REVIEW ARTICLE

THE MODE OF ACTION OF LOCAL ANAESTHETICS

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It is exactly a century since Niemann first isolated the alkaloid cocaine and noticed its local anaesthetic properties. He reported that it "benumbs the nerves of the tongue, depriving it of feeling and taste." In 1884 Koller introduced cocaine as a local anaesthetic in ophthalmology, and its use spread later to general surgery.

Various attempts have been made to explain the mode of action of such drugs, but no clear cut case has yet been made out. Claude Bernard[1] in 1875 insisted that all agents which depress nerve cells, including heat and asphyxia, do so by producing the same modification in the cell, i.e. there is a single and universal mechanism of narcosis. Seventy-five years later, Butler[2] expressed dissatisfaction with this concept: he believed that when two different drugs produce the same apparent effect, it is not justifiable to assume, without further evidence, that the same mechanism is involved in both instances.

Between these two observations many investigations have been performed upon general anaesthetics, and various hypotheses have been advanced about their mode of action. It follows that, as both general and local anaesthesia relate to drug action on nervous tissue, theories about the former should be applied to local anaesthesia, and must merit study. It is also apparent that attention should be paid to the transmission of nerve impulses, as there may well be factors involved in it which affect the action of the drug in producing a local anaesthetic effect. In the present review both these aspects are considered, together with other factors which appear to be important in local anaesthesia. This is followed by an examination of various problems which arise.

Transmission of the Nerve Impulse

It is not the purpose of this study to discuss the various theories of transmission at length, but certain features are pertinent.

Current Theory

There is a potential difference across the membrane of resting nerve, the protoplasm being negative and the interstitial tissue fluid positive. Bernstein[3,4] observed that the concentration of potassium ions is much greater inside nerve fibres than outside: he considered that, if the membrane is impermeable to anions and sodium ions, the difference between the internal and the external concentrations of potassium would explain the resting potential. He suggested that, during activity, the selective permeability for potassium collapses, and the membrane potential subsequently approaches zero.

This concept forms the basis of the present commonly accepted theory of nerve conduction. Some doubts about the hypothesis arose from observations by Hodgkin and Huxley[5,6] and Curtis and Cole[7,8] that the membrane

potential of squid giant-axon does not merely drop to zero at the peak of the action spike, but is substantially reversed.

Resting nerve exhibits a continuous energy expenditure which is directed towards the uptake of potassium: opposing this is a high intracellular potassium concentration which tends to escape from the nerve fibre at a rate determined by the concentration gradient and by the permeability of the membrane. The surface concentration of potassium is low, but escape of it from within, such as occurs during the passage of a nerve impulse, activates a restoration mechanism at the surface.

In 1949 Hodgkin and Katz[9] suggested that action potential is due to the movement of sodium ions, thus the rising phase of the spike results from a specific increase in the sodium permeability of the membrane, whilst potassium permeability is more than normal during the falling phase. Measurements of ionic movements during nervous activity, both by indirect methods and by using radioactive tracers, confirm this: details are given in Hodgkin's review[10] and by Hodgkin and Keynes[11].

Most of the original work was performed on squid tissue but the general pattern of behaviour is seen in other tissues and species. On stimulation, activity is triggered by the spread of electrical current from a neighbouring region. It is accompanied by permeability changes in the membrane, which breaks down partially or completely. The process is marked by definite electrical changes: the initial positive deflection observed is probably due to sodium ion entry, as it is absent in sodium-free solutions, and the changes in current are subsequently continued by an outward movement of potassium ions. The sodium movements provide the current required to depolarise the resting membrane ahead of the active region. After the change the nerve fairly quickly returns to normal, and can conduct another impulse, the fibre having gained a small amount of sodium, and having lost a similar quantity of potassium. The ionic movements are the immediate energy source for impulse conduction, and are reversed later by a slow process requiring metabolic energy.

Calcium concentration has also been implicated in nerve processes. Low calcium values produce an increase in excitability, and Keynes and Lewis[12] have shown that the total internal concentration of this ion may be about 1/30th of that outside. This suggests that there may be some control of calcium values around the nerve. In his comprehensive review, Hodgkin[13] suggested that calcium ions are not directly involved in the conduction of impulses, but are important in influencing the permeability and excitability of the membrane. Regarding the former, he suggested that there are special channels for sodium and potassium: when the nerve conducts an impulse the channels open up, allowing first sodium, and then potassium ions to move down their concentration gradients. These movements generate the action potential.

Nerve has been represented as a cored conductor, the axis cylinder being surrounded by a more or less insulating layer of lipid (the membrane), beyond which there is an external longitudinal conductor (the interstitial tissue fluid), and functional models, such as the iron wire one of Lillie[14], have been constructed. Hirschfelder and Bieter[15] believed

that alterations in the surface layer of this model upon "stimulation" are analogous to the passage of the action current in a nerve fibre, where an increase occurs in the permeability of the lipid layer. They concluded that, in general, substances which increase the permeability of the lipid layer (in non-destructive concentrations) might increase nerve actions and sensations, whilst those which decrease the permeability to water and ions might act as anaesthetics.

Structural Factors

The differential effects of local anaesthetics raise the question of whether peripheral nerves differ anatomically or physiologically. The possession of myelin may create a major distinction, although it is usually claimed that its function is purely an acceleratory one.

Gasser and Erlanger[16] stated that the important variables are the threshold of excitation and the velocity of conduction, both of which depend upon fibre size. Hence the order in which fibres are blocked may be a linear function of their diameter. Experimental results suggest that there is much truth in this assertion.

Another important structural feature is the connective tissue sheath around nerve. Lorente de Nó[17] believed that it could be ignored in surface potential analysis, despite claims that it distorts the potential difference obtained. His view deserves comment, as it depends on the thesis that the tissue sheaths provide little or no electrical or diffusion resistance, and this is opposed by considerable evidence that the sheath *is* a diffusion barrier and has a high electrical resistance. Bishop, Erlanger and Gasser[18] in 1926 suggested that many 'nerve' properties might be features of this non-nervous structure. In 1949 Rashbass and Rushton[19] compared intact and desheathed frog nerves. They revealed a close conformity to exist between nerve and the simple cable theory of conduction, when the epineurium was removed. Later histological work by Krnjević[20] suggested that the perineurium, rather than the epineurium, is the barrier to diffusion, but he demonstrated that desheathing always removes the perineurium. They believed that much of the earlier work on nerve resistance, permeability, and polarisation needs re-interpretation in the light of the possible effects of a highly polarisable resistance being interposed between the nerve axon and the electrode. Despite this, Lorente de Nó clung to his contention, describing desheathed nerve as "abnormal". Krnjević[21] obviated this criticism by using perfused nerves, thus bringing drugs into intimate contact with nerve tissue, and simultaneously evading any effects produced by the sheath. His results clearly show, as did those of Rashbass and Rushton with a long electrode inserted below the sheath, that the latter is a very significant barrier.

Consequently, it is inevitable that the views of Lorente de Nó should be treated with reserve. He rejected the concept of a porous membrane whose permeability might be influenced, and he repeatedly[17,22] emphasised metabolic effects. It is true that metabolism must supply energy for nerve function and for the maintenance of the resting membrane potential, but it does not follow that it is the sole key to the production of block.

Indeed, Lorente de Nó himself reported that nerve cannot be stimulated unless the resting membrane potential is above a critical excitability level, and he admitted that lack of sodium ions, or anaesthetics of the cocaine type, may prevent the production of the action potential without lowering the resting membrane potential. The last is an important admission.

Acetylcholine and the Nerve Impulse

It has been suggested that acetylcholine may propagate the nerve impulse along the axon, and this idea has influenced certain researches on local anaesthetics.

The chief protagonist of the theory, Nachmansohn[23,24], claimed that acetylcholine is released from an unknown precursor and depolarises the membrane, thus establishing a local circuit which excites neighbouring regions. The acetylcholine is immediately destroyed by cholinesterase, and is re-synthesised into the inactive precursor during the recovery period, by the enzyme choline acetylase. Acetylcholine and the two enzymes do exist in nervous tissue, but the action outlined above is strongly contradicted by considerable experimental evidence, chief amongst which is that acetylcholine, even in massive concentration, does not depolarise nerve[25]. Nevertheless, Nachmansohn continued to assert the validity of his theory. Feldberg[26] has stated that Dale's words of 1948 are still true . . . "the ingenuity of its (the theory) supporters is sorely taxed to discover even plausible ways of escape from the facts which contradict it".

Saltation

Erlanger and Blair[27] suggested that the impulse is transmitted along the nerve in a jumping (saltatory) fashion, each segment between two nodes of Ranvier behaving as a unit. Restriction of excitability to the nodes has been confirmed, and Rashbass and Rushton[19] envisaged that impulses pass quickly along myelinated sections of nerve and receive a "boost" at the nodes. Huxley and Stämpfli[28] regarded myelin as a conductor which increases conduction velocity by making local circuits act at considerable distances ahead of the active region. They supposed that the action potential process is generated at the nodes. This view is favoured by the fact that agents producing stimulation or depression of conduction have a stronger action on the nodes than on the internodes. Blocking an internode stops the impulse, presumably due to the interruption of current flowing forward in the axis cylinder and back in the fluid of the myelin sheath. A similar arrangement seems to exist in unmyelinated fibres.

Impulse Initiation

Katz[29] studied the depolarisation of sensory terminals and the genesis of impulses in frog muscle spindles. Stretching depolarises the endings, and a local potential change can be recorded from the afferent axon at a point close to the spindle. The potential varies with the rate and amplitude of stretching, and generates repetitive impulses in the sensory nerve.

Depolarisation increases with velocity of stretching until it attains a maximal value. On analysing the stretch two phases were revealed. The first coincides with the period of initial lengthening, and is due, Katz suggested, to changes in membrane capacity. In the second, the depolarisation and rate of discharge are maintained at a lower level, which may be due to a change in membrane permeability. It is noteworthy that the membrane is implicated in both phases. In 1951 Albert[30] stated that the cellular membrane plays an essential rôle in the propagation of excitation, and is therefore a likely site for the action of hypnotics.

The question of impulse transmission is not entirely straightforward, and this is true when transmission is applied to sensation. Intensity of stimulation has to be expressed, and this can be done by frequency of impulses[31]. If all sensation were equally anaesthetised the problem would be simpler. This is not the case, hence the suggestion[32] that some lines are preferentially paralysed.

Such difficulties are bound to cloud any clear appreciation of local anaesthetic action, and are difficult to overcome completely.

Theories of General Anaesthesia and their Relevance to Local Anaesthesia

The Overton-Meyer Theory

The first essays in this field were made at the time of the clinical introduction of ether and chloroform. Their solvent properties produced the suggestion that narcosis is caused by dissolving some fatty constituents of the brain which are re-deposited in the liver. The concept fails to explain the reversible effect of the anaesthetics, unless there is a rapid re-synthesis of the constituents, or a re-transfer of the original ones from the liver back to the brain.

Overton coined the term "lipoid"[33] to describe the true fats, and the more elaborate fat-like substances, such as lecithin and cholesterol. In analytical research it proved impossible to employ brain tissue or even the extracted and denatured brain lipids: consequently a simple model substance was often employed, the original and commonest one being olive oil.

The basic concept was to relate anaesthetic activity of a drug to its partition coefficient, making use of this model. It is relevant to note here that both local and general anaesthetics are usually more soluble in fat than in water. The full theory, endorsed by Overton, was published in 1899 by Meyer[34], and propounded that drugs which are lipid soluble, and can become distributed in protoplasm, act as narcotics: the partition coefficient is important as it determines the relative distribution of the drug in the mixture of body constituents, including water and lipids.

The shortcomings of the theory were later defined[35]. It was suggested that inhibition mechanisms vary, that is to say different drugs may act at different points, and produce depression in a variety of ways, and the theory only applies to the "indifferent narcotics" (an ill-defined group of fat-soluble organic compounds which includes alcohol and chloroform). Such propositions necessarily limit the theory.

The theory has been criticised because the critical lipid phase of neurones is relatively unknown, and because many anaesthetics fail to conform to it. The latter was true when Löfgren tested local anaesthetics on a water and oleyl alcohol model[36] which has often been regarded as a better representation of the cellular lipids involved in narcosis. (A lecithin in gelatin one has been used with fair success for local anaesthetic studies). Löfgren concluded that local and general anaesthetics are not strictly comparable. In his opinion, a major drawback to the theory is that compounds with widely differing activities may have the same coefficients.

From the literature it seems that many workers have attempted to apply the concept too rigidly with a model which was chosen for convenience and simplicity. On the other hand, Collander[37] commented that lipid molecules are orientated in layers in membranes, whilst in bulk phases, as in models, they are randomly distributed, and may behave quite differently. He saw little theoretical advantage in studying coefficients with solvents that are chemically more closely related to the membrane than is olive oil. Failure to imitate cell membrane lipids, for example, cholesterol, may explain the anomalous drugs which have high partition coefficients but are not depressants[30]. Attempts to rectify this have only been limitedly successful.

Cell constitution is very important in the application of this theory. Cellular protoplasm is separated from the environment by the plasma membrane, and any substance entering the cell must pass the membrane. Work and Work[38] commented that the degree of correlation of lipid solubility and penetrant powers of a drug is noteworthy, but stressed that some lipid-insoluble substances may penetrate into cells. They suggested that the membrane might be a continuous lipoprotein skin, or a sieve-like structure. The second would allow the entry of lipid-insoluble substances without requiring the agency of an active transfer mechanism.

Burger[39] admitted that the true anaesthetic is preferentially absorbed by cells containing large amounts of lipid, but argued that the composition of nerve invalidates the theory, since only half of the solids are lipids, whilst up to 90 per cent of the nerve is water. This is ludicrous: nerve may contain a mere 5 per cent of lipid, but even if there was only a fraction of this amount present, there is no reason why it should not govern anaesthetic action, provided that it is situated at strategic positions for producing block.

In 1948 Löfgren[36] stated that many experiments supporting the theory had, in some way or other, been inadaequately performed. In his own work especial care was taken to maintain pH constancy. He found that various general narcotics may act as local anaesthetics, when they probably conform with the Overton-Meyer rule: with his own local anaesthetic compounds, including lignocaine, he concluded that their minimum effective concentrations cannot be a function of the distribution coefficient alone. However, he did not reject the rule completely, and he reported experiments to support the view that local anaesthetics behave ideally in the lipid phase. From this Butler[2] made the far-reaching suggestion

that physical properties might merely be a factor regulating the access of the drug to its site of action.

Barlow[40] conceived that drugs have to penetrate the neurilemma, hence fat solubility may be necessary for the entry of a drug into the axon, although Hansteen[41] maintained that lipid solubility and penetration are not related as it occurs in denatured lipids. Skou[42,43] carried the concept further. Using local anaesthetics and extracts of nerve lipids from desheathed frog sciatic nerve he concluded that a correlation exists between anaesthetic potency and penetration of a monolayer of nerve lipids. Like Löfgren, he was careful to use a constant pH in his experiments.

