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New Considerations About the Structure of the Membrane of the Living Animal Cell

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Abstract: The philosophy and assumptions behind the research are summarized. The cell membrane in life is likely to be solid, of unknown thickness and of uncertain biochemistry; the myelin lamellae are artifacts and can not be regarded as sheafs of cell membranes. It is very unlikely that the cell membrane is covered with anatomically distinct receptors or channels. The physiological and biochemical properties attributed to them as structures are quite independent of their anatomy. The ionic channels originally proposed as a result of studies of excitable membranes have now been detected in virtually all membranes in the cell, including endoplasmic reticulum, liposomes and vacuoles. Physiologists believe that channels only open during excitation of nerve and muscle cells, which implies that they are permanently closed in non-excitable cells, although it has been known for more than 50 years that small ions cross the membranes continuously. Explanations are given for the appearance of artifacts.

THE FOLLOWING ELEMENTS of the philosophy of research have been used in this paper.

1. In biology, truth is a description of structures, events and relationships in intact living organisms in their natural environments, unaffected by the procedures used to examine them. It follows that findings made under the latter conditions are *prima facie* more valid than those made in dead organisms, or in fixed, frozen, dehydrated, embedded, sectioned, centrifuged or homogenized tissues, or in those to which have been added powerful unnatural chemicals or natural chemicals in unphysiological concentrations.
2. All true findings must obey the natural laws, for example, of geometry, thermodynamics and physical chemistry.
3. Theories and hypotheses must be provable and disprovable to be of value.
4. Findings must be repeatable, but repeatability alone is not a criterion for validity.

5. A hypothesis or theory derives its value from the accuracy with which it forecasts the results of future experiments.
6. In evaluating opposing views, evidence should only be adduced if it is crucial to the belief in, or the denial of, either view.
7. Whenever a procedure changes a tissue significantly, control experiments must be carried out to exclude possible effects of the procedure in masking or exaggerating the results of the experiments.
8. One should not adopt an agnostic view about a hypothesis or a theory central to one's belief. Either one is satisfied that the evidence is sufficient and cogent enough to accept it, or one should not accept it.
9. Protagonists of particular views take upon themselves not only the responsibility for the validity of their own findings, but also that of all other findings which they quote in support of their announced views.
10. The idea that a simple hypothesis is more likely to be true than a complicated one (Occam's Razor) is a useful device.
11. Research workers have an unlimited duty to discuss any findings, theories or hypotheses, which they have already published or seek to publish, with any interested parties, unless and until the authors have retracted their views publicly.
12. The desire to approach the truth is the main motive driving the scholar.
13. Any published evidence is the property of the whole community, and may be quoted by any interested party.

Assumptions of present paper

1. Diagrams of structures, movements, pathways or relationships are only of value in so far as they are supported by observations and experiments, or suggestive of testable hypotheses.
2. Findings from studies of prokaryotes, naturally occurring single cells and cells in tissue culture, are relevant to the understanding of cells in metazoa.
3. Whereas lumens of vessels, ducts and glands may be empty when examined, there are no empty spaces *within cells* in living intact organisms. Vacuoles in living animals contain solutions, suspensions, particles and secretions.

Notes on presentation

Statements about either gender apply with equal force to the other. Original references to generally held views, which can be found in popular textbooks of biology will not be cited, but will be indicated by an asterisk.* Hypotheses and assumptions will be indicated in *italics*. Considerations, which have been dealt with in detail in previous publications, will not be repeated here.

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The structure of the cell membrane

The popular view is that the cell membrane is trilaminar, * 7–10 nm thick, * is covered by receptors* and punctured by channels.* The receptors often have extracellular, membrane and intracellular domains.* The trilaminar appearance is either due to two layers of protein enclosing a lipid layer, according to *the Davson-Danielli hypothesis** or the membrane is fluid, and has protein molecules protruding from its surface, according to *the Singer-Nicolson hypothesis.** It is believed to be perforated by ion channels.* The thickness of the cell membrane and other trilaminar membranes measured by electron microscopy is likely to be inaccurate, because in life it is believed to contain a large proportion of water, and thus it would shrink from dehydration during preparation for electron microscopy. In addition, the heavy metal stains observed by the electron microscopists are deposits on the outside of the cell membranes. It is widely believed that the 'trilaminar' appearance indicates two layers.* Simple geometry dictates that a real layer, however thin, has two surfaces, so that any single real membrane—if magnified enough—must appear as two lines. If the heavy metal stains were to *dissolve* the membrane, there would be no layer on which they could deposit, so it would still not appear as one discrete straight line.

