bility product of the respective $Me(OH)_2$-compounds is not exceeded under the conditions chosen here. However, in some cases (e.g. at $[Mg]_t > 10$ mM and pH > 10 (Refs. [6] and [10]) a correction of the $[Me]_t$-value with respect to the formation of $Me(OH)^+$ and $Me(OH)_2$ may become necessary.

In order to give hints for the practical use of two-metal-ion buffers, the pH-range of the validity of eq. (27) and the suitability for use in a two-metal-ion buffer are listed in Table III for each chelator discussed in this paper. The item on the suitability is based on the pH-independence of the Me_1^{2+}-level and the buffer capacity within the pH-range covered by eq. (27).

Zusammenfassung. Es werden Puffersysteme für divalente Metallionen beschrieben, die im Bereich von etwa pH 6–10 gegenüber den herkömmlichen Metallpuffern eine bemerkenswert niedrige pH-Abhängigkeit der Metallionenkonzentration und der Pufferkapazität zeigen.

Die Puffersysteme bestehen aus einem starken Komplexbildner (vorzugsweise einer Polyaminocarbonsäure) und zwei verschiedenen, divalenten Metallionen, die bezüglich der Bindung durch den Komplexbildner in Konkurrenz stehen. Dasjenige Metallion, dessen Konzentration gepuffert werden soll, wird als Primärion bezeichnet. Das zweite Ion, das Sekundärion genannt wird, soll vom Komplexbildner mindestens um den Faktor 10–100 schwächer gebunden werden.

Die Gesamtkonzentration des Primärions ist geringer als diejenige des Komplexbildners, während die Summe der Gesamtkonzentrationen der beiden divalenten Metallionen höher ist als diejenige des Komplexbildners.

Die Konzentration des freien Primärions kann in den beschriebenen Puffersystemen auf drei verschiedene Arten variiert werden: 1. durch Änderung der Gesamtkonzentration des Primärions, 2. durch Änderung der Gesamtkonzentration des Sekundärions, und 3. durch die Wahl eines passenden Komplexbildners mit entsprechenden Metallionen- und Protonendissoziationskonstanten.

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Abbreviations and symbols

t-CDTA, *trans*-Cyclohexane-1,2-diamine-tetraacetate.

t-CPDA, *trans*-1,2-Cyclopentylenedi-[iminodi(acetate)].

EDTA, Ethylenediamine-N, N, N', N'-tetraacetate.

EGTA, 2,2'-Ethylenedioxybis[ethyliminodi(acetate)].

HEDTA, N'-(2-Hydroxyethyl)-ethylenediamine-N, N, N'-triacetate.

OBDA, 2,2'-Oxybis[ethyliminodi(acetate)].

o-PDTA, *o*-Phenylenediamine-N, N, N', N'-tetraacetate.

Me^{2+}, divalent metal ion.

Me_1^{2+}, 'primary' metal ion.

Me_2^{2+}, 'secondary' metal ion.

$[Me^{2+}]$, concentration of the free metal ion (not bound by a chelator).

L, chelator.

n, number of dissociable protons of L.

K, dissociation constant.

$K_{MeL}{}^{(n-2)-}$, dissociation constant of the complex $MeL^{(n-2)-}$.

$K_1, K_2 .. K_n$ } proton dissociation constants (definitions see text).

$K_{MeHL}{}^{(n-3)-}$, proton dissociation constant of the complex $MeHL^{(n-3)-}$.

i, total index.