The strict interpretation of the theory immediately creates difficulties, since all fat-soluble substances are obviously not narcotics, and it also fails to explain why some drugs with normal[2], or "sub-depressant"[30], partition coefficients are convulsants: it is tempting to presume that inadequate lipid penetration, with consequent accumulation outside the tissue, is the cause.

Finally, careful note should be made of, (1) the admission[44] that the lipid content of brain cells must influence the surface tension theory, (2) the contention[41] that denatured lipid behaves like the normal substance, hence adsorption is the salient feature, and (3) the belief, expressed by Meyer himself[45], that the presence of the narcotic in the lipid changes the permeability of the cell wall.

Clearly, a link exists between this and other factors.

The Traube Hypothesis

The basis of this theory is that a change is caused in the surface tension of the cell. Traube[46] noted that substances which lower surface tension, including many narcotics, pass most rapidly into the cell, and concluded that this must be an important measure of their ability to pass the cell membrane.

The theory was formulated in two papers[47,48], and involved the measurement of capillary activity of the drug at an air: water interface. Commenting upon the hypothesis, Toman[49] suggested that it has not been subjected to a rigorous test. This seems to be a fair criticism. Moreover, Albert[30] has emphasised that air:water interface results are largely limited to members of homologous series of drugs, and merely signify the non-wettability of the substance, and do not imply any specific adsorbability on a cellular receptor.

As alkalisation increases the surface activity of alkaloids, Traube[50] attributed the increased potency of local anaesthetics in alkaline solution to this cause. Luduena and others[51] corroborated this.

Henderson[52] remarked that the importance of the theory lies in its connection with the adsorption one, and it is evident that they are closely related. It is clear, too, that it has important links with penetration and permeability.

Although some local anaesthetics may lower surface tension *in vitro*, many good narcotics do not lower the interfacial tension between oil and

water[53]. Results from water must be treated with caution in view of the contention[44] that the clinging intensities of narcotics to lipids and to water are often completely opposite. Correlations are obtainable, but the significant ones have always been derived from homologous series of drugs[17,30,51]. Moreover, some substances lower the interfacial tension between oil and water, for example, soaps and detergents, but have no narcotic properties[54].

Finally, the observation[36] that chloroform and lignocaine increased the surface tension of an ergosterol film completely contradicts the theory, but this may be due to the model chosen.

The Warburg Theory

Narcotics are readily adsorbed on surfaces *in vitro*, and a similar effect may occur at the cell surface. Warburg[55] showed that narcotics could displace amino acid from a charcoal surface, and claimed that narcosis is caused by adsorption, and this effect is independent of the chemical nature of the narcotic.

Henderson[52] commented that all the models used in examining the theory dealt with enzymatic effects, chiefly oxidation ones: this is important, as he was convinced that narcosis and depression of oxidation are not the same thing.

The Warburg and the Overton-Meyer theories have been linked by the work of King and others[56] using homologous drugs on a water and paraffin oil model, and Rider[57] claimed that the intact local anaesthetic salt is adsorbed by nerve cells, possibly by structures of a lipoid nature. Löfgren[36] suggested that, *in vivo*, the anaesthetic is strongly retarded at the node surface: consequently a highly concentrated layer of drug is formed, and when it has reached a critical value the disturbance of the film may reach the anaesthetic stage. Höber[58], however, pointed out that some narcotic substances are apparently not adsorbable upon any cell structure or constituent, and there are substances which are exactly the opposite.

Höber and others[59], later proposed that the adsorption layer protects the surface film of the nerve from the changes which are features of excitation, that is, it produces a form of membrane stabilisation.

In adjudging the Warburg theory it is likely that adsorption can potentially influence two processes. Firstly, it may decrease metabolism by "blanketing" oxidative processes, and secondly, it may affect permeability by decreasing porosity. Support and criticisms have been found for both views, but the decisive point is that both processes are covered by other theories. Adsorption, by itself, is unlikely to explain anaesthetic action.

The Permeability Theory

As normal nervous excitation involves changes in the membrane (including increased permeability to ions), a substance which can stabilise the membrane should prevent them, thus producing a conduction blocking effect.

The theory was formulated by Lillie[60], and he envisaged narcotic adsorption on, followed perhaps by dissolution in, the membrane, thus producing a change in its physical state.

Although the theory was advanced for the central nervous system, peripheral nerve fibres are more accessible for study of the effects of drugs on polarised membranes. In them the blocking of impulse depolarisation by a stabilising action has been demonstrated. Bishop[61] employed potassium chloride (KCl) and cocaine as blocking agents, and claimed that nerve block may occur without depolarisation. He decided that if depolarisation occurs it is a sign of the condition produced rather than the means of block.

Höber and others[59] concluded that narcotics are cytolytic, so normal selective cation permeability is lost, and all ions can pass through, with consequent depolarisation. Later, Höber[58] suggested that the interfacially-active narcotics produce a *decrease* in permeability by forming an adsorption layer upon the pore walls. This obstructs, or even completely blocks, the channels, so that even small molecules, such as water, may be excluded.

Observing block without depolarisation, Bennett and Chinburg[62] were "forced" to the view that anaesthetics fix membrane conditions: the prevention of the calcium-induced shift in resting potential by pretreatment with procaine strongly supported this opinion. They also considered that the depolarisation by procaine and cocaine reported by Höber and others[59] was due to the use of excessive concentrations. (They were seven times stronger than those used by the authors to produce 90 per cent block in frog nerve: as many of Höber's results came from crab nerve, which is even more drug sensitive, the discrepancy may be greater still.)

Shanes has consistently supported the theory, and produced a comprehensive publication in 1958[63]. He based his work on the modified Bernstein theory, and recognised the importance of metabolism in ionic movement, designating it "active transport", but he did not believe that it contributes directly to membrane potential.

He did not regard permeability restriction as a surface obstruction effect. He visualised the membrane as a semi-rigid, semi-fluid, structure, with lipid molecules held together by intermolecular forces. He prescribed some flexibility in membrane molecule spacing, depending upon both physico-chemical factors and temperature.

He endorsed his earlier classification of two types of active substances, the stabilisers and the labilisers. The former, including calcium, procaine, and cocaine, reduce the electrical effectiveness of sodium, potassium, and other ions, whilst the latter, including low calcium and veratrine, accentuate the ionic effects on membrane potential. He defined stabilisers as agents blocking nerve or muscle impulses without any change in resting potential. Such agents "dissolve" by molecular displacement in the interpore regions, and thereby increase lateral pressure on the pores, thus decreasing channel size and permeability: the last prevents depolarisation on stimulation. About labilisers he was indefinite, suggesting that they

may reduce lateral pressure by adsorption on inter-channel regions rather than by being dissolved in them.

Certainly, Shanes' conception of stabilisation harmonises with observed results in the light of Hodgkin's description of nerve transmission[13].

Hardt and Fleckenstein[64] used various stabilisers to show that the prevention of depolarisation which they caused was accompanied by potassium retention: sodium was not investigated.

If a muscle is placed in Ringer solution where part of the sodium has been replaced by potassium, a swelling occurs, but treatment with cocaine reduces the causative entry of potassium chloride. Moreover, in a treated muscle, the normal depolarisation effect of KCl is reduced[65]. Shanes[66] extended the work to other local anaesthetics with similar results. Manipulations with sodium ions, using procaine and cocaine as stabilisers, yielded identical conclusions[67].

According to Shanes[63], the theory raises the factor of rate of action. It partly depends upon the speed of arrival at the site of action in the nerve, but could also be influenced if physiologically-active substances are emerging from the channels but cannot escape, and hence accumulate around the fibres. This may occur even in single fibres unless they are well irrigated. This feature might well be important in isolated tissues, although in the intact animal natural factors, such as the blood system, probably obviate it.

Calcium can act as an anaesthetic agent, whereas low calcium concentrations have a labilising effect which can be prevented by cocaine or procaine. Shanes rejected suggestions that modifications in the calcification of the nerve fibre surface can be produced, but he admitted that intracellular calcium might be important. He believed that calcium, and also low temperature, produce stabilisation, not by lateral pressure on the pores, but by increased rigidity of the inter-pore region: this reduces flexibility, and hence permeability, of the membrane.

Shanes finally considered that any weakness or lack of effect of stabilisers generally might be due to an inability of the channels in the membrane to be further compressed.

Lastly, it has been claimed[54] that narcotics variously affect permeability, the contention being that this factor might determine the entry of an agent into a cell without accounting for its action inside. This is not fully in accordance with the theory of membrane stabilisation, but is of interest, supposing as it does that permeability represents a phase, rather than the complete mechanism, of narcosis.

The Colloid and Protein Coagulation Theories

Bernard[1] showed that cell colloids may aggregate during anaesthesia, this process being reversible, and he conceived the view that narcosis consists of a reversible semi-coagulation of the substance of the nerve cell. This led to the formation of two related concepts of anaesthesia.

The basis of the first is that narcosis is a non-specific action, as also is simple surface adsorption, due to a change in the colloidal dispersion of protoplasmic components. This was alleged to be visible under the

ultramicroscope, to be reversible, and to involve proteins as well as lipids[68]. Barlow[40] rejected the theory on several counts. His criticisms arose from the observation that the narcotic concentrations of anaesthetic are much smaller than those needed to flocculate the colloids, and some narcotics apparently decrease the dispersion of the colloids.

Butler[2] regarded the theory as the product of over-simplification which showed a tendency to discount the complexity of living systems. This comment seems to be a justifiable one.

The protein theory was founded on the concept that narcotic concentrations of drugs produce a reversible coagulation of essential cell proteins, thus inhibiting normal function. Conflicting evidence was produced, but Henderson[52] presumed that the toxic effect of high concentrations of narcotics may be attributable to precipitation intracellularly, or at the cell surface. This seems to be drawing rather a thin line between this and the full precipitation events which occur, for example, in *rigor mortis*.

Several workers have demonstrated synergisms between various proteins and local anaesthetics, but serum proteins have been unsuccessful in this context. No conclusions about mode of action are forthcoming from them.

The two theories are so similar that joint criticism is justified. Neither has much supporting evidence, and neither appears to allow the speedy reversibility characteristic of local anaesthesia. Although colloid or protein coagulation may be produced by general or local anaesthetics there is no reason to suggest that these are in any way associated with the production of narcosis.

The Dehydration Theory

This states that anaesthesia is due to water loss from the cell or cells concerned. Kochmann[69] considered that anaesthetics reversibly dehydrate or stabilise the cell colloids, and thus reduce membrane permeability, which leads to metabolic inhibition and functional arrest. Winterstein[70] disputed this because dehydrated muscle can become very irritable.

The evidence for the theory is bald and unconvincing, and fails to substantiate the concept[15] that the action potential is accompanied by dehydration changes in certain areas of the nerve. As a theory of general anaesthesia, let alone local anaesthesia, it is quite unacceptable as it stands.

Acetylcholine Theories

The origin of these is difficult to trace. The concept of acetylcholine activity in nerve transmission is nowadays discredited, but its rôle in autonomic transmission is undisputed. Much evidence has been obtained from muscle preparations: this does not preclude it, but it must be treated with reserve.

Wilson and Wright[71] claimed that procaine inhibits acetylcholine release at the neuromuscular junction, whereas Thimann[72] concluded that procaine and similar local anaesthetics block the acetylcholine receptors

at sensory nerve endings. The tissue difference, motor versus sensory site, must be remarked. Conclusions about the relationship between procaine and the neuromuscular-blocking drugs have been very conflicting. Some of this may stem from species differences of the test animals.

Much the same difficulty arises in connection with anticholinesterase activity, as widely divergent results have been recorded. The involvement of esterases in local anaesthesia was propounded by Bieter[73] because di-isopropylfluorophosphonate (DFP) and eserine can abolish conduction without depolarisation or transient excitation. Toman, Woodbury and Woodbury[74] reported similar results with these drugs and procaine. They attributed the effect to an increase of the nerve conduction threshold above a critical value, although the Nachmansohn theory demands that anticholinesterases should produce an enduring nerve depolarisation. They suggested that the nerve block is due to side-effects which are independent of anticholinesterase activity: large doses of DFP are needed to produce block, whilst relatively low concentrations antagonise cholinesterase. Furthermore, all anticholinesterases do not act on nerve like these two: such discrepancies would not occur if acetylcholine accumulation is the basis of conduction block. Skou[75] studied local anaesthetics in the electric eel, and also concluded that cholinesterase inhibition is not related to peripheral nerve blockade. Any attempt to produce linear correlations was unsuccessful.

Although this work primarily referred to local anaesthesia, the results further confirm the rejection of the acetylcholine theory of nerve transmission.

Greig, Holland and Lindvig[76] introduced a novel concept by relating penetration to cholinesterase inhibition. They suggested that surface-active local anaesthetics, like butacaine, penetrate mucous membranes by inhibiting cholinesterase, while inactive ones, such as procaine, can produce anaesthesia here only if the enzyme is first blocked by eserine. They anticipated the obvious criticism by demonstrating that eserine alone was ineffective on the rabbit cornea tissue used. Their hypothesis, despite various assumptions they had to make, has not been seriously discredited, and should merit further investigation.

Many allusions have been made to the similarity between atropine and local anaesthetics[49], particularly procaine, and De Elió[77] has held that atropine has a local anaesthetic effect which is about 50 per cent that of procaine. Sinha[78], however, rejected these views. Moreover, in all his comprehensive studies he noted that procaine seems to play a lone rôle, and this observation may be important.

In the autonomic system little evidence is at present available from the synapse, though this field might be informative. With neuromuscular junctions results are obtainable that local anaesthetics are curariform, but there is no general agreement that the modes of attaining block are similar. That such effects may be side reactions is far from improbable, particularly when it is remembered that the *in vivo* use of local anaesthetics does not produce any obvious autonomic or neuromuscular actions, despite various means of administration.

The Histamine Theory

This hardly ranks as a theory, but a note of it is desirable. Many nerve fibres have been shown to contain histamine[79], and it has been proposed that it participates in nervous conduction. This view has failed to gain acceptance. Although many antihistamine drugs show local anaesthetic properties, Sinha[78] concluded that the two activities are not related. As, therefore, the relationship seems to be fortuitous, further consideration is unnecessary.

Metabolic Theories

Their basis is that narcotics interfere with cell oxidations, and that anaesthesia is essentially a type of asphyxia caused by a loose union between the drug and the oxygen-carrying groups in the cell. Much supporting evidence has been forthcoming, but reports have been made of no change in, or even increased oxygen consumption with some narcotics[70,80].