The 'trilaminar' appearance of the cell membrane, the nuclear membrane and the mitochondrial membrane suffers from another geometrical disability. One can nearly always follow the 'tramline' all the way round the nuclear and mitochondrial membranes, and most of the way round the cell membrane. Since these membranes would be randomly orientated in a tissue, one would expect a full repertoire of widths to appear from the tramline to the flat plate. Indeed, solid geometry permits one to calculate the incidence at which one would expect to see the membranes normal to the plane of section, if one knows only the apparent width between the tramlines and the thickness of the section (Hillman and Sartory, 1980, pp. 37–41). One sees the nuclear, mitochondrial, red cell and synaptic membranes many times more frequently in transverse section than solid geometry would permit. Even without such a calculation, one should see the latter membranes face on as frequently as one sees them in transverse section. This is not seen.

Explanations for two-dimensional appearance of cell membranes

The following explanations have been offered for this apparent anomaly:

(i) many authors select micrographs showing a clear transverse section of the membrane, because it looks more elegant in their publications. That is not acceptable, because one can look through any electron microscope at sufficiently high power magnification, or in published micrographs chosen to show other features, and one still sees too many transverse sections;

(ii) a second possible explanation is that—like a door made of optical quality glass—one would not see it from the side when it was closed; one would only see its edge when the door was open at right angles, because enough glass would then be in one's line of vision. If one could only see it in this rare condition, one should see a *space* corresponding to the width of the partially or fully closed glass door, much more often than the door open at right angles to one's line of vision. No electron microscopist has ever responded to requests made—in private and in public—to send me a micrograph or a publication showing a large enough part of a cell in which a random selection of these orientations can be seen in the same picture;

(iii) it has been said that one can see the membranes in all orientations, if one tilts the stage of the electron microscope.* Of course, if the membranes were cut while they were randomly orientated, one would *not* have to tilt the stage to see the full range of thicknesses; the light microscopists do not. Indeed, the necessity for electron microscopists to use this maneuver represents a tacit admission that there is a geometrical anomaly;

(iv) it has even been asserted that the cutting of the sections for electron microscopy somehow orientates the membranes at right angles to the electron beam. No mechanism for this has been suggested;

(v) the problem has been ignored or denied, although no one has questioned the calculations demonstrating it.

In addition to seeing an interface, there is independent evidence for the likely existence of a solid cell membrane. The extracellular fluid and the cytoplasm are chemically different but miscible,* therefore, there has to be a barrier between them. If the barrier were fluid, the shapes of the cells would change as the pressure on them varied, due to movements of the body, contractions of muscle and blood pressure. When a cell is penetrated by a salt-filled glass electrode, it records a sudden change of direct current voltage,* *which appears to be occurring in the region of the interface*. Of course, one can not know for certain whether the potential difference arises between the extracellular fluid and the interface, across the interface, or between the interface and the cytoplasm. The same is true for potential differences across the nuclear and mitochondrial membranes.

Living cells, such as red cells, swell if placed in hypotonic solutions.* This suggests, but does not prove, that the cells are surrounded by membranes, which allow the more rapid entry of water than other molecules or ions. Furthermore, Hyden, Cupello and Palm (1984a,b) have cut open isolated neurons and washed out the cytoplasm. They covered a fine hole with flattened membranes, and measured fluxes of γ-aminobutyric acid across them. It is very unlikely that a liquid interface—as opposed to a solid membrane—would behave in this way.

Electron microscopists often point out that one can not see the cell membrane by light microscopy, because they see it as 7–10 nm thick,* which is beyond the resolution of the light microscope. Of course, the cell and nuclear membranes were detected about 250 years before the electron microscope was used to study biological tissue. The 7–10 nm thickness is derived from measurements of dehydrated and extracted membranes. Yet the cell membrane may be beyond the resolution of the light microscope, even if it is significantly thicker in the fully hydrated state. The presence of the interface is detected by light microscopy not only because it appears as a line of apparently higher refractive index than the liquids on either side of it, but also because the latter liquids differ in their refractive indices, absorbtion of visible light, and reactions to stains. However, the electron microscopists are also unable to see the cell membrane. They deposit a salt of a heavy metal on either side of the membrane and examine this deposit.

