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.

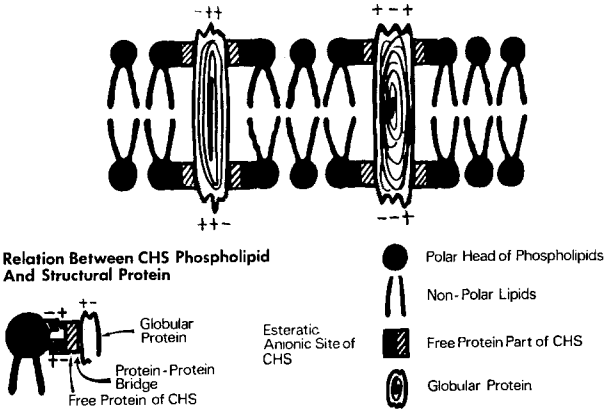

Fig. 2. Hypothetical structure of cell membrane incorporating acetylcholinesterase(CHS) (see text).

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

[1] Supported by a grant No. MA-4181 from the Medical Research Council of Canada. All mass spectra were recorded by Mr. L. MARAI on the Varian MAT CH-5 gas chromatograph-mass spectrometer at the Best Institute, Toronto, an MRC regional facility.
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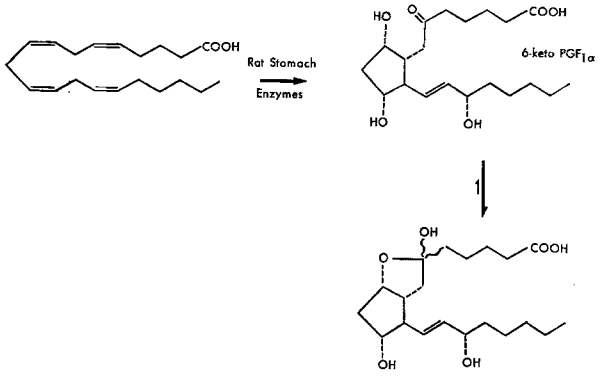
20 m*M* EDTA. The fundic portion (3.9 g) was dissected and homogenized in 20 volumes buffer (Polytron, top speed, 10 sec, 0°). A small portion of the homogenate (5 ml) was incubated with tracer arachidonic acid ($^3H_8$-20:4, 4×10^6 dpm, S.A. 93 Ci/mmole, New England Nuclear); the rest of the homogenate (73 ml) was incubated with unlabelled 20:4 (300 µg/g, Mann 99%). Incubation (20 min, 37°) was terminated by the addition of diethyl ether (2 vol), water (1 vol) and the mixture was rapidly acidified to pH 3 with *N* HCl. The ether phase was separated, washed to neutrality with water and evaporated in vacuo to complete dryness. The residue was transferred to a thin layer plate of silica gel G and after development in chloroform/methanol/acetic acid/water (90:9:1:0.65 v/v) the zone on the plate corresponding to PGE_2 was removed, washed with methanol, and the methanol eluate was taken to dryness. The residue, containing some PGE_2 formed during incubation, was dissolved in methanol (2 ml) and treated at 0° with sodium borohydride (15 mg). After stirring for 30 min at room temperature, the mixture was acidified to pH 3, and extracted with diethyl ether. The ether extract was washed to neutrality with water and taken to dryness. In other experiments PGE_2 was resolved from the mixture by conversion into PGB_2 after treatment with a solution of methanol: N KOH (1:1 v/v). After reaction the residue was purified again on thin layer plates of silica gel G (same developing solvent) and the zone on the plate corresponding to PGE_2 was again removed and eluted as described above. The eluate was taken to dryness and converted to the methyl ester derivative with a freshly prepared solution of ethereal diazomethane: methanol (9:1 v/v). Aliquots were converted to the methoxime and benzyloxime derivatives and analyzed by mass spectrometry after conversion of the hydroxyl groups to the corresponding trimethyl silyl ether (TMS) derivatives.



Structure proposed for novel metabolite of arachidonic acid formep by the rat stomach.

Results. Arachidonic acid is converted in good yield (> 60% of tracer 20:4) into a mixture of compounds with chromatographic properties similar to PGE_2. The major portion of compounds in the mixture are unreactive towards alkali and sodium borohydride, both of which convert PGE_2 into other products. Thus, PGE_2 in the mixture is easily removed through reaction with these reagents. That the residual compounds contained a keto group was determined by reaction of the methyl ester derivative with methoxylamine hydrochloride in pyridine. The resulting product was converted to the TMS derivative with Tri Sil Z (Pierce Chemical Co) and analyzed by combined gas chromatography-mass spectrometry. Only 1 major peak was observed (carbon value 25.2, 3% SE-30, Gas Chrom Q, T: 250°) with intense fragment ions at *m/e* 629 (M^+), 614 ($M-CH_3$), 598 ($M-OCH_3$), 558 ($M-C_5H_{11}$), 539 (M—TMSOH), 508 (M—(TMSOH + OCH_3)), 468 (M—(+C_5H_{11} + TMSOH)), 449 (M—(2×TMSOH)), 378 (M—(C_5H_{11} + (2×TMSOH)), 217 ($TMS\overset{+}{O}=C-CH=CH-OTMS$), 173 ($TMS\overset{+}{O}=CH-C_5H_{11}$) and 115 ($C_4H_8COOCH_3$, base peak). Further confirmation of the identity of the compound was obtained through analysis of the mass spectrum of the methyl ester benzyloxime-TMS derivative. This derivative showed 2 isomers on gas chromatography due to the bulky benzyloxime grouping (29.5, 29.9 carbon values) and showed characteristic shifts in the spectral fragmentation pattern when compared with the methoxime derivative. The base peak (1st isomer) was also found at *m/e* 115 corresponding to the C_1-C_5 fragment.

These findings are in agreement with the structure proposed in the Figure. The isolated compound appears to exist mainly in the cyclic form, a lactol, based on the following evidence:1. lack of reaction with sodium borohydride in methanol; 2. lack of reaction with *n*-butylboronic acid in dimethoxy-propane except after prior reaction with methoxylamine hydrochloride in pyridine (C. PACE-ASCIAK, to be published). In this case an MO-NBB derivative is formed. 3. Positive reaction with methoxylamine hydrochloride and benzhydroxylamine hydrochloride in pyridine. Further studies are in progress to determine the mechanism of formation of this compound.

Effect of PGE_1 on Lipogenesis in Perfused Rat Liver

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29 September 1975.

Summary. In perfused livers of 24 hour-fasted rats, PGE_1 (prostaglandin E_1) infused continuously into the perfusate, was found to cause a 45% increase in the incorporation of 1-^{14}C acetate into liver fatty acids. PGE_1 was found to have no effect, however, on the activity of the key lipogenic enzymes.

Although several studies of the effect of PGE_1 on lipogenesis in rat adipose tissue have appeared[1–4], only one has been published concerning this effect in rat liver[5].

In a study on glycerol esterification[6], it was shown that PGE_1 caused a significant increase in the esterification of 1-^{14}C glycerol into hepatic glycerides of perfused liver obtained from rats fasted for 48 h. It might therefore be expected that PGE_1 would cause an increase in fatty acid synthesis in perfused rat liver. This was in fact observed by CALANDRA and MONTAGUTI[5] in liver slices, using low concentration of PGE_1.

In the present study, perfused rat liver was used to determine the effect of PGE_1 on the ^{14}C acetate incor-