Warburg[81] showed that narcotics inhibit the oxidation of amino acids and similar substances. In much of his work charcoal was used as a model of the surface. The choice of both model and substrate cast some doubt on the cogency of his conclusions.

The chief advocate of metabolic theories has been Quastel. He demonstrated[82] that anaesthetic concentrations of narcotics reversibly inhibit the oxygen consumption of brain slices. This arrested the oxidation of several substances, but not succinate. Watts[83], however, was able to inhibit succinate oxidation in brain by using local anaesthetics: cinchocaine was the most effective compound, and cocaine and procaine the least effective ones. Earlier, Sherif[84] investigated several anaesthetics on isolated rabbit sciatic nerve: cocaine and procaine both reduced oxidation, and the effect increased with concentration. The most powerful agent for reducing metabolism was eucupinotoxin, but this has a negligible effect upon nerve conduction. 5 per cent urethane was roughly equivalent to 2 per cent procaine. Sherif considered that urethane affects nerve oxidations only in relatively high concentrations, though it has a marked effect on the oxidation processes of brain tissue. This is a notable idea, as it suggests a difference in reaction between the two types of nervous tissue: it keeps recurring.

Lorente de Nó[17] rated oxidative processes more highly than any ionic concentrations in maintaining membrane potential: he recognised the importance of sodium and potassium ions only as far as they directly or indirectly participate in enzymatic reactions. Nevertheless, from his work on cocaine he admitted that a drug might restrict metabolism in such a way that the membrane potential is maintained with a reduced oxygen consumption. Gerard, also,[54] declared that functional anaesthesia is not always attended by depressed respiration. He felt that narcotics probably act along oxidation-inhibition lines, but commented that the most convincing evidence of interference with cell metabolism is usually obtained from complicated systems rather than simple ones. Consequently much information has accrued about suspected locations

of block, while more fundamental considerations may have been omitted. Many sites have been proposed for blocking activity, but as Gerard rightly stressed, the limitation on all the work is that locations were determined by exclusion rather than by positive proof. He suggested that narcotics might inhibit complete metabolic systems by some relatively non-specific physico-chemical action, rather than by blocking a postulated link.

Gerard's views were not shared by others. Burger[39] favoured the idea of the high susceptibility of nervous tissue to oxygen lack, and named an enzyme link in the oxidation chain as a possible site of action. Barlow[40] also believed in enzyme inhibition, and drew attention to speed of onset, emphasising that an enzyme would probably be affected instantaneously. Much work on enzymes has utilised models, or simple mixtures of enzyme and narcotic. The drawback to this, and related approaches, is that it is certain that even the complete tissue *in vitro* may behave very differently from the same material *in vivo*. Barlow cited narcotics which, *in vitro*, lower creatine phosphate levels, whilst *in vivo* they have exactly the opposite effect. Presumably the basic physiological difference between nervous tissue *in vivo* and *in vitro* is that the former is still actively working. McIlwain[85] devised a method of electrical stimulation of cerebral cortex slices *in vitro*, and found that the levels of metabolic activity were then the same as in similar tissue *in vivo*. This technique may prove useful in future research, but could well be more applicable to the brain than to peripheral nerve. Potassium stimulation has also been used to overcome the difference, and it is claimed[86] that local anaesthetics can depress respiration in cerebral cortex slices thus treated.

Butler[2] has most relevantly indicated that neither inhibition of enzyme nor of brain tissue oxygen consumption are effects peculiar to anaesthetics. Indeed, some inhibitors are convulsants, like picrotoxin and leptazol, and he regarded the inability of the theory to distinguish between anaesthetics and convulsants as a serious deficiency. He also discounted the idea[87], derived from work with cocaine, that, although the total brain oxidation might show little change, small areas may suffer from oxygen shortage. Such a concept would be very difficult to prove satisfactorily, but it will require further substantiation if it is to be accepted.

Undoubtedly, inhibition of oxidation often accompanies anaesthesia. In the intact brain this might be due to a reduction in the number of neuronal discharges which occur continuously, and which, naturally, consume oxygen in their recovery phases.

Experiments with other nerve tissues have been most revealing. Larrabee, Posternak and Bronk[88] used narcotics in concentrations sufficient to block sympathetic-synaptic transmission. Sodium pentobarbitone and alcohol reduced resting metabolism, even in sub-blocking concentrations. The concentration of the former had to be raised 5 to 10 times to block B and C fibres. Cocaine, on the other hand, could be given in concentrations of five times the blocking dose, and yet it failed to depress resting metabolism. This is important as it provides clear evidence of a divergence of metabolic effects when general and local anaesthetics are employed.

Because of Quastel, much of the work on metabolism has dealt with oxygen utilisation, but inhibition of other mechanisms is a distinct possibility. Butler[2] noted that most of the quasi "narcotic" drugs can inhibit the breakdown of adenosine triphosphate (ATP), even in disrupted or dead cells, and he emphasised how little is known of the effects of anaesthetics upon phosphate metabolism. Recently it has been claimed that clear evidence exists suggesting that ATP is the energy source for sodium ion extrusion[13]. Caldwell and Keynes[89] made the relationship quantitative by claiming that four phosphate bonds are broken for each sodium ion ejected. Whittam[90] used human red blood cells, and concluded that ATP may be linked with active cation transport. This trend is both new and important. It is possible that hesitation had been felt previously about applying results which hold for muscle energy relationships to nerve.

In considering the influence of metabolism it must be appreciated that it is linked with the maintenance of the ionic states inside and outside the nerve. Interference with it must ultimately influence ion relationships, and hence conductivity. Conversely, if conditions in the nerve are altered it is likely that metabolism will be affected also.

One of the leading questions is whether the work done against the steady ionic leak from nerve is aerobic or not. Brain is very susceptible to oxygen lack, but it is conjectural if peripheral nerve is similarly affected[91]. Lorente de Nó[17] reported that anoxia alone does not totally depolarise, hence metabolism only depends indirectly upon respiration. His concept of a reserve of oxidised material seems reasonable, especially as there is an equivalent in muscle: it seems to be more typical of peripheral nerve than elsewhere, and it might create the impression of partial respiratory independence.

Ionic Influences

These have never been raised to the status of a theory, but the current views on nerve transmission indicate that ions are potentially important. The stimulation by small doses, and blocking by large doses, of potassium chloride have already been described. Sinha[78] claimed that the inhibition of KCl stimulation by means of local anaesthetics is a fairly accurate guide to their relative anaesthetic activities.

A sign of the complexity of the situation is the potassium loss reported in mammalian heart during vagal stimulation, as this ion effect must be linked with acetylcholine release[63].

Potassium studies probably evolved from local anaesthetic synergisms: the empirical surgical use of potassium sulphate with cocaine and procaine has long been practised. Hoffman and Kochmann[92] demonstrated a species difference effect using equivalent strength mixtures of local anaesthetics and potassium sulphate which were injected intravenously into guinea pigs and intradermally into man: the toxic effects were less in the guinea pigs.

Adriani has emphasised[93] that pain and oedema after injection may limit the usefulness of potassium potentiation. Other synergisms have

been reported, involving, amongst others, agents which have proved permeability-increasing or metabolic effects, which suggest links with other mechanisms in order to achieve the blocking effect.

Rider[57] investigated synergisms, especially that of butacaine with other local anaesthetics. That one local anaesthetic may behave like an inorganic substance towards kindred drugs seems quite anomalous, and yet butacaine can, like the others, be synergised by potassium sulphate. This sign of individuality should not be allowed to go unremarked.

Rider also showed that butacaine hydrochloride is more active than the sulphate, and concluded that this indicates potentiation by the chloride ion. This idea is surprising, because Rider himself furnished an alternative explanation. The hydrochloride is less soluble than the sulphate: he visualised that when two drugs are in contact with nerve cells, the less soluble one will tend to escape from water to any structure capable of receiving it. Furthermore, the presence of abundant body chloride may hinder sulphate as it may have to be adsorbed first, and may lose activity in so doing. This rôle of body chloride is important, because it creates the possibility that a drug may have completely different modes of action *in vitro* and *in vivo*. This leads to the whole field of pH effects, whether due to body constituents or to external manipulations. It is feasible that solubility effects such as Rider outlined may, in some way, explain the striking success of potassium sulphate as a synergistic agent.

Bein[94] claimed that optimal cell functioning depends on ionic ratios, especially of potassium and calcium, rather than on absolute amounts of them but that, in local anaesthesia, the situation may be sometimes completely reversed. Both potassium and calcium alone produced variable or indeterminate effects, but when combinations of one or other of them with procaine were made, the two ions appeared to be antagonistic. This illustrates how easily an effect can be modified, the difference here being between the ion effects in normal cells and in cells influenced by procaine.

Lorente de Nó[17] reported that excess of calcium ions caused irreversible deterioration of nerve fibres and, peculiarly, damaged the rapidly conducting A fibres more than the C ones. He ascribed this to penetration and accumulation of calcium ions in the myelin which causes a swelling due to an osmotic effect. This leads to myelin disintegration and consequent destruction of the conduction faculties. He alleged that excess of potassium has similar effects.

Gasser[95] suggested that after-potentials are signs of recovery processes following impulse conduction, and the modified Bernstein theory supports this. Lorente de Nó claimed to have found them in areas which have not been activated, and this could seriously upset the present views on the recovery period. However, Shanes has interpreted these potentials as signifying inadequacies of the metabolic recovery processes: if this is correct they need not be confined to nerve that has recently been activated.

McDowall and Soliman[96] suggested that many drugs produce sodium accumulation at specific receptors. In the light of present knowledge about transmission this effect might be expected if the action of the drug

is to facilitate membrane stabilisation: this affects membrane permeability, and hence the movement of sodium ions inwards at excitation.

Electrical Theories

Burge (see 92) reported a change of electrical polarity in fish brain cells from negative to positive under the influence of narcotic drugs, and attributed this physical change directly to the drugs. However, since sodium, potassium, and chloride ions carry the electrical charge in nerves[13,17], changes in ion distribution will produce electrical effects, and thus Burge may have seen the results, rather than the cause, of block.

A different approach is the production of narcosis by electrical currents. It is not, however, a practical means of producing local anaesthesia, nor has the work upon it been at all explicit.

Indeed, it is questionable how far the entire electrical approach can be taken. Bishop[61] has asserted that nerve block is not primarily due to altered potentials but to changed irritability. This appears to be true, hence the observation of electrical conditions, for example, membrane potential and action potential, can furnish important information about the events caused by local anaesthetics, but should not be regarded as a means of explaining modes of action.

Thermodynamic Activity

This is an estimate of the molecular work needed to transfer the narcotic from the pure liquid phase to the unknown one of locus of action in the narcotised cell. It is derived from vapour pressure determinations. The theory was advanced by Ferguson in 1939[97], and was based upon the supposition that narcosis depends on a physical mechanism: any major deviation from the thermodynamic activity range would denote a chemical rather than a physical action.

Brink and Posternak[98] clearly recognised that thermodynamic methods of analysis will not reveal molecular mechanisms of narcosis. They proposed their use to measure narcotic effectiveness, but had to admit that ether often fails to conform to the theory, despite its clinical effectiveness. Butler[2] commented that no anaesthetics show regular relationships between potency and any physical property. He considered that physical measurement not only fails to predict anaesthetic doses quantitatively, but even fails to predict reliably the qualitative nature of the pharmacological action.

Evidently, classing this as a theory of anaesthetic action, even for general anaesthesia, as has often been done, is a misnomer. Like electrical activity, thermodynamic studies may be useful for predictions and observations of behaviour and effect, but they do not clarify the means of local anaesthetic action at all.

Other Factors in Local Anaesthesia

Several topics are closely related to the present problem and deserve further consideration.

Effects of pH

Investigations of pH have often provided information about the active form of local anaesthetics, whilst the study of the latter has frequently been governed by the pH. It is not thought desirable to divorce such information here, as the two are so clearly inter-related.

Bignon in 1892[99] showed that alkalisation increases the activity of cocaine solutions, and introduced an alkalised suspension of cocaine ("cocaine milk") into clinical practice: its activity was, in fact, very little greater than normal cocaine hydrochloride. Gros, however, confirmed the observation on other local anaesthetics and suggested[100] that the greater potency in alkaline solutions is caused by the free base being the only active constituent: alkalisation potentiates anaesthesia due to the increased amount of base liberated. Trevan and Boock[101] repeated this work and corroborated this theory, as did other authors[15,57,102]. Gros suggested that the free bases of all anaesthetics have much the same activity, but Löfgren[36] has strongly criticised this.

Sollman[103,104] showed that sodium bicarbonate increases the efficiency of cocaine and procaine about 8-fold on motor fibres, and 2 to 4 times on sensory fibres, of isolated nerve. He suggested that the alkali helps the liberated anaesthetic base to penetrate into the nerve trunks.

Régnier and David have consistently claimed that all the aqueous forms of cocaine and procaine, ions, base, and salt, are active, and that the alkali acts directly on the tissues concerned, because the addition of alkali to a saturated aqueous solution of cocaine base increased its anaesthetic action[105]. Although Trevan and Boock[101] explained this as a buffering effect by the alkali, allowing more of the poorly-buffered cocaine to be effective, Régnier and David remained adamant in their refusal to accept this, saying that release of the free base plays a minor part only in the phenomenon compared with the direct effect of the alkali[106,107].

In 1931 Gerlough[108] noted that the presence of acid seems to inhibit local anaesthesia, for example 0·25 per cent butacaine poorly anaesthetises rabbit cornea at pH 5·5, whereas at 7·4 the same concentration gives a considerable duration of anaesthesia. He suggested that this might explain why local anaesthetics often fail to act in acutely infected areas, as in abcesses. Bieter[73] attributed the acidity effect to decreased hydrolysis of the anaesthetic.

Hirschfelder and Bieter[15] considered that the free bases of alkaloids are usually more active than the salts because the latter are more soluble in water, whilst the former are more soluble in fat, lipids, and organic solvents. They felt that local anaesthetic potency should be a function of the degree to which the anaesthetic is hydrolysed, provided that as the free base it is sufficiently soluble to remain in solution or in a finely divided suspension. (Furthermore, anaesthetic effectiveness must also be a linear function of the pH of the tissue, hence a basic local anaesthetic is more effective in alkaline than in acid media.) Consequently, the weaker the base, the more free base is released, and the greater should be the anaesthetic potency. This is best achieved, at least in homologous

series, if the soluble anaesthetic salt is made from the base and a weak acid instead of hydrochloric or sulphuric acids. The findings of the authors held for the conjunctiva and for intradermal injection, but not in infiltration anaesthesia, or in the urethra. They believe that modifying factors may arise here: in deep tissues, or in the presence of urine, the anaesthetic salt reacts with sodium chloride which tends to decrease the hydrolysis and, by ionic interchange, to return much of the anaesthetic to the form of the hydrochloride, regardless of the acid used to form the salt. That is to say a buffering action occurs.