Definitions.

$$\overline{\overline{[L]}}_t = [L^{n-}] + [LH^{(n-1)-}] + \cdots + [LH_{n-1}^-] + [LH_n] .$$

$$\overline{[L]}_t = \overline{\overline{[L]}}_t + [MeL^{(n-2)-}] .$$

$$[L]_t = \overline{\overline{[L]}}_t + [Me_1L^{(n-2)-}] + [Me_2L^{(n-2)-}] .$$

$$P = 1 + \sum_{i=1}^{i=n} \frac{[H^+]^i}{\Pi K_i} .$$

$$R = \frac{K_{Me_2L}{}^{(n-2)-}}{K_{Me_1L}{}^{(n-2)-}} \frac{[Me_2L^{(n-2)-}]}{[Me_1L^{(n-2)-}]} .$$

$$\Delta = [Me_1]_t + [Me_2]_t - [L]_t .$$

Lipid Mobility and Function in Biological Membranes

Recent advances in bioenergetics[1–7] and immunology[8] show that the fluidity of membrane lipids is of prime physiological significance. The recent 'fluid mosaic' model of cell membranes envisages membrane proteins as floating like icebergs in fluid lipid[9] and lipid-protein interactions can increase substantially the collapse pressure of such film components. For example, rhodopsin can rotate freely in the retina membrane which has a viscosity of about 2 poise[10]. It seems that the above properties are linked with conformational changes in the membrane[4,5].

Any membrane reaction between protein 'icebergs' and an external reactant which expands the protein would tend to compress the lipid film since the protein would function like the moveable barrier in a surface balance. Energy would thereby tend to transfer from the reaction to the compressed lipid and from there to another process. Alternatively, lipid compression could be relieved by expansion of the membrane. Hence the compression would normally be transient. Lipid therefore provides a mechanism for the storage and transmission of energy. The mechanism proposed is more generally applicable than my earlier concept of 'lipid rubbers'[4] but the two concepts are not mutually exclusive.

If there were much cholesterol, or ceramides, in a lipid film, as in some nerve and plasma membranes, the film would inherently be substantially compressed[11] and its viscosity is high (5–20 poise[10]). A small expansion of a protein 'iceberg', or penetration of the film by a lipo-

protein[12], would then tend to produce a sharp rise in surface pressure[13]. Such films are therefore ideal for providing restoring, or initiating forces for membrane conformational changes. For example, in action potentials dilation of the membrane to the Na^+ – permeable state liberates heat[14] and membrane ordering increases[15]; bleaching causes rhodopsin to sink further into the retina membrane lipid[16] and membrane birefringence reduces[17]. These phenomena suggest the presence of lipid restoring forces. They are also likely to participate in reversible crosslinking reactions in membrane pumps[4].

Substantial 'condensation' of lipid would tend to occur if membrane lipid of the 'liquid-expanded' type was sufficiently compressed[11]. These film properties are favoured by lipid unsaturation and a low cholesterol content, as in mitochondria[18]; such films would serve best for biological energy conservation since the rate of development of surface pressure with film compression reduces sharply once condensation commences, and condensation conserves substantial energy[11]. Energy stored in one fully immobilized C_{14} lipid chain at 25°C ($T \Delta S \approx 3$ JK mol^{-1} per $> CH_2$)[19] is adequate for thioester formation; such an energy source has been postulated in oxidative phosphorylation for forming a fatty acid thioester to mediate electron flow from NADH into the respiratory chain[5].

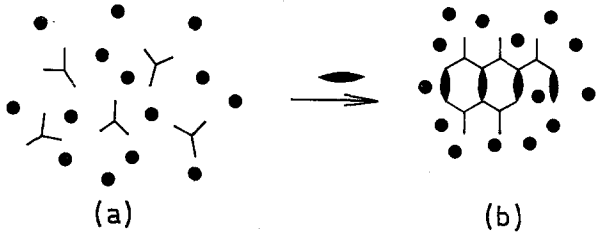

Fig. 1. When bivalent antibody (>—), floating on the surface of a fluid lipid membrane (a), reacts with multivalent antigen (⬬) a crosslinked lattice begins to form which is postulated to expel and compress lipid (b) until the surface pressure and rigidity of the latter prevents further polymerization (● represents the polar heads of the lipid). The Fc arm of the antibody may well be anchored ionically to the polar groups of lipid.