The concept of the unit membrane

Most cytologists believe that the 'unit' membrane is the electron microscopic manifestation of the lipid protein layer of Davson and Danielli.* Yet they know that during the embedding, it was subjected to several different concentrations of ethanol, and to propylene oxide,* both of which extract lipids powerfully, so that it is extremely unlikely that they are looking at any structure which originally contained a substantial proportion of lipids. Indeed

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The myelin sheath

Many people regard *the myelin sheath of peripheral nerves as being composed of a scroll of cell membranes.** They believe this on the grounds that Geren (1954) claimed in tissue culture that the Schwann sheath wraps round axons. Her demonstration was by no means convincing. One can observe that the lamellae of the myelin sheath seen on electron microscopy are remarkably uniformly distant apart, and do look similar to the 'trilaminar' membranes.* However:

(i) neither in the literature, nor in our own studies of the myelination of the sciatic nerve did we see the Schwann sheath wrapping round the axon (Hillman and Jarman, 1989);

(ii) as the cell membrane has a higher refractive index than both the extracellular fluid and the cytoplasm, and the membrane is visible by light microscopy, it follows that the myelin sheath as a whole should have a higher refractive index than the axoplasm or the extracellular space. Phase contrast microscopy shows it to be lower (Hillman and Jarman, 1989; 1991);

(iii) if one were to split a myelinated fiber precisely down the middle in its long axis, it would be a reasonable expectation that one should see the maximum diameter of the axon, and that all the lamellae would appear equally spaced from each other. However, a longitudinal section which cut off a segment of the myelinated fiber, say, half its radius deep, or a section anywhere except through the middle of its long axis, should show the lamellae as splayed—that is, any longitudinal section in which the axon was not visible, should show the lamellae spaced different distances apart. I have never found that the above expectations of solid geometry have been respected in any electron micrograph of a myelin sheath;

(iv) the myelin lamellae are only seen in longitudinal or near perfect transverse sections in electron micrographs. There seems to be an extraordinary dearth of oblique sections;

(v) the periodicity of oblique sections was originally measured by low angle diffraction on hydrated tissue (Schmidt, 1936; Schmitt, Bear and Palmer, 1941), and later by electron microscopy on dehydrated tissue (Fernandez-Moran, 1950; Finean, 1960), so that one would expect these two measurements to be different. Nevertheless, their similarity is usually regarded as evidence of their accuracy.

One must conclude that the lamellae of the myelin sheath—as opposed to the myelin sheath itself—are probably metal deposits on the dehydrated sheath contents, and should not be regarded as models of cell membranes.

Receptors, ion channels and transmembrane molecules

There is a vast literature on receptors, ion channels, carriers enzymes and transmembrane molecules* *believed to be either within the membranes, or attached to them from either side, or protruding from them, or spanning them with an extracellular and intracellular domain* (Table I). Many of the macromolecules have been identified, isolated and sequenced, and their dimensions and configurations have been worked out.* Hardly any issue of 'Nature' or 'Science' fails to include a paper showing the newly recognized sequence and structure

of one of these molecules. Almost without exception, the widths of these molecules at right angles to the plane of the cell membrane exceed the thickness of the membrane two to three times (Kistler, Stroud, Klymkovsky *et al.*, 1982; Strange, 1988; Stein, 1990; Kandel, Siegelbaum and Schwartz, 1991; Kandel and Siegelbaum, 1991; Kandel and Schwartz, 1991; Kubo, Baldwin, Jan *et al.*, 1993). This is expressed in diagrams and computer models in every paper, textbook and popular article on these molecules.* The thickness of the cell membrane is within the resolution of the electron microscope.* Why, then, are the transmembrane molecules, the Singer-Nicolson membrane proteins or the receptor molecules, hardly ever seen by transmission electron microscopy? The only one of these molecules claimed to have been seen at all—and this by scanning but *not* by transmission—is the acetylcholine receptor on the Torpedo end-plate (O'Brien, Eldefrawi and Eldefrawi, 1972; Moore, Holladay, Purett *et al.*, 1974; Toyoshima and Unwin, 1988), yet nearly all the molecules in Table I are clearly within the resolution of the cytologists' hypothesis.

The electron microscopist looks at a deposit of heavy metal or salt, and has no way of knowing for certain whether it precipitated by itself, on a membrane, a receptor, a vesicle, a granule, or even a gas bubble.