Krahl, Keltch and Clowes[109] used simple cells (*Arbacia punctulata* eggs and larvae) to investigate the form in which local anaesthetics penetrate the cell, and the probable form in which, once inside, they enter into chemical reactions giving anaesthesia. The total local anaesthetic concentration required to produce a 50 per cent reduction in cell division at pH 7·0 was 100 times greater than that needed at 9·1. They also reported an extraordinary tendency for the cations to escape from solution in the intracellular aqueous phase to adsorb on, or combine with, cellular constituents. They concluded that the anaesthetics penetrate only in the form of undissociated molecules, and considered that, unlike many other anaesthetic substances, it is the intracellular concentration of cation, and not the undissociated molecule, which causes the physiological effect. They further suggested that basic anaesthetics are local ones because cells at the site of application require so much anaesthetic to satisfy the laws of membrane penetration that relatively little is left to produce general anaesthesia.

Dawes carried this trend further. His work on the heart[110] supported the contention that local anaesthetic and quinidine-like properties characterise the free base. Not only are the most powerful local anaesthetics most active upon the auricle, but conversion into the quaternary salts, which stabilises the cation, of some of the "cardiac" compounds could abolish their activity, just as a similar conversion of local anaesthetics abolished their effects. The importance of this cannot be ignored. He decided that the free base can readily penetrate nerve and muscle, and once inside the cell it equilibrates with its cation again according to this equation.

$$\underset{\text{cation}}{R_3NH^+} + OH^+ \rightleftharpoons \underset{\text{base}}{R_3N} + H_2O$$

In this way cations of these substances could get inside the cells: previously it was thought that the free base of local anaesthetics, and quinidine substitutes, is the active constituent because of its penetrant powers. However, Dawes' concept makes it feasible that the function of the free base is merely to facilitate the entrance of the active cation. This conception is a most notable one.

Löfgren[36] maintained that much early work on minimum effective concentration is useless because of the use of unbuffered biological material, and lack of control of pH. Some authors appreciated this and took steps to obviate it[42,43,61,111]. A striking feature was the repetition

by Mongar[112] of work on guinea pig wheals[77] using buffered instead of unbuffered solutions: cinchocaine was 34 times as powerful as procaine, as against the earlier estimate of only 10 times.

In surgery, the natural body buffering probably conceals any variations in efficiency due to pH, but pH changes are obviously most important in surface anaesthesia. Mucous membranes have poor buffering capacities, so the usual surface anaesthetics owe their efficiency to a high activity: such drugs are often so toxic that they cannot be injected. Consequently Löfgren dismissed the idea that these substances have a special affinity for mucous membranes. Procaine is reputedly inactive on surfaces, but this is incorrect: since procaine solutions, compared with lignocaine, are not stable for long at pH values beyond 5, the surgical solution has a pH of less than 5, and *this* solution does not anaesthetise mucous surfaces. If, however, the pH is increased to 7 or more, the solution has such an increased effect on the surface that it even surpasses that of an equal concentration of cocaine.

Gray and Geddes[113] stressed two important features. The pH requirement varies with each drug and its concentration, since better precipitation of a free base occurs with a strong solution than a weak one. With inflamed tissue, the increased vascularity of the area as well as pH may lessen the effect.

Since the body represents one of the best systems of buffers than can be obtained, the effect of pH upon the mechanism of local anaesthesia and its influence upon the availability of the active form of the drug deserves serious consideration.

Differential Effects

That local anaesthetics elicit effects in various tissues has already been stated, but further examination of this is desirable.

Nervous tissue. It is well known that local anaesthetics can produce sensory anaesthesia without motor paralysis. Gasser and Erlanger[16] suggested that thresholds vary amongst sensory fibres, so the time for blocking depends on the functions mediated. They claimed that small fibres are usually blocked before large ones, but blocking is not effected with any precision. As small fibres have a relatively greater surface area than large ones, the smaller the fibre the greater should be the accessibility. On this basis washing should allow the smaller fibres to recover first: this is not so, and recovery proceeds in the reverse order to blocking. They justified their theory, however, by saying that in small fibres protoplasmic chemical combination with the anaesthetic goes far beyond the point of block into stages of disorganisation: on washing re-organisation is necessary before recovery can begin. Such a mechanism should cause fibre block on a systematic size basis: as this does not occur their explanation can only be a partial one.

Heinbecker, Bishop and O'Leary[114] demonstrated that local anaesthetics first block action potentials in unmyelinated fibres, and then the smallest myelinated ones, progressing up to the largest myelinated ones. Obviously

some structural modification tends to delay blocking in the second two types, provided that it is accepted that local anaesthetic efficiency is fundamentally the same for all nervous tissue[15]. Myelin is immediately suspect, and will be considered later. Hirschfelder and Bieter listed fibre activity loss as, vasoconstriction, temperature, pain, touch, joint and pressure sense. With intraspinal injection the curious fact emerged that motor activity disappeared before joint and pressure sense. This may be because, intraspinally, motor nerves are more bare and hence more vulnerable.[73] This concept is questionable: a more likely factor is that of accessibility to the, unmyelinated, motor nerve cells. Frumin and others[115], demonstrated that dorsal root blockade can be achieved with procaine doses which are too small to affect radically the passage of impulses in the root. They ascribed this to the lack of myelin on the ganglion cells which they therefore assumed to be more sensitive than either the dorsal root fibres or the spinal cord. In clinical doses motor paralysis frequently occurs, suggesting involvement of the ventral root as well.

There have been reports of block in A and B fibres before C fibres with KCl, anoxia, and the nerve narcotic phlorizin[17] as well as with cocaine[49]. There is no reason to doubt the accuracy of these observations, and it is apparent that there is more than one way of producing block, and even differential block.

Löfgren[36] pointedly remarked that local anaesthetic action is readily compared on isolated motor nerves, but that conditions for practical anaesthesia, sensory block in a complicated biological milieu, are difficult to survey in detail.

Toman[49] reasoned that different fibres might act by various chemical mechanisms, so long as the general explosive system occurs, and this would naturally allow different types of block to exist. The flaw in this view is the apparent lack of specificity, for types of nerve, of blocking agents, and the general conformity of nerve to the current transmission theory.

Barlow[40] considered that there might be different enzyme systems in sensory and motor fibres. He implied that the susceptibility of the particular enzyme system determines the rate of production of nerve block. He saw no reason why transport at mucous membranes and nerve surfaces should be identical, hence a local anaesthetic may be feeble on the eye, but quite active elsewhere. This may well be due to strictly local factors such as pH or cholinesterases.

Muscle tissues. In myasthenia gravis, procaine may sometimes accentuate the general muscular weakness, an observation which led to the idea that it either affects neuromuscular transmission or the muscle directly.

MacGregor[116] used both procaine and cocaine in skeletal muscles *in vivo*, and remarked a reduction in tension with both, cocaine having the more powerful effect. As pre-curarisation potentiated the effects it was considered that local anaesthetic and curariform actions are similar. MacGregor suggested that local anaesthetics may directly reduce excitability or contractility of muscle fibres, and considered that the action is

partially upon the motor nerve endings, and partially directly upon muscle fibres.

Sollman and Estable[117] investigated the action of procaine on the excitability of frog muscle and nerve tissues. They claimed that the depression is reversible only within rather narrow limits, and also that it depresses the excitability of skeletal muscle nearly as much as that of motor nerve fibres (given effective penetration, which depends on prolonged exposure to the drug). They concluded from this that nerve depression by local anaesthetics is not a distinct phenomenon but a manifestation of general "protoplasmic" depression, which is useful in practice because of the favourable ratio of anaesthetic action to local irritation and systemic toxicity. They felt that clinical practice contributes to the specialisation effect by ensuring that drug and nerve are in close contact. Procaine hydrochloride in relatively high concentrations, over quite long periods, induced irreversible paralysis in excised muscle and nerve: clinically this is not attained, because lower doses are used and the drug is removed by the circulation. Attempts to induce irreversibility in animals have failed indicating the wide margin of practical safety with procaine. The danger of comparing *in vivo* and *in vitro* results too closely is again apparent.

It has been claimed[118] that afferent proprioceptive fibres from muscle spindles are highly procaine-susceptible. However, Matthews and Rushworth[119] showed that procaine paralyses the large afferent and efferent fibres of the soleus muscle simultaneously, but the γ-efferent fibres, to the intrafusal fibres[120], much earlier. In a single afferent fibre from a frog muscle spindle, Matthews and Rushworth[121] claimed that there is a two-stage response when cocaine is applied to the nerve supplying the muscle. First, the frequency of spindle discharge falls suddenly to a new level similar to the one after ventral root section, apparently due to γ-fibre paralysis, and, second, the spindle afferent itself is affected, and before its complete paralysis will not transmit high frequency impulses. This appears to accord with the findings of Katz[29]. Procaine and cocaine therefore produce a reversible γ-de-efferentation of the intrafusal muscle fibres. Such evidence opposes the concept of direct muscular excitability depression, and emphasises that the situation is not as simple as was once thought.

Other factors. Action sites may be significant, because in excised nerve the anaesthetic soaks inwards from all points of the circumference, whilst in man, as probably in all *in vivo* experiments, a concentration gradient may exist across the width of the nerve. The situation of the nerve, and the site of drug application may well be the cause of this. Allied to the latter, Henderson[52] has suggested that the various differentials between tissue affinities for anaesthetics might be largely attributable to blood flow differences. This is certainly a possibility, and one that is known to be important for general anaesthesia.

Onset and duration of activity have been related to adsorption equations[52], but it is likely that they are related to concentration of the drug, time of contact, and the surface area of the exposed region. Kato[122] considered that the minimal effective concentration (C_m) of local anaesthetic

for a single fibre differs little from that of a nerve trunk, and this would be unlikely if adsorption were the basic cause. If a fibre is exposed to an anaesthetic slightly above its C_m value, block sets in almost immediately. From this Löfgren[36] decided that diffusion into the nerve is critical in onset of block. Good local anaesthetics should possess high activity coupled with a high diffusion coefficient: the criteria of usefulness are latency, duration, activity, and toxicity.

Anaesthetic Structure and Properties

A general recapitulation of evidence which has already been quoted is not proposed, but rather a consideration of any especial features which seem to relate to the present problem.

Löfgren[36] stressed that the common clinical local anaesthetics have a typical composition. They are pronounced basic esters or amides of aromatic carbonic acids. Their general formulation runs,

amino group—intermediate chain—aromatic residue.

The amino group, usually secondary or tertiary, is of great importance for specificity, and he claimed that virtually no usable anaesthetics omit this group. He explained some exceptions as a replacement of the hydrophilic amino group by another hydrophilic group, like hydroxyl, as in benzyl alcohol. Substitution of the aromatic residue by an aliphatic one results in a considerably inferior effect. Löfgren envisaged the hydrophilic group of the anaesthetic to make contact with a suitable polar hydrophilic group in the membrane (polar or "head" association), while the lipophilic part probably helps to form the complex (the "tail" acts on the membrane film by van der Waal forces) so that penetration can occur. Because of this Löfgren was not surprised that local anaesthetics do not follow the Overton-Meyer rule, since the distribution coefficients are important, but not dominantly so. Although many narcotics have local anaesthetic properties, the typical aromatic amine type of local anaesthetic cannot be used for narcosis. They are purely local, even on central nervous organs[39]: moreover, dosage increase does not give general anaesthesia, but only systemic poisoning as a toxic level is reached. In doses of a quarter to one half of the lethal one, local anaesthetics usually produce (cerebral) convulsions: there is a rough parallel between anaesthetic activity and convulsive power[123]. Benzyl alcohol produces an even narcosis, without convulsive activity, and Löfgren believed that this is attributable to the hydrophilic hydroxyl group.

Surface tension effects have been implicated in both local anaesthetic activity and irritancy. Luduena and others[51] investigated all three aspects with several local anaesthetics, and finally concluded that, although local anaesthesia may be independent of surface-tension lowering activity, the irritancy which can be caused by these drugs is caused by this, or a related, mechanism. Irritancy is a difficult thing to estimate, especially as that related to pH effects may be modified by the buffering action of the tissue fluids. The whole question must await further investigation. The same authors envisaged that local anaesthetic activity may result from a very

high affinity for some specific structure in the nerve fibre. Some characteristics may increase the activity of the molecule without modifying the physico-chemical affinity, for example the presence of a long carbon chain increases the lipid solubility of the molecule, provided that it does not interfere with the attachment of the polar group, or groups, in the drug, to the receptors (that is, the hydrophilic end).

Butler[2], however, declared that no specific chemical structure is necessary for general anaesthetic activity: he claimed that in the aliphatic alcohol series anaesthetic activity is almost equally correlated with vapour pressure, oil:water distribution coefficient, surface activity, and water solubility. Höber[58], too, had similar views, claiming that narcotics are chemically "indifferent", and that they do not react chemically with cell components.

The over-riding impression about structure-property relationships is that, super-imposed on the basic "local anaesthetic structure" of the molecule there is a series of structural modifications which may influence, directly or indirectly, the local anaesthetic effect.

Structural Features of Nerve

The modifying effects of the connective tissue sheath have been examined in an earlier section, but an observation by Lorente de Nó[17] requires comment. He claimed that the penetration of connective tissue sheath and nerve by veratrine-like substances is exceedingly rapid, and regarded this as evidence in favour of lack of sheath resistance. But Shanes[63] has described veratrine as a labiliser, that is to say, it destroys the permeability of the membrane, hence speed of penetration here is a characteristic of veratrine and not the sheath.

Another structural feature is myelin. Its insulating properties have been known for some time, and comprehensive work by Kato[122] has clearly endorsed the concept of saltation. Furthermore, studies on single fibres revealed a striking phenomenon, namely that nerve conduction can be blocked instantly by a drop of relatively dilute narcotising drug, or of isotonic sugar solutions, or even of distilled water, when applied to a region where nodes of Ranvier are exposed. If the sheath alone was exposed to cocaine or urethane in Ringer, conduction was often retained for well over an hour, but if a node was similarly exposed conductivity disappeared within one second. Kato concluded that narcotics diffuse into the axis cylinder only through the nodes, and spread in both directions along the fibres. Sub-threshold concentrations produced a sudden change in threshold, reducing excitability, and recovery on removal was similarly abrupt.

Lorente de Nó[17], however, categorically denied that myelin is an obstacle, and rejected the idea of substances acting on the nodes. He felt that such an assumption would be justified if test substances acted first upon myelinated fibres, and quoted some that act first on myelinated ones, none of which, it should be added, were local anaesthetics.

In his comprehensive treatise Löfgren[36] recapitulated Kato's views fairly fully, and the fact that he had no criticisms to make cannot be ignored. Lussier and Rushton[124] confirmed Kato's work, and produced

evidence that nerve threshold is about 30 per cent higher at the mid-internode than elsewhere. From this it is plain that myelin must not be mistaken for a perfect insulator, although its influence is great.