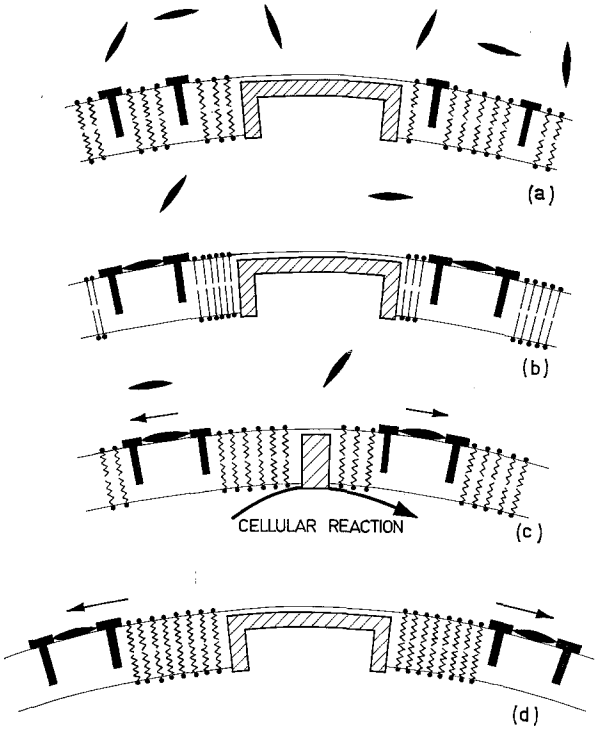

Fig. 2. Surface formation in the lymphocyte membrane of floating antigen-Ig (a) expels and transiently compresses lipid (b) until a control lipoenzyme collapses sufficiently into a compacted configuration to initiate a critical cellular reaction (c); antigen-Ig now moves through the resulting fluid lipid to form a 'cap' which is expelled from the membrane when the lipoenzyme expands (d).

The elasticity of lipid films is influenced profoundly by anaesthetics[7], steroids[6], or complex formation with lipid components (e.g. oestradiol-progesterone[20]; cholesterol-tetrodotoxin[13]), which alter lipid mobility and surface pressure[4]; such changes could therefore modulate processes inducing membrane conformational changes[4].

In the immune response of lymphocytes, reaction between antigen and an immunoglobulin (Ig) in the membrane stimulates cell division and production of antibody (Ig). Antigen-Ig reaction produces a marked expansion which increases exponentially with the association constant of the reaction; this unusual effect is attributed to hydrophobic interactions[21]. The ladder structures in antigen-Ig complexes[22] suggest that molecules of Ig floating in the lipid membrane, perhaps through attachment of the Fc arm to the lipid surface, might combine with multivalent antigens so as to sweep lipid out of the interior of the complex (Figure 1); the surface 'sweep' could be the oligosaccharide attached near the Ig 'hinge' since this would lie flat on the surface[11]. Such formation would tend to compress the lipid (Figure 2).

Triggering of the immune response would follow if there were present, in the inner side of the lipid membrane, a critical lipoenzyme 'iceberg' in an expanded, unreactive conformation (Figure 2). As antigen-Ig reaction compres-

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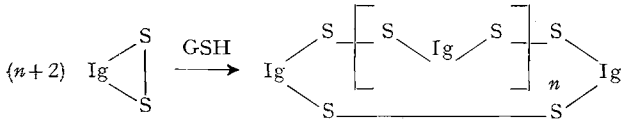
ses lipid the surface pressure would tend to rise and contract the lipoenzyme. When the latter contracts sufficiently into a reactive conformation it could trigger off a poised cellular reaction. Lipid compression (Figure 2b) would therefore be transient. Such a critical conformational change in a cellular surface molecule other than the Ig receptor provides a convenient, common pathway for lymphocyte stimulation. Any reaction with various membrane receptors which tended to indice comparable lipid compression could trigger off the same cellular response and synergistic effects would be possible. For triggering to occur antigens reacting with Ig must be multivalent, occupy substantial area, and liberate sufficient energy to induce adequate expansion of the 'barrier'. 'Tolerance' would occur if small multivalent antigens occupied the specific antibody receptors but induced insufficient expansion.