Difficulty of seeing macromolecules

The following explanations have been offered to me personally for the difficulty of seeing macromolecules protruding from membranes, but the question seems to have been ignored in the literature:

(i) the acetylcholine receptor has been seen in the Torpedo (please see above);

(ii) the macromolecules are not seen because they do not take up the heavy metal stains. If macromolecules, which do not take up stains were present, one should see deposits around unstained areas of the expected size of the molecules in the extracellular, membrane and intracellular 'domains';

(iii) it has been suggested that the concentration of these macromolecules in the membrane is so low that one would not expect to see them, although calculations suggesting this do not seem to have reached the literature. Such an explanation seems very unlikely in view of the huge number of such macromolecules alleged to be on or in the membrane (Table I), including families of receptors for nicotine, muscarine, epinephrine, gaba, glutamate, histamine, dopamine etc. (Lamble, 1981; Hucho, 1982; Scholefield, Davison, Fujita *et al.*, 1987; Bowery, Bottiger and Olpe, 1990; Hulme, 1990);

(iv) many of the members of the 'family' of receptors have been found by the use of ligands,* which have affinity for homogenates, microsomal or synaptosomal preparations. The use of such preparations implies unavoidably that *the highly energetic subcellular fractionation procedures do not alter the affinity of the receptors, not only for the transmitters, hormones and drugs, but also for all the ligands mimicking them.* The Second Law of Thermodynamics make this extraordinarily unlikely (Hillman, 1972);

(v) *it has always been assumed that ligands bind to the same receptors as the substances with which they compete** (Rang, 1973). Therefore, they can be used to detect the location of the receptors. One reason given for using the ligands is that, for example, a transmitter would be broken down rapidly by enzymes in their vicinity, before it could be detected. Like the physiologists studying acetylcholine or epinephrine, those who study receptors could inhibit the enzymes which break them down.* A second reason for the

TABLE I. Macromolecules believe to have extracellular, transcellular and cytoplasmic 'domains', or to protrude from the cell membrane.* It should be noted that in both the Davson-Danielli and the Singer-Nicolson models, the cell membrane is full of the structural lipids and proteins.

Several ion channels each for sodium, potassium, chloridee and calcium ions*
Singer-Nicolson's membrane proteins
Multiple receptors to: transmitters, including families of receptors to acetylcholine, muscarine, nicotine, epinephrine, dopamine, glutamate and gaba.
Membrane carriers and transporters, including pumps
Membrane antigens

use of ligands is that they bind the receptors sufficiently firmly that they are not detached during isolation. The stronger binding is itself *prima facie* evidence that they adhere to additional or different sites.

Patch clamps and channels

The idea that the cell membrane might be punctured by ionic channels came when the employment of radioactive isotopes showed that cations were in continuous flux in and out of tissues (Brooks, 1940; Cold Spring Harbor, 1940; Mullins and Brinley, 1969). A later finding was the detection of minute step-like current at the tip of electrodes 'patch-clamping' many membranes (Neher and Sakmann, 1976). These currents depended on the presence of different ion species and were voltage-sensitive. The use of ligands and molecular biological techniques enabled a large number of receptors and channels to be isolated and sequenced. Their structures could then be worked out (Scholefield, *et al.*, 1987; Catterall, 1988; Bowery, *et al.*, 1990; Kutswada, Kashiwabuchi, Mori *et al.*, 1992; Ho, Nicholls, Lederer *et al.*, 1993; Kubo *et al.* 1993). It was also found that fractions believed to contain channel molecules could be injected into frog oocytes, and endow them with the 'patch clamp' characteristics attributed to ionic channels (Leonard, Nargeot, Snutch *et al.*, 1987; Umbach and Gundersen, 1987; Dascal, Lotan, Karni *et al.*, 1992).

A recent volume of 'Methods in Enzymology' (edited by Rudy and Iverson, 1992) shows the vast range of ionic channels currently believed to exist.* Table II is made up from lists of two groups. Cahalan and Neher (1992) say that "instead of the handful of channels recognized before the mid-1970s, hundreds of distinct channel subtypes that are regulated by a variety of mechanisms have now been described". In the same volume, Saimi, Martinac, Delcour *et al.*, (1992) add that, "It appears that all plasma membrane and organelle membranes, including those of microbes, are equipped with ion channels". (See also Keller and Hendrich, 1992).