The fact that many nerves are not uniformly susceptible, owing to structural features, is something which may well influence local anaesthesia, if the word "local" is interpreted in its strictest sense.

Problems which arise

Inevitably, the primary question relates to the validity of applying theories of general anaesthesia to local anaesthesia. The answer is relatively simple: there is little choice, as all attempts have aimed at translating these theories into local anaesthetic terms, rather than formulating special theories *per se*. This is not altogether unreasonable, since the basic material in both instances, brain and peripheral nerve, is nervous, although in different locations.

The looseness of the terms used is, however, unfortunate, for example, "narcotics" may include hypnotics, general, and local anaesthetics, hence caution has to be exercised in much of the interpretation.

The thesis presented here is that local anaesthesia is not explicable in terms of any one theory, not from any particular inadequacy of these, but because it is considered that anaesthesia can be produced in different ways.

Conduction failure may arise from increased threshold, fall in spike amplitude, or sub-critical resting potential. The last implies that the normal ionic balance is lost: this depends on metabolic processes as well as ion concentrations, diffusion gradients, and membrane penetrability. Interference with any of these factors is potentially capable of producing block.

Toman[49] has rightly remarked that the mechanism of nerve conduction does not fit into any of the schemes devised for ganglia and neuro-effector systems. This again underlines that results and theories must not be too readily transposed from one part of the nervous system to another.

The present discussion is divided into two sections. In the first, features which have limited either results or theories will be examined: in the second, an attempt will be made to resolve various topics which appear to be significant in this field.

Limiting Factors

The effect of pH. The practice of potentiating local anaesthetics with alkali indicated that the two are related, provided that the drug is a basic one; the neutral compound, benzyl alcohol, is not influenced by hydrogen ion concentration. The contention by Sollman[103,104], that alkalisation increases the effect on sensory fibres less than on motor ones is curious: it may well be that the drugs were more effective on the former in the first place, and this could easily limit further improvement.

The buffering by the body is obviously important, and it is impossible to emulate such conditions in isolated tissues. The lack of adequate pH

control may have influenced the results of many experiments found in the literature, although workers like Löfgren and Skou have stressed the need to minimise variations in such tissues by keeping the pH constant. Löfgren suggested that in some of the common local anaesthetic test methods, an alteration of one pH unit may change the minimum effective concentration value tenfold. The link between pH and procaine stability, instanced by the same author is interesting. Earlier, Bullock and Cannell[125] showed that at pH 4·3 about 2·5 per cent of the drug is available, whereas at 7·5 about 75 per cent is available.

Finally, Höber's statement[58] that the paralytic effect increases with rising pH in local anaesthetics, is independent of pH in the alcohols, and decreases with increasing pH in general anaesthetics of the barbiturate type, is most notable in view of the close parallels sometimes sought between the three classes.

Species differences. That these exist is undisputed, for example the observation[92] that equivalent local anaesthetic mixtures are more toxic to man than to guinea pig. The gap is especially wide in places, stretching as it does across the division between invertebrates and vertebrates. The surprising thing is that invertebrate results, such as those of Lillie[126], which formed the basis of the permeability theory, have been successfully applied to many other animals. On the other hand, Krnjević has somewhat disturbingly suggested that actions in frog preparations may vary in animals obtained in the autumn from those found in frogs taken in the spring[21]. Quite evidently the application of observations from one animal to another is accompanied by considerable uncertainty.

Tissue differences. Much the same limitation occurs here, too, despite the claim by Lorente de Nó[17] that the electrical phenomena, at least, in muscle and nerve are identical. Shanes[63] believed that vertebrate muscle is more like invertebrate nerve, especially crab, than vertebrate nerve. The claim that anaesthetic action on skeletal muscle and motor nerves, given effective penetration[117], is comparable, is not too serious: the authors admitted that muscle has to be well soaked, and formulation of such conditions must damage the comparison. Moreover, penetration is probably of less importance in sensory nerves and, in particular, the unmyelinated pain fibres. The difficulties are well illustrated by the anti-acetylcholine activity displayed by local anaesthetics in rabbit intestine and ear vessels, whereas extremely variable results, ranging from antagonism to synergism, were forthcoming from cardiac muscle of the same animal[77].

The effect of drugs like procaine on muscle γ-efferent nerves[121,127] may lead to further confusion, as this nervous effect could be misinterpreted as a muscle reaction.

Tissue distortion. This is an important factor, and much of the evidence about the connective tissue sheath and the barrier effect of myelin has already been described. Lorente de Nó's claims, based on the effects of veratrine[17] must inevitably cast doubts about the validity of his arguments in this sphere, and, particularly, his view that myelin is not a barrier to penetration.

Recently the proofs of distortion of results by nerve connective tissues have been listed by Shanes[63]: the presence of such factors must surely affect drug action, in particular its onset and reversibility of action.

The experimental phase used. Differences between local anaesthetics *in vivo* and *in vitro* were tentatively suggested in 1930[57]. The evidence[117], quoted earlier, that procaine can cause irreversible paralysis in excised muscle and nerve, whereas, *in vivo*, the safety margin is unlimited, powerfully supports this.

Watts[83] has claimed that the results of oxidation inhibition by various local anaesthetics on a brain homogenate show a correlation between *in vivo* and *in vitro* results. However, the discrepancies in creatine phosphate levels with central narcotics, quoted by Barlow[40], were only remedied by electrical stimulation of the isolated material. It is hard to see why local anaesthetics apparently worked better on brain oxidations than central narcotics: practical usage does not reflect these results. It could be that, unlike peripheral nerve, brain is unconcerned with phosphate metabolism.

Welch and Bueding[128] have claimed that the only enzyme action seen *in vitro*, which has been confirmed *in vivo*, is that of eserine. This is an assertive remark, but the gulf between the intact animal and isolated tissue has frequently been confirmed. Nevertheless, to preclude results on these grounds would eliminate most of the information there is, hence they must be utilised, but with reserve.

Homologous series. The convenience of using related compounds is undoubted, especially to minimise errors from factors such as differences in diffusion and detoxication rates[129], and they have often been employed. This is perfectly acceptable, and it is not surprising that such series have shown some regular features which allow predictions to be made (for example concerning the Overton-Meyer theory[39]), or which show certain correlations (thus, between surface activity and local anaesthetic potency[30]), but their use must stop there. It is wrong to carry conclusions from them to unrelated substances. Luduena and others[51] admitted that the surface tension relationship, cited above, exists in homologous series, but they emphasised its absence when comparing compounds with radically different structures. This must be true of the wide variety of substances embraced by the term "local anaesthetic".

Use of models. Models have been used frequently, the most famous one being Lillie's iron wire. The Overton-Meyer theory was derived from a simple one which has been criticised. The difficulty of constructing models of nerve has been emphasised[17], not merely in creating the concentric layers of axon, sheath, and external medium which are involved, but in giving it a resting membrane potential: it has to be dynamic.

Notwithstanding, models have been, and must be, an important, and sometimes the only bridge between a theory and the complete tissue or system.

Temperature. Löfgren[36] felt that the effect of temperature should be considered, although rating it of less consequence than pH. It is certainly important in the contemporary topic of freezing anaesthesia. Little

work has been done on the mode of action of low-temperature anaesthesia, though Lorente de Nó has claimed[17] that hypothermia, oxygen lack, and depolarising agents all act identically on action potential. No conclusions can be drawn from this electrical reaction alone, and the possibility exists that refrigeration produces its effect in a similar fashion to the widely quoted means of obtaining local anaesthesia (often accidentally produced) of anaemia. The latter presumably arises from metabolic effects, although not necessarily direct ones upon aerobic mechanisms: van Harrevald and Christensen[130] have suggested that a slow depolarisation is produced, owing to a reduced metabolism which cannot maintain the membrane potential. Alternatively, the effect may be related to van't Hoff's Law, producing a physico-chemical activity depression to subfunctional proportions. However, Shanes[63] has prescribed an increase in membrane rigidity, and this seems to be a more likely approach. It must not be overlooked that the effect of temperature, *per se*, is potentially important, apart from variations in it produced deliberately.

Anaesthetic ratings. Potency ratios are often used in these studies. These are based on several test methods, some involving minimum effective concentration, others effective duration, and yet others, latent period. Such lack of uniformity has been criticised[36,131] and must surely influence quantitative results.

Qualitative ratings, too, are important, as few drugs have a single effect. Atropine, procaine, quinidine, pethidine, and papaverine have been listed[30] as having the following common properties, local anaesthetic, spasmolytic, analgesic, cardiac retardation, and anti-acetylcholine effects, in a number of tissues. Each drug has one property highly developed at the expense of the others but, even so, such multiplicity may lead to confusion. These remarks certainly apply to other drugs as well, and the view that benzyl alcohol is a general, rather than a local, anaesthetic[123] illustrates this point clearly.

Procaine. Despite its wide use experimentally, there are grounds for doubting whether procaine can be regarded as a standard local anaesthetic. For example, it can be injected intravenously, a property shared only by lignocaine, although, from the literature available (including [36,132,133]) it seems that lignocaine acts in a "normal" local anaesthetic fashion in other respects.

Other experiments have been described[78,134,135] pointing to different behaviour by procaine compared with other local anaesthetics. Toman has especially underlined[49] its activity on muscle tissues which seems to be more highly developed than is usual in local anaesthetic drugs. Examples of this have been instanced above, as also its anti-acetylcholine activity. Toman remarked that death from overdosage is usually caused by cardiac arrest.

Evidently, many limitations restrict the results of local anaesthetic researches. It is problematic how much weight attaches to each aspect, but it is clear that, if taken to their logical conclusions, virtually every result would be invalidated on one score or another: that local anaesthetic

theory does show some relation to practical activity of the drug indicates that this must not happen. Rather, the results must be taken and used cautiously in lieu of further proof, which, it is to be hoped, will be forthcoming.

Features for Resolution

It remains to be seen if any points can be resolved, within the limits of available information, which bear upon local anaesthesia. An attempt will be made to answer certain definite questions.

Is Local Anaesthesia a Surface Activity?

Lillie's nerve model showed characteristic surface changes during "activity", and the membrane has been named as a key site in transmission[13,30,36,63]. Consequently, it is a likely situation for drugs to act. The attempt[16] to relate differential blocking effect to fibre size is an expression of a belief that surface area, at least, is important. Several of the theories of anaesthetic action are linked with surface phenomena and the important work by Bennett and Chinburg[62] cited the cell membrane as the probable site of action of local anaesthetics.

Velocity of reaction, too, suggests an effect at the surface, and this has aroused comment with both local anaesthetics[18] and with calcium ions[136]. Finally, with many local anaesthetics, the permeability theory of nerve transmission is worthy of very close attention, and its relation to activity at the surface is undisputed.

On balance, therefore, it is probable that many local anaesthetic drugs act at, or around, the surface of the cell.

What is Known About the Membrane?

It has been conjectured that the membrane need not be anatomically distinct, but could be a fluid entity[17]. However, as early as 1932 it was envisaged as a continuous layer of fat, and alternative suggestions of an emulsion containing fat and protein[15], and a strongly organised lipoprotein film combined with metal ions[36], have been put forward. Furthermore, von Muralt[137] claimed to have photomicrographed the membrane in ultra-violet and polarised light.

Shanes[63] has recently emphasised that myelin sheaths, Schwann cells, and connective tissue sheaths are relatively rigid, and must not be mistaken structurally for the membrane which is found only at the nodes. Notwithstanding he cited evidence that myelin and the physiological membrane have similar dimensions, comparable electrical characteristics, are depolarised by KCl, and, significantly, both react similarly to local anaesthetics[138,139]. Skou[43] used local anaesthetics to demonstrate that stabilisation is associated with a tendency of the lipid phase of the membrane to expand: myelin and nodal membrane behaved like each other. Shanes quoted studies with X-rays and polarised light indicating that the lamellar structure of the membrane is essentially the same as the physiological membrane, that is a double layer of lipid molecules, perhaps

bounded at each aqueous interface by a layer of protein[140]. The active membrane is relatively thick, probably being up to about 100 Å.

Barlow's[40] comment that neurilemma contains fat provides a timely reminder that myelinated CNS fibres have no neurilemma, in contrast with similar peripheral fibres. It is probable that most work on peripheral nerve employed medullated fibres, and it is consequently an unavoidable inference that the possession of a neurilemma must be regarded as a factor, distinguishing central from peripheral nervous tissue, which could influence drug actions, no matter whether they are general or local anaesthetics.

What is the Rôle of Myelin?

That myelinated fibres are most excitable through the nodes has been clearly demonstrated. The threshold of stimulation is up to 30 per cent higher in the mid-internode region, and it is thus clear that myelin exercises some form of protective function, apart from any acceleratory one with which it invests nerves. It is therefore to be expected that unmyelinated nerves should be more susceptible to drug action than myelinated ones.

What Causes Differential Effects?

Evidence for differential block has been advanced, and this is important in view of the present concept[31,32,120] that receptors can signal stimulus intensity by means of impulse frequencies. The block of high frequency impulses would surely lead to differential effects, and Bennett and Chinburg[62] attempted an explanation of how this block occurs. Moreover, Granit[120] has claimed that the sensations of touch and pressure are merely distinguished by the numbers of stimuli involved, and he also asserted that warmth and cold are identified by patterns of impulses. The scope for "selective" effects thus appears to be quite wide.

The work by Katz[29] is of interest because he reported that the action of procaine upon the receptors showed the normal blocking effect without any depolarisation. It raises the possibility that the sensory arc may be more sensitive to local anaesthetic in the vicinity of the receptor than in its nerve. Certainly it should be borne in mind that many local anaesthetic injections are made into regions, particularly subcutaneous ones, which are rich in receptors. Even with medullated nerve there is bound to be a gap between the myelin sheath and the receptor itself: it is quite possible that at this gap, and perhaps the receptor itself, are points of hyper-susceptibility to anaesthetics.

The cases of preferential blocking of A and B fibres[17] may have two explanations. These faster conducting fibres may be more susceptible to metabolic upset, for example to oxygen lack, than C ones, or Toman's theory[49] of different conduction mechanisms may be substantially correct. However unacceptable the latter appears it must be admitted that central and peripheral neurohumours vary, just as nerve conduction and end-plate transmission probably differ. The fact that synapses are more

susceptible to drug action than nerve cells, and neuromuscular junctions less than either[39] lends some support to this.

In all the results, it should be remembered that it is easier to obtain normal reactions from motor than sensory nerves, and this must inevitably colour the conclusions.

Finally, the results of Forbes and others,[141] deserve mention: they claimed that, in general anaesthesia, electrical representation of sensory stimuli reaches the cortex with undiminished, or even augmented, intensity, but due to the depression they are not recognised. Clearly the drugs involved were in no way local anaesthetic, and if this is substantiated it must raise doubts about the activity which is thought to be common to both local and general anaesthetics, although action sites are obviously implicated.

