{2\alpha}$ viz. 6(9)oxy-11, 15-dihydroxy-prosta-7, 13-dienoic acid and 6(9)-oxy-11, 15-dihydroxy-prosta 5, 13-dienoic acid[10,11]. This report deals with the isolation of another new compound formed from tritiated arachidonic acid whose structure is 6-keto-$PGF_{1\alpha}$.

Materials and methods. Stomachs from 12 male adult rats (Wistar, approx. 200–250 g) were removed, freed from surrounding tissue, cleaned, washed thoroughly with ice-cold 0.05 *M* KH_2PO_4-NaOH (pH 7.4) buffer containing

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