It is useful to identify the assumptions inherent in 'patch clamp' experiments. Firstly, the stepped currents originate in the cell membranes and not the electrodes. Secondly, they arise in the channels within or near the receptors. Thirdly, the individual patch clamp characteris-

TABLE II. List of membranes with ion channels, A, from Cahalan and Neher (1992); B, from Saimi *et al.* (1992)

A	Neurons		Cells in brain slices
	Cells of sensory transduction		Mitochondria
	Glial cells		Nuclei
	Muscle cells, including skeletal, cardiac, smooth		Endoplasmic reticulum
			Liposomes
	Endothelial and epithelial cells including, vasculature, respiratory system and kidneys		Voltage dependant Na^+, K^+, Ca^{2+}, Cl^-
	Secretory cells, including pancreatic acinar, lachrymal gland and juxtamedullary cells		Transmitter gated channels and receptors
			Liver cells
	Hepatocytes		Vacuoles of sugar beet
	Pancreatic β cells		Lipid film vesicles
	Keratinocytes		Liver mitochondria
	Osteoblasts	B	Paramecium blisters
	Osteocytes		Paramecium detached cilia
	Cells of haemopoietic system, including erythrocytes, lymphocytes, macrophages, mast cells, neurophils blood platelets		Yeast spheroblasts
			Yeast mitochondria
			Yeast vacuoles
	Oocytes		E coli giant spheroblast
	Plant protoplasts		E coli giant cells
	Yeast		Vesicle and liposome blisters
	Bacteria		Toxin + azolectin

tics in the presence of different ions in different concentrations each derive from different anatomical channels. Fourthly, the channels which the molecular biologists isolate with ligands have not undergone significant changes in their properties during the isolation. Fifthly, ionic channels exist in the membranes of intact living organisms.

The list of channels (Table II) gives clues as to some of the anomalies of the idea:

(i) it is generally agreed that nerve, muscle and a few plant cells (see Bingley, this volume) are excitable, and that the ion channels only open when the latter cells are excited (Hille, 1992; Stein, 1990, p. 190). Thus the channels of non-excitable cells must be closed all the time. Yet as Ling (1992, p. 300) has pointed out, it has been known for 50 years that sodium, potassium and chloride ions are in continual flux in and out of cells (Heppel, 1939; Steinbach, 1940; Cold Spring Harbor, 1940);

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(ii) the belief that vacuoles are surrounded by double membranes originates from observations by electron microscopy (De Duve, 1969; Daems, Wisse and Brederoo, 1969). However, it has also been claimed that milk droplets have trilaminar membranes (Wooding and Kemp, 1975; Keenan, Franke, Mather *et al.*, 1978), although one can increase the number of fat droplets considerably by simply shaking the bottle;

(iii) there is no way of knowing whether the tip of the micropipette has a membrane stretched across it, or it is blocked by poorly conductive tissue, which increases its noise. Only recently, the relevant control experiments have been done, namely examination of the behavior of the properties of the patch clamp electrodes in the absence of biological membranes. Such experiments have shown: rapid transitions between a conducting and a non-conducting state, selectivity of ion flow, and inhibition by divalent ions and protons (Lev, Korchev, Rostovtseva *et al.*, 1993; Sachs and Qin, 1993);

(iv) with the exception of the acetylcholine receptor with the tube for the sodium ion channel (op cit), like the receptors, none of the channels have been observed by transmission electron microscopy on endoplasmic reticulum, sarcoplasmic reticulum or vacuoles.

Macromolecules on the membranes

With the exception noted above, so many of the molecules reported to be in or protruding from the cell membranes are not seen by electron microscopy, it would be useful to discuss other possible explanations for their absence:

(i) although the macromolecules were originally at those sites, they were lost during the preparation of the specimens. If this were so, one would have to explain why the acetylcholine receptor manages to stay;

(ii) the macromolecules do not have extracellular and intracellular domains. However, many of them have been isolated, cloned and their sequences worked out (see above). They are drawn with extracellular and intracellular domains, because it is realized that the structures deduced are, indeed, too large to be accommodated in the thickness of the cell membranes;

(iii) there are few or no receptors or channels in life. It is generally agreed that rapidly acting anesthetics, such as thiopentone or diazepam, or other drugs, such as epinephrine, theophylline or curare—when injected intravenously—act as soon as the circulation takes them to the brain or target organs.* The membranes of the cells will not previously have been exposed to the above substances except for epinephrine, so that they will not have had time to generate receptors to which the drugs could bind, therefore, it is supposed that either they act on other receptors which were previously there,* or that they do not act by binding to receptors.* If drugs, transmitters or hormones can produce effects without receptors, one must ask how persuasive is the evidence for 'specific' receptors for every natural ligand and drugs. Antacids, osmotic purgatives, diuretics and chelating agents do not have receptors (Rang and Dale, 1991);