What is the Influence of the Structure of the Drug?

The importance of structure was appreciated early[142]. Various features are associated with drug action phases.

The union of the drug and the receptive surface. Löfgren[36] has clearly shown that the possession of a hydrophilic grouping in the molecule is of great importance, although excessive hydrophilic properties may produce interaction with the membrane and different anaesthetic activities as a result[39].

Lipid solubility characteristics of the molecular have been remarked[51], but they are clearly secondary to hydrophilic properties.

Its fate there. Some sort of blanketing effect has been suggested as many drugs act in the form of undissociated molecules[94]. The largest body of opinion favours the concept of hydrolysis of the drug.

Its destiny thereafter. Assuming that changes of a hydrolytic nature occur, the free base commands attention. Increased anaesthetic activity with alkalisation supports this[100], and the relationship has been recognised, to some degree, at least, in muscle and nerve[15,51,101,116]. On balance it seems quite clear that the importance of the free base lies in its penetrant powers into the nerve. A different view, based on procaine, is that the cation is involved[67]. It is likely that this conclusion was based on an anomalous observation, but it serves as an introduction to the concept[109] that the efficacy of local anaesthetics depends on cations acting intracellularly. The loss of potency accompanying stabilisation of the cation[110] strongly supports this view.

How Important is Penetration?

It was early suggested that rates of action and diffusability might be as important to local anaesthetics as to other drugs[143]. If local anaesthetics act by film penetration they might display some haemolytic activity. Gessner, Walter and Reinhardt[144], investigated about fifteen of them: all produced haemolysis, and a fair correlation was obtained between local anaesthetic potency and haemolytic power. Since then penetration has been cited frequently, and significantly, in connection with various effects including surface tension[46], adsorption[145], anticholinesterase[76],

potassium synergism[15], and permeability[63], the last author stating that the high lipid solubility of many compounds used in narcosis is consistent with this view. Skou[111] has claimed that the anaesthetic content of nerve lipid is directly proportional to the free base, but that the cation may have a part to play. The view, expressed in 1955[146] that, once through the membrane, the free base dissociates and acts on axoplasm seems to accentuate the importance of penetration, and to link this section with the preceding one.

Conclusions

Various theories have been said to be valid for local anaesthesia. Most authors appear to have recognised the shortcomings of such a policy: the danger lies, not in applying a theory to local anaesthesia, but in taking results from general anaesthesia of the central nervous system and applying these indiscriminately to peripheral nerve. It has rightly been said . . . "most axonology seems considerably removed from problems of central nervous function, except by broad analogy"[49]. This statement is not upset by the observation that, just as general anaesthesia is inexplicable by one theory alone (and the use of such diverse agents as progesterone[147] and xenon[148] amply confirms this), the same is applicable to local anaesthesia. Many substances can stabilise the membrane or inhibit metabolism, but are not local anaesthetics. On the other hand, many local anaesthetics seem to act in ways explicable in terms drawn wholly, or partially, from several theories: that the same theories are not named each time strengthens the belief that various mechanisms are involved, and block by such dissimilar agencies as refrigeration and oxygen under high pressure[149] confirms this.

The fact that a model, or isolated tissue, is no substitute for the living cell or tissue in its natural surroundings is inescapable: that models, at times, provide information which would be entirely lacking otherwise is true, but, as with general anaesthetics, they must not be used as a basis for generalisations about local anaesthesia.

Finally, it is possible to compile a list of salient features, most of which have been fairly clearly proved.

1. The scientific investigation of local anaesthesia and its practical application are often unrelated. A striking example of this is the effect of hydrogen ion concentration upon the action of procaine at mucous surfaces.

2. The current views on nerve impulse transmission give added prestige to concepts of nerve block being caused by changes in permeability. It is worthy of note that many local anaesthetics stabilise membrane conditions.

3. That some agents block by depolarisation is undeniable, but as the nerve impulse is accompanied by a wave of depolarisation, it is clear that, if "conventional local anaesthetics" behaved in this way, their action would be preceded by a stimulatory effect. This is not borne out in clinical practice.

4. From studies of conduction it is to be expected that inhibition of metabolism may ultimately cause block, but it is a fairly slow process, owing to such factors as the anoxic reserve.

5. Permeability or metabolic effects occur at, or in, the surface, and this must be partially governed by lipid solubility (Overton-Meyer theory), and also by adsorption (Warburg theory).

Sooner or later the drug penetrates the cell, and in most local anaesthetics, of the basic type, the free base is the agent which achieves this. Owing to this action of the free base, the pH of the anaesthetic solution, and the modifying effect of the surrounding medium, are important in the efficacy of the drug.

There is a growing body of evidence that, after securing penetration into the cell, the free base is converted into the cation, which is the true "nucleus" of anaesthetic activity.

6. Considerable variation in drug activity is caused by impedance from connective tissues around the nerve fibres. This, coupled with site of injection, and pH, may explain many of the delays in onset of drug action. Myelination constitutes a further barrier to drug action, as the drug has to effect an entry first at the nodes of Ranvier. It effectively ensures that, under normal conditions the unmyelinated fibres, including the pain ones, block first. Furthermore, although it is not vital to the present study, the peculiar susceptibility of the nodes of Ranvier to drug action lends support to the saltatory theory of nerve conduction.

7. Further differentiation is provided by partial blocking, which disrupts some impulse frequencies in nerves, whilst leaving others unchanged. By virtue of this, certain intensities, and perhaps patterns, of sensation may be selectively eliminated before other ones.

There is some evidence for preferential local anaesthetic effects in the vicinity of the sensory nerve ending, that is, at the receptor, or immediately adjacent to it. This could well allow a further differentiation of effect if some endings are more susceptible than others.

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RESEARCH PAPERS

THE CRITICAL MICELLE CONCENTRATION OF CETOMACROGOL 1000

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Received January 12, 1960.

The critical micelle concentration of cetomacrogol has been determined by the iodine and solubilisation methods, and also from surface tension measurements. Results from the three methods were 0·0070, 0·006 to 0·008, and 0·0063 per cent respectively. The presence of sodium chloride in the solutions decreased the critical micelle concentration. From surface tension data, an area per molecule at the air:water interface of approximately 120Å^2 was calculated.

Non-ionic surface active agents generally form micelles at low concentrations. This, together with their non-conductance in solution, has led to some difficulty in determining their critical micelle concentrations (CMC), as the solutions are so dilute that only small differences in physical properties are observable in the immediate pre- and post-CMC regions. Recently, a new method has been used by Ross and Olivier[1] and by Becher[2] for the determination of the CMC of non-ionic detergents. The method is applicable at very low concentrations.

Cetomacrogol 1000 is a non-ionic detergent of general formula $CH_3[CH_2]_{15 \text{ or } 17}OCH_2[CH_2OCH_2]_{19-23}CH_2OH$. In this work Ross and Olivier's method has been used to determine the CMC of this detergent in water and in sodium chloride solutions of various concentrations. Values of the CMC have also been obtained from solubilisation and surface tension experiments.

Experimental

Materials. The cetomacrogol used was a commercial sample based on cetyl alcohol. The molecular weight calculated from the above formula lies between 1121 and 1297, depending on the number of ethylene oxide residues in the chain. The measured molecular weight, from freezing point depression in benzene was 1210. The sodium chloride, iodine, and dimethyl yellow used were Analar materials.

Iodine method for determining CMC[1]. Spectra were measured on a Hilger and Watts' Uvispek spectrophotometer, and certain of the optical densities on a Unicam SP600 spectrophotometer. A stock solution of iodine was made up to contain 30 mg. $l.^{-1}$ iodine, and this solution was used to dilute a 0·1 per cent solution of cetomacrogol containing the same concentration of iodine. After a rough experiment to determine the CMC approximately, solutions were prepared to contain detergent concentrations close to the CMC. The optical densities of these solutions were measured in 1 cm. cells at 360 and at 390 mμ. By plotting optical density against detergent concentration, two straight lines were obtained

which intersected at the CMC (except for concentrated sodium chloride solutions, see later).

Solubilisation experiments. Dimethyl yellow was shaken with solutions of cetomacrogol of varying concentrations for 14 days at 20°. The solutions were centrifuged, 5 ml. samples withdrawn and diluted to 25 ml. with absolute ethanol, and their optical densities measured at

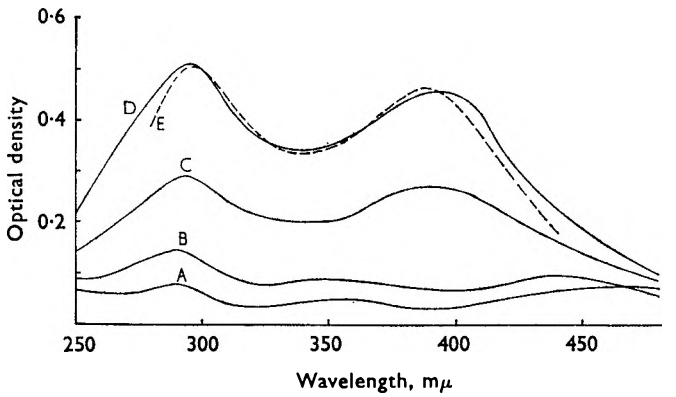

FIG. 1. Spectra of solutions containing iodine 30 mg.l.$^{-1}$ and varying concentrations of cetomacrogol.

A. Iodine alone.
B. Iodine + 0·002 per cent cetomacrogol.
C. Iodine + 0·01 " " "
D. Iodine + 0·02 " " "
E. Iodine + 0·02 " " " + 0·6667N sodium chloride.

413 mμ, according to the directions of Kolthoff and Stricks[3]. To ensure that equilibrium was attained, the more concentrated solutions were treated in this way after 5, 8, 11 and 14 days. The measured optical density did not increase after the eighth day, showing that the solutions were saturated with dye.

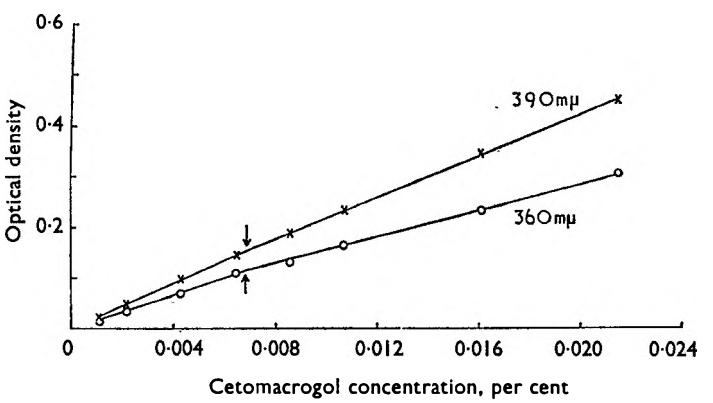

FIG. 2. Optical densities of cetomacrogol solutions in water containing iodine 30 mg.l.$^{-1}$

Surface tension measurements were made using the ring pull method, the usual corrections being applied to the results[4]. The surface tension of each solution was determined repeatedly until a constant result was obtained. Adsorption effects on the walls of flasks were avoided by making up the solutions, allowing them to stand for two hours, discarding them, and draining the flasks thoroughly. Fresh solutions were then made up in the same flasks. The dish which held the solution for measurement was treated similarly. Measurements were made at 20°.

Results and Discussion

In Figure 1 the spectra of solutions containing the same amount of iodine (30 mg.l.$^{-1}$) and varying concentrations of cetomacrogol are shown. The presence of a small concentration of detergent causes a

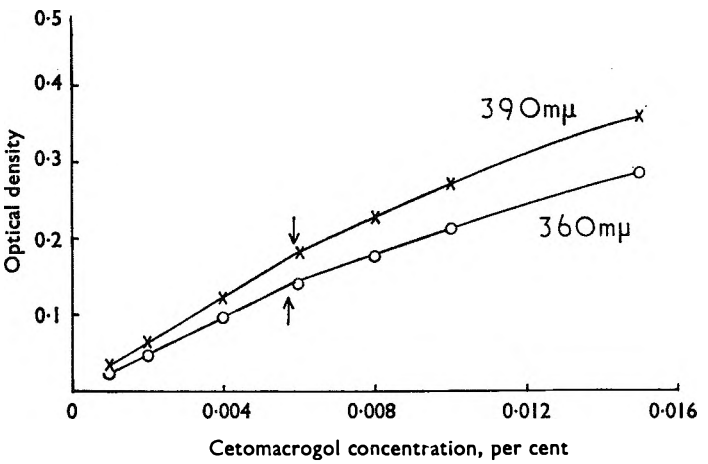

Fig. 3. Optical densities of cetomacrogol in N sodium chloride containing iodine 30 mg.l.$^{-1}$.

large increase of optical density compared with the solution of iodine in water. The curve run for iodine–0·02 per cent cetomacrogol–0·6667N sodium chloride has the same general shape as the curve for iodine–0·02 per cent cetomacrogol. The curves are generally similar to those recorded by Ross and Olivier[1], who found that the spectra of different detergents in the presence of iodine had a λmax at 360 mμ; they chose this wavelength to make measurements for the CMC determination. In the present work the λmax appeared to be at 390 mμ, so measurements were made at both these wavelengths. The values obtained for the CMC were the same, within experimental error, at both wavelengths, e.g., Figure 2 shows that the CMC in water determined at 360 was 0·0068 per cent, while at 390 mμ it was 0·0069 per cent. A further experiment gave 0·0071 per cent and 0·0070 per cent at the two wavelengths respectively. The iodine method provides a rapid means of determining the CMC.

It has been shown[2,5] that the CMCs of polyoxyethylene derivatives of alcohols and phenols can be expressed by an equation of the type:

$$\ln c_o = A + B.R$$

where c_o is the CMC in units of per cent $\times$ 10^4, R is the ratio of the number of moles of ethylene oxide to the number of moles of alcohol or phenol present in the detergent. A and B are constants. A appears to depend on the nature of the alcohol or phenol present, while B depends

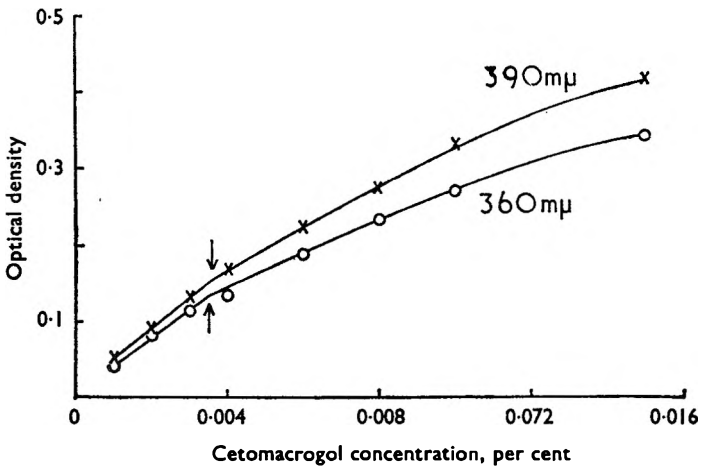

FIG. 4. Optical densities of cetomacrogol solutions in 2N sodium chloride containing iodine 30 mg.l.$^{-1}$.

on the electrolyte concentration of the solution[2]. For polyoxyethylene derivatives of lauryl alcohol, Becher[2] gives the equation:

$$\ln c_o = 3{\cdot}72 + 0{\cdot}038\ R$$

while for these derivatives of stearyl alcohol:

$$\ln c_o = 3{\cdot}69 \pm 0{\cdot}0068\ R$$

Lauryl and stearyl alcohols esterified with twenty-two ethylene oxide units gave calculated CMCs from these equations of 0·0095 and 0·0046 per cent respectively. The observed value for cetomacrogol of 0·0070 per cent is of the correct order as it falls between these values.