Since IgG contains only 1, but IgM contains 5, and IgA 2 subunits linked by disulphide bridges[22], IgG will be least effective for compressing a membrane film when reacting with antigen; IgG can form a ladder lattice only with multivalent antigen whereas the polymerized antibodies found in membranes have many more reactive sites. Glutathione (GSH) stimulates[23], but dehydroascorbic acid (DHA) inhibits cell division[23]. Stimulation of cells by hormones is preceded by a decrease in ascorbic acid. The appearance of GSH in high concentrations coincides with mitotic activity[23]. Oxidized GSH promotes membrane swelling but the disulphide hormones (e.g. oxytocin, vasopressin and insulin) are 10^7 times more effective[24]. Because the swelling reactions are enhanced by small concentrations of GSH, and other thiols which enhance disulphide exchange reactions, it is thought that the swelling arises from such exchanges[24]. Perhaps ascorbate reduces oxidized GSH, and GSH so liberated (perhaps with help from an enzymic disulphide hormone from T cells) then catalyses polymerisation of Ig subunits for incorporation into membranes to produce effective 'sweeps' on reaction with antigen:



By preventing reduction of oxidized GSH, DHA might inhibit such polymerization and so influence the immune response.

Under some conditions at 37°C Ig-antibody complex in lymphocytes moves along the membrane to form a cross-linked 'cap'[8]. This event shows that antigen-Ig complex can occupy a substantial area of the membrane. Such movements could arise from the surface pressure gradient generated by expansion of the lipoenzyme (Figure 2d). Antigen-Ig would then be swept from the membrane (pinocytosis) since antigen-Ig occupies more space than Ig.

Cooling to 0°C, or adding a metabolic inhibitor, prevents antigen-Ig movement but not formation of antigen-Ig complex[8]. If lipid compression tended to induce a marked rise in film rigidity because of the presence of cholesterol, such rigidity could be stabilized by a metabolic inhibitor which prevented contraction of the lipoenzyme, perhaps by suppressing removal of a metabolite bound to the latter.

Analogous principles probably apply in plasma membranes where different polypeptide hormones, inducing membrane conformational changes at separate receptors, via sulphide-disulphide exchange reactions[24], trigger c-AMP production at one common site[25]. Perhaps amocrine cells use similar principles to modulate information between various synapses via pressure changes in lipid.

The hypothesis suggests a number of experiments including the following. The postulated change in membrane surface pressure from antigen-Ig reaction, and the predicted influence on it of membrane stabilizers, labilizers and glutathione, should be observable in a surface balance. Spin labelling and microcalorimetry could be applied to study lipid mobility and entropy changes in lymphocyte reactions.

Zusammenfassung. Es wird vorgeschlagen, dass die Reaktion des Thiol/Disulpid Austausches zwischen Antigen oder Polypeptid-Hormon und dem Mambran-Antikörper oder Hormon-Rezeptor (in Lymphozyten oder Plasma Membranen), der im beweglichen Membran-Lipoid suspendiert ist, einen vergrösserten Oberflächen-Komplex bildet, der das Lipoid komprimiert und dadurch ein Enzym in eine kritische Konformation zusammendrückt, welch letztere die Immun-Reaktion oder c-AMP-Bildung auslöst.

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PRO EXPERIMENTIS

A Method for Dissecting Yeast Asci Without Micromanipulator

Saccharomyces cerevisiae is an organism suitable for genetical studies since, amongst other reasons, it is amenable to tetrad analysis. The dissection of yeast asci is generally accomplished with the aid of a micromanipulator (Winge and Laustsen[1]). An important improvement in technique resulted from the introduction of snail digestive extract for the enzymatic digestion of the ascus wall (Johnston and Mortimer[2]). In this report I wish to describe a method, amenable to modifications, which allows the dissection of yeast asci without the aid of a micromanipulator.

Two simple instruments are used. Firstly a micropipette is made from glass tubing, the form and approximate dimensions of which are indicated in the Figure (a, b). To the large end of the pipette, a piece of transparent plastic

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