(iv) it may be untrue that each different ligand which competes with a transmitter, hormone or drug, occupies a single or 'family' of receptors. Usually the actions of one of these substances has been found by accident, and their chemistry has been modified slightly

to make them more active, to inhibit their actions or to make them less toxic. It is perhaps not surprising that substances of similar chemistry should 'compete' with each other, and thus be assumed to be acting on the same receptors. The concept of receptors should be completely examined in the light of partial competition, partial inhibition, cooperativity, efficacy, multiple conformations of a receptor, etc. (for review, see Colquhoun, 1973; Lamble, 1981; Black, Jenkinson and Gerskowitz, 1987);

(v) it is extremely unlikely that a tissue which has been homogenized, centrifuged and to which powerful unnatural chemical reagents have been added—for example during a procedure in which microsomal, ribosomal or synaptosomal fractions have been prepared — will have the same affinity for a transmitter, hormone or drug as it did in the intact animal. Add to this the likelihood that the unnatural ligands and solubilization will also affect the relationships significantly, it becomes increasingly dangerous to try to derive quantitative relationships intended to be relevant to the intact animal from any observations involving disruption. The experiments to test this question are at least half a century overdue. If one eschews an agnostic position, one must reserve acceptance or any conclusions from them until the crucial assumptions have been tested.

Until then, one must doubt whether there are a harvest of receptors and channels on and in the cell membrane,* and thus it would be useful to propose an alternative explanation for the large number of experiments, which have been interpreted in the light of the current popular hypotheses.

The rapidity with which transmitters, aminoacids and drugs, act when applied near the surface of cells (Curtis, Phillis and Watkins, 1960; Mayer and Westbrook, 1987), coupled with the assumption that resting and action potentials occur across the cell membrane, makes it likely that these substances do, indeed, act on the membrane. I propose the following theory. The peculiar property of all intact living tissues is that they have a wide spectrum of affinities for transmitters, hormones, drugs and toxins, the sign of their effect being the reactivity of the membrane. The latter molecules also have affinities for chemicals in the extracellular fluid, the cytoplasm, the mitochondrial membranes, the mitochondrioplasm, the nuclear membrane, the nucleolonema and the pars amorpha.

Elsewhere, 101 different phases with which a substances would come in proximity in its passage from a cerebral blood vessel to a neuron have been listed (Hillman, 1988). It would be reasonable to suppose that the chemical should have a different affinity for each of these phases, not just the cell membranes. There is absolutely no reason whatsoever to suppose that substances added to tissues react only with the cell membranes, and not the other components of the tissue, with which they come into contact. Troshin (1966) and Ling (1992, p. 31–155) have pointed out that the particular properties of any chemical mixture depend upon every chemically active species at the particular concentration at which they occur at that particular instant. The location(s) of action of the transmitters, hormones and drugs, will only be found for certain by the use of non-disruptive procedures.

'Specific' affinity?

When crystals are deposited on a desiccating dish, no one says that particular sites on the dish have a 'specific' affinity for the crystalloids. Yet when a granule or particle appears on a membrane as a result of dehydration during histology, histochemistry, im-

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munocytochemistry or electron microscopy, it is generally regarded as a ligand having a particular affinity for that site on the membrane.

This paper is concerned with the structure of the cell membrane in relation to its physiology. All the reservations expressed here concern the existence of receptors and ion channels *as anatomical structures* on the membranes, and therefore, the assumptions that the physiological and pharmacological events occur at such sites are unsound. Nevertheless, there is little doubt that natural ligands act at low concentrations on several particular organs, but it is not known precisely at which subcellular or molecular sites, because the procedures used for examining them are themselves likely to change their location.

The unsparing use of Occam's and Popper's Razors to shave off unlikely, unproved and unprovable hypotheses results in the following view of the membrane. It is one layer thick and its precise chemistry and molecular chemistry in life are unknown. There are no specific anatomical receptors or channels protruding from its surface. For reasons which Ling (1992) has detailed, it is unlikely that it houses transport enzymes. It is not connected to any cytoskeleton or to the nucleus (to be submitted for publication).

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