The curves for the determination of the CMC in N and 2N sodium chloride solutions are shown in Figures 3 and 4. Although a straight line was obtained for the optical density: concentration plot below the CMC, a curve was obtained above it. This makes the determination of the CMC in concentrated salt solutions uncertain, although the curve flattens considerably as the CMC is approached.

As the salt content of the solutions was increased, the CMC decreased. The effect is small compared with that on ionised detergents; the CMC of sodium dodecyl sulphate in water[6] is 10 mM.l.$^{-1}$, in 0·2N sodium chloride[7] it is 0·8 mM.l.$^{-1}$, a twelve-fold decrease. Cetomacrogol shows a decrease from 0·0070 per cent in water to 0·003 per cent in 2·5N sodium

chloride (Fig. 5). Non-ionic detergents are believed to possess a weak positive charge in aqueous solution[5], and presumably the presence of sodium chloride decreases the charge, thus causing a lessening of the intermolecular repulsive forces which will oppose aggregation of monomers

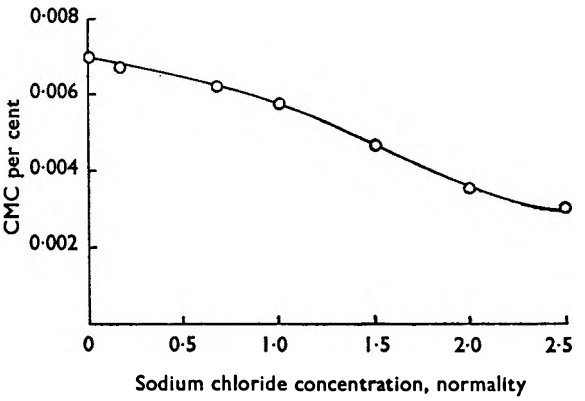

FIG. 5. Variation of CMC with sodium chloride concentration

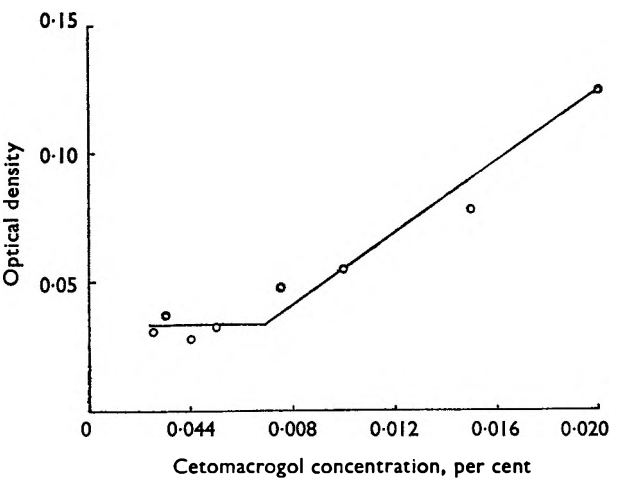

FIG. 6. Solubilisation of dimethyl yellow; optical density versus cetomacrogol concentration per cent. See Text.

to micelles; the CMC will shift to a lower concentration. The effect of salts on cetomacrogol solutions is being investigated further.

A small but roughly constant amount of dimethyl yellow appears to be solubilised in the pre-CMC region (Fig. 6). At concentrations above the CMC, the amount of dye solubilised increases, due to uptake in the micelles. The solubilisation experiments are rather inaccurate, due to the smallness of the optical densities to be measured, and to the difficulty

of removing suspended dye particles from the solutions, even with prolonged centrifugation. From these experiments, the CMC in water can be estimated to be in the region 0·006 to 0·008 per cent, which agrees with the results from the iodine method.

Both these methods involve the addition of a foreign substance to the detergent-water system. In view of this, it was desirable to check the results against a third method where no foreign substance was present. Consequently the surface tensions of a series of solutions were measured. The results are shown in Figure 7 as a surface tension : log concentration

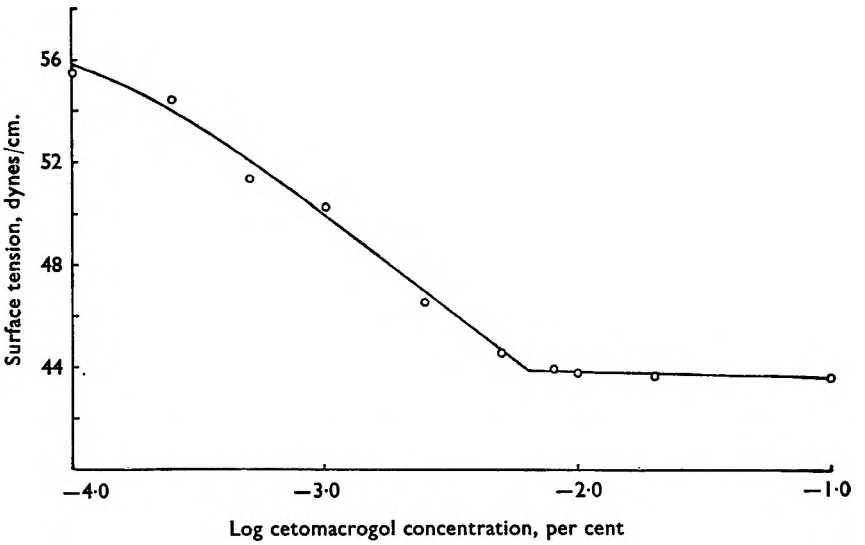

FIG. 7. A plot of surface tension versus log concentration of cetomacrogol (per cent).

plot. As expected the surface tension is almost constant at concentrations greater than the CMC. Below the CMC, the surface tension steadily decreases with increasing concentration, showing that an increasing number of molecules is being adsorbed at the air : water surface. In the region from log concentration of −3 to the CMC, the plot is linear, and the value of the CMC is 0·0063 per cent, in good agreement with the results from the other methods, and indicating that the addition of iodine or dimethyl yellow to the solutions does not affect the CMC appreciably.

From the slope of the surface tension-log concentration graph, the area/molecule can be calculated using the Gibbs equation. In the log concentration −3 to −2·4 region a value of approximately 120 Å^2 was calculated. The structure of the film at this interface is likely to be one in which the chain of polyoxyethylene units lies in the aqueous phase, while the hydrocarbon part of the molecule is forced up above the surface. The area/molecule is greater than the cross-sectional area of the polyoxyethylene chain (roughtly 20 Å^2 from molecular models), leading to the idea that the chain either lies along the surface, or is

considerably curled up. A more detailed study would be required to elucidate the complete structure of the surface film.

Acknowledgements. I should like to thank Professor J. P. Todd and Dr. J. B. Stenlake for their interest, and Glovers Chemicals Ltd. for the gift of the cetomacrogol sample.

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GLYCYRRHETINIC ACID—A TRITERPENE WITH ANTI-OESTROGENIC AND ANTI-INFLAMMATORY ACTIVITY*

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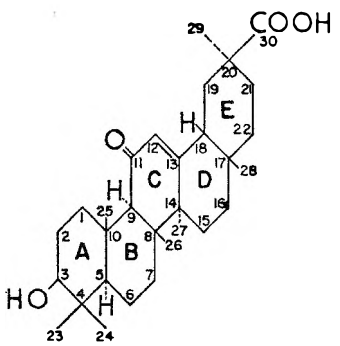
Received January 20, 1960

Glycyrrhetinic acid was found to resemble the Δ^4-3-ketosteroids in antagonising uterine growth response to exogenous oestrogen in doses which did not interfere with somatic growth or the oestrogenic effect on pituitary trophic hormones. The anti-oestrogenic effect was not mediated through gonadal inhibition, adrenal stimulation or potentiation of endogenous adrenal corticoids. Glycyrrhetinic acid is neither androgenic, oestrogenic nor anti-androgenic. However, it does resemble the glucocorticoids in depressing granuloma tissue formation in adrenalectomised rats.

Glycyrrhetinic acid is the aglycone[1] of glycyrrhizinic acid, the constituent of crude licorice extract affecting electrolyte balance[2–4]. Glycyrrhetinic acid is a triterpene[5] resembling allopregnane and androstane in the orientation of the hydrogen atom on C(5). (I). The $\alpha\beta$-unsatura-



(I)

ted 11 ketone group in ring C morphologically relates glycyrrhetinic acid to the Δ^4-3-ketosteroids (testosterone, progresterone, adrenal corticoids). The similarity in chemical configuration between glycyrrhetinic acid and corticoids is probably the basis for the mineralocorticoid activity[4,6,7] and pituitary-adrenal inhibition[2,8] previously reported. Derivatives of glycyrrhetinic acid which had anti-inflammatory action in the intact animal[7,9] did not influence liver glycogen deposition[10].

This report is concerned with the effect of glycyrrhetinic acid on the inhibition of growth induced by a steroid of the oestrane series, an action of the Δ^4-3-ketosteroids[11–14], and the anti-inflammatory activity of the acid in the absence of the adrenal gland.

* A preliminary report was presented to the American Physiological Society, September, 1959, and an abstract of this work appeared in *The Physiologist*, August, 1959, **2**, 72.

METHODS

CFN rats, maintained on Purina chow, were used. Normal rats received water *ad libitum*, while adrenalectomized animals received 1 per cent saline instead. Drugs were administered subcutaneously either in a vehicle of 1 part ethanol and 9 parts sesame oil for β-glycyrrhetinic acid* and glycyrrhetinic acid,† or in sesame oil for the sex hormones.‡

β-Glycyrrhetinic Acid and Sex Hormones

Rats, 22 to 23 days old, weighing 35 to 40 g., were treated for three days. Each rat received two injections (at different sites) twice daily of 0·1 ml. of vehicle with or without β-glycyrrhetinic acid as well as sesame oil with sex hormone. All doses were expressed as the total dose administered over 3 days. 72 to 75 hours after the first dose the animals were killed by decapitation. Organs were weighed on the Roller-Smith balance, and a comparison of relative weights was made by analysis of variance.

The increase in uterine weight in intact and bilaterally adrenalectomised rats (adrenalectomised 1 hour before the first dose) to 0·30 μg. of oestradiol benzoate was determined with and without β-glycyrrhetinic acid. Intact rats received a total of 0·60 to 6·0 mg., and adrenalectomised rats 6·0 mg. of β-glycyrrhetinic acid.

The effect of β-glycyrrhetinic acid on the response of the uterus to oestradiol benzoate was assessed in a 6-point assay. The oestradiol dose response curve of vehicle-treated rats was compared with that for the animals treated with β-glycyrrhetinic acid (6·0 mg.) for parallelism and potency.

Studies were then made to determine whether the anti-oestrogenic effect was limited to the uterus.

Somatic growth of immature female rats which had been individually housed was assessed by weighing the animals before and after the period of treatment. The adrenals, thymus and ovaries were weighed in addition to the uterus. The four groups each of ten individually housed rats consisted of controls, oestradiol-treated (0·30 μg.), β-glycyrrhetinic acid treated (6·0 mg.), and oestradiol (0·30 μg.) plus β-glycyrrhetinic acid (6·0 mg.) treated animals.

The effect of β-glycyrrhetinic acid on testicular atrophy induced by 75 μg. of oestradiol benzoate in immature male rats was also determined. Weights of the testes of controls, oestradiol treated, and oestradiol and β-glycyrrhetinic acid (6·0 mg.) treated animals were compared.

To determine whether the acid had androgenic or anti-androgenic activity, seminal vesicle and testes weights of rats treated with testosterone

* Supplied by S. B. Penick and Co., N.Y.

† Supplied by Dr. T. E. Weichselbaum, Washington University Medical School, St. Louis, Missouri. (m.p. 275°; $[\alpha]_D + 112°$; solubility at room temp., 20 mg./ml. in ethanol.) *See* Carlat, Margraf, Weathers and Weichsel baum, *Proc. Soc. exp. Biol., N.Y.*, 1959, **102**, 245.

‡ Estradiol benzoate and testosterone propionate supplied by Organon Inc., Nutley, N.J.

propionate (1·20 mg.), β-glycyrrhetinic acid (6·0 mg.), testosterone propionate (1·20 mg.) and β-glycyrrhetinic acid (6·0 mg.) were compared with controls.

Depression of Granuloma Tissue Formation in Bilaterally Adrenalectomised Rats

The anti-inflammatory action of β-glycyrrhetinic acid and glycyrrhetinic acid (in total doses of 4·0 and 8·0 mg.) was measured by depression of granuloma tissue formation by the cotton pellet technique[15]. Two cotton pellets (10 to 12 mg.) were subcutaneously implanted in the dorsal region

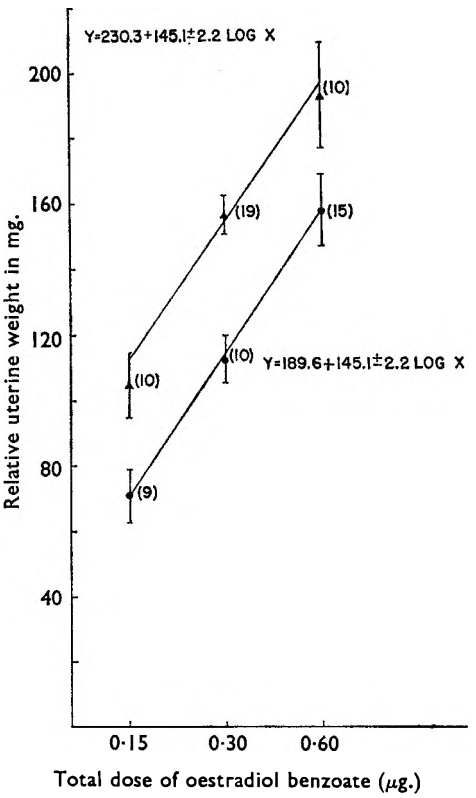


FIG. 1. Dose response curve for oestradiol benzoate in immature rats based on relative uterine weights. Oestradiol and β-glycyrrhetinic acid or vehicle administered subcutaneously for 3 days in 6 doses. Potency of oestradiol in the presence of 6·0 mg. of β-glycyrrhetinic acid (total dose) is 0·53 ± 0·05 ($P < 0·001$ based on analysis of variance).

▲ Oestradiol. ● Oestradiol + 6 mg. of β-glycyrrhetinic acid.

of male rats of 100 g. weight at the time of bilateral adrenalectomy. Either the acid in 1 ml. of vehicle or the vehicle alone was administered for 4 days after adrenalectomy. Pellets with granulation tissue were

removed 24 hours after the last dose, oven dried overnight at 80°, and weighed.

RESULTS

β-Glycyrrhetinic Acid and Sex Hormones

The increase in weight of the uterus in response to exogenous oestrogen indicated that β-glycyrrhetinic acid was a potent oestrogen antagonist (Fig. 1, Table I). β-Glycyrrhetinic acid in doses from 1·20 to 6·0 mg. produced a 23 to 33 per cent decrease in the weight response of the uterus to 0·30 μg. of oestradiol benzoate with no significant differences between

TABLE I

DEPRESSION BY GLYCYRRHETINIC ACID OF THE UTERINE WEIGHT RESPONSE OF IMMATURE RATS TO 0·3 μg. OF OESTRADIOL BENZOATE

	Total dose in mg.	No. of animals	Uterine weight mg./100 g.	Per cent decrease	P value
Intact	Control	19	156·9 ± 6·2	—	—
	0·6	10	141·0 ± 9·0	10·1	N.S.
	1·2	6	105·0 ± 10·1	33·1	<0·01
	1·8	6	120·0 ± 12·2	23·5	<0·02>0·01
	2·4	4	104·6 ± 9·9	33·3	<0·01
	3·0	5	107·7 ± 4·5	31·4	<0·001
	6·0	10	113·0 ± 7·3	30·0	<0·001
Adrenalectomised ..	Control	9	143·1 ± 11·1	—	—
	6·0	9	109·4 ± 4·4	23·5	<0·001

doses (Table I). This decrease in response quantitatively agrees with the previously reported results for progesterone and desoxycorticosterone[12]. Bilateral adrenalectomy 1 hour before the first dose did not affect the anti-oestrogenic potency of 6·0 mg. of β-glycyrrhetinic acid (Table I). There were no significant differences between the responses of intact and adrenalectomised rats similarly treated (Table I).

TABLE II

THE EFFECT OF GLYCYRRHETINIC ACID ON THE UTERINE WEIGHTS OF IMMATURE RATS

Total dose in mg.	No. of animals	Uterine weight mg./100 g.
Control	17	41·2 ± 4·5
1·2	5	55·9 ± 2·5
1·8	5	54·6 ± 3·5
2·4	5	57·4 ± 7·4
3·0	5	38·3 ± 2·1
6·0	11	36·9 ± 10·0
15·0	5	52·5 ± 5·1
30·0	4	37·3 ± 8·6

A comparison between the dose response curves of rats given oestradiol alone and those given also β-glycyrrhetinic acid (Fig. 1) indicated that 6·0 mg. of the acid caused a significant reduction in the potency of the oestradiol, twice the dose of which was required in the presence of the acid to obtain a response equivalent to that of the oestrogen alone.

β-Glycyrrhetinic acid had no effect upon the immature uterus in the absence of exogenous oestrogen (Table II). There were no significant

differences between the uterine weights of untreated and glycyrrhetinic acid treated rats.

β-Glycyrrhetinic acid restricted the uterine weight increase in response to oestrogen in doses which did not interfere with somatic growth or depress gonadal size (Table III). Moreover, adrenal hypertrophy and thymic involution induced by 0·30 μg. of oestradiol benzoate were not affected by the acid (Table III). Testicular atrophy (Table IV) induced

TABLE III

A COMPARISON OF INCREASES IN BODY WEIGHT AND RELATIVE ORGAN WEIGHTS OF IMMATURE FEMALE RATS (10 PER GROUP) TREATED WITH 0·3 μg. OF OESTRADIOL BENZOATE OR 6 mg. OF GLYCYRRHETINIC ACID OR BOTH

Group No.	Oestradiol benzoate μg.	Glycyrrhetinic acid in mg.	Increase in weight	Adrenals mg./100 g.	Thymus mg./100 g.	Ovaries mg./100g.
1	Control	Control	8·5	20·6 ± 1·00	235·1 ± 13·68	18·1 ± 0·65
2	Control	6	10·2	20·1 ± 1·00	281·9 ± 25·70	17·9 ± 1·11
3	0·3	Control	8·0	23·4 ± 1·43	185·5 ± 20·20	16·5 ± 0·72
4	0·3	6	7·4	27·1 ± 2·00	149·1 ± 4·36	16·0 ± 0·65

TABLE IV

THE EFFECT OF GLYCYRRHETINIC ACID ON THE SIZE OF THE TESTES OF IMMATURE RATS GIVEN VEHICLE , TESTOSTERONE PROPIONATE OR OESTRADIOL BENZOATE

Group No.	Hormone and dose	Glycyrrhetinic acid in mg.	No. of animals	Testes mg./100 g.
1	Sesame oil only	Vehicle only	12	592·8 ± 18·0
2	" " "	6	4	669·6 ± 64·6
3	Testosterone 1·20 mg.	Vehicle only	16	594·4 ± 24·6
4	Testosterone 1·20 mg.	6	8	568·1 ± 22·9
5	Oestradiol 75 μg.	Vehicle only	7	319·6 ± 17·5*
6	Oestradiol 75 μg.	6	8	439·1 ± 15·5

* Decrease in testes size significant, P = 0·02.
† No significant difference from group 5.

TABLE V

EFFECT OF GLYCYRRHETINIC ACID ON THE RESPONSE OF THE SEMINAL VESICLES TO TESTOSTERONE IN IMMATURE RATS

Group No.	Testosterone propionate mg.	Glycyrrhetinic acid in mg.	No. of animals	Relative weight of seminal vesicles mg.
1	Sesame oil only	Vehicle only	4	20·8 ± 2·9
2	" " "	6	4	24·7 ± 2·7
3	1·2	Vehicle only	8	39·3 ± 2·4
4	1·2	6	8	35·9 ± 1·6

by 75 μg. of oestradiol benzoate was also not affected by 6·0 mg. of β-glycyrrhetinic acid. Apparently the drug did not antagonise the effect of oestrogen upon the pituitary-trophic hormones.

β-Glycyrrhetinic acid had no androgen activity (Table V) in immature rats nor did it interfere with the proliferative response of the seminal vesicle to 1·20 mg. of testosterone propionate. It also had no effect upon the size of the immature testes (Table IV).

Depression of Granuloma Tissue Formation in Bilaterally Adrenalectomised Rats

The two preparations (β-glycyrrhetinic acid and glycyrrhetinic acid) significantly depressed granulation tissue formation in doses of 4·0 and 8·0 mg. (Table VI). The differences in decreased granulation tissue formation between doses as well as between preparations were not significant.

TABLE VI

DEPRESSION OF GRANULOMA FORMATION BY GLYCYRRHETINIC ACID IN THE COTTON PELLET TEST IN ADRENALECTOMISED MALE RATS

Treatment	Total dose mg.	No. of pellets	Mean granuloma dry weight mg	Per cent decrease	P value
Controls	—	23	21·1 ± 2·00	—	—
β-Glycyrrhetinic acid	4	12	9·9 ± 0·86	53·1	<0·001
" "	8	10	8·6 ± 0·91	59·2	<0·001
Glycyrrhetinic acid	4	11	13·6 ± 1·48	35·3	<0·01
" "	8	22	13·5 ± 2·45	35·8	<0·02>0·01

DISCUSSION

Glycyrrhetinic acid resembles the Δ^4-3-ketosteroids in antagonising oestrogen. β-Glycyrrhetinic acid was found to be a potent inhibitor of oestrogen-induced uterine growth in doses which did not interfere with somatic growth nor the oestrogenic effect on pituitary trophic hormones.

The antagonism of oestrogen-induced uterine growth does not appear to be mediated through gonadal inhibition, adrenal stimulation or potentiation of adrenal corticoid action. This effect of the acid was not altered by adrenalectomy. It is unlikely therefore that the observed action in depressing oestrogen-induced growth of the uterus was due to the ability of the acid to interfere with the metabolism of hydrocortisone[16] and desoxycorticosterone[17]. The antagonism may be through the same mechanism suggested for the naturally occurring steroids. Testosterone, progesterone and adrenal corticoids antagonise the oestrogenic response of the uterus by alteration of enzyme systems[18–21]; consequently, uterine cell permeability changes induced by oestrogen are affected, and therefore the degree of proliferative response[21].

The two parallel dose response curves obtained with oestradiol benzoate with or without glycyrrhetinic acid indicated that even a 4-fold increase in exogenous oestrogen could not decrease the degree of inhibition induced by 6·0 mg. of β-glycyrrhetinic acid. The anti-oestrogenic effect obtained with the minimum effective dose of the acid (1·20 mg.) was not increased by a 5-fold increase in dosage (to 6·0 mg.). Apparently the restriction of uterine weight response had no relation to the oestradiol-glycyrrhetinic acid ratio.

There has been much speculation about the requisite structural factors in steroid activity. Mardones[22] has reported that oxidation of C(11), C(17) or C(21) diminished anti-oestrogenic potency and increased corticoid potency. When the 3-ketone group was present, neither the

absence of a double bond from testosterone[23] nor the addition of another double bond to cortisone and hydrocortisone[10] altered the anti-oestrogenic activity. However, androstenediol which does not have a ketonic oxygen did not have anti-oestrogenic activity[23]. The ketonic oxygen itself regardless of position may be necessary for anti-oestrogenic activity since its presence in glycyrrhetinic acid endows the acid with the ability to antagonise oestrogen.

Glycyrrhetinic acid resembles the glucocorticoids in anti-inflammatory action, which is not dependent upon the adrenal gland. Depression of granulation tissue in adrenalectomised animals must be attributed to the similarity in chemical configuration between glycyrrhetinic acid and the corticoids. The absence of a correlation between dose and response in this work was probably due to the poor absorption or rapid excretion of the drug or both.

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THE CONTRIBUTION OF SURFACE CHARACTERS TO THE WETTABILITY OF LEAVES

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Received December 30, 1959

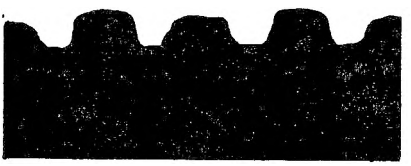
A method of preparing carnauba wax positive replicas of leaf surfaces is described and these have been used with other techniques to study some of the factors which govern the wetting of leaves by water. Leaves from four species of plants were used. It was found that macroscopic surface roughness was the chief factor preventing the wetting of *Festuca pratensis*. The contribution of surface chemistry and surface roughness is discussed also for results obtained with leaves of *Agropyron repens* and two species of *Papaver*.

The distribution and retention of chemicals, which are applied to crops, is affected by the morphology of leaves, in particular the detailed structure of the surface. Variation of surface structure between plants affects the wetting when sprays are applied and this in turn affects the efficiency of the formulation[1]. Experiments have been made to assess the effects of various physical factors on the retention of spray droplets like surface tension of spray liquid, size, velocity and angle of incidence of impinging droplets[2]. Fundamental studies have shown that the magnitude of the contact angle for a liquid on a solid surface is influenced by the surface chemistry[3], the surface roughness, and the presence or absence of air films between droplet and surface[4]. According to Adam[3], the effect of roughness is to *increase* the apparent contact angle if the true (advancing) angle is greater than 90°, but to *decrease* it if the true angle is less than 90°. It is believed that the sub-microscopic surface roughness of some leaves, attributed to minute wax extrusions[5], is responsible for high contact angles (greater than 90°) in most plants[6] and the waxy coverings of leaves have been isolated, fractionated and analysed[7]. Reflection of droplets from surfaces or "droplet bounce" has been shown to occur with contact angles of 140° and the roughness of waxy leaf covering has been held responsible for reflection from pea leaves[2]. The contribution of macroscopic surface roughness as distinct from microscopic roughness of leaves has not been fully investigated. Furthermore, the separate effects of macroscopic surface roughness and surface chemistry of leaves in relation to wetting have not been studied and the object of the present work was to devise a method which could be used for this purpose.

Experimental

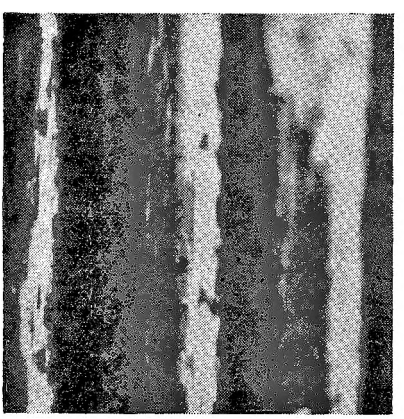
Leaves were chosen of four species which showed marked macroscopical differences. Two of the species showed macroscopical differences between upper and lower surfaces. All were collected from plants cultivated in the same plot. The measurement of advancing contact angle was selected as a criterion of the wetting of leaf surfaces by water droplets

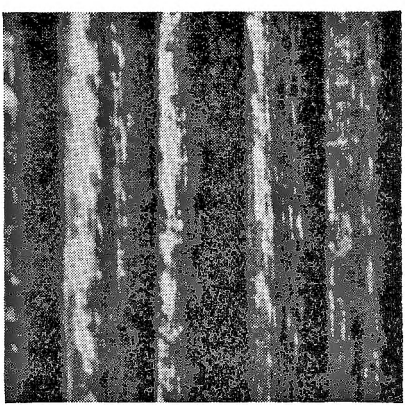
and the method described by Fogg[8] was used. Determinations were made on water droplets of standard size placed on four different surfaces: (*a*) fresh leaves; (*b*) ether-washed leaves; (*c*) positive replicas of leaves prepared from carnauba wax the normal contact angle of which is 84°; and (*d*) carnauba wax replicas coated with beeswax the normal contact angle of which is 90°. Measurements were not attempted near major veins on leaves or near impressions of major veins on replicas. Leaves were washed in ether as described by Martin and Batt[7]. Carnauba wax



FIGS. 1 and 2. Wax replicas of the upper surfaces of *Festuca pratensis* and *Agropyron repens* respectively (end view × 35).

replicas, prepared as described below, were coated with a thin film of beeswax by immersion in a solution of white beeswax 5 per cent w/v in chloroform and then allowed to dry. With all wax replicas coated with beeswax the film was thin and did not alter the macroscopic roughness of the surface, although fine epidermal detail was not as clear as in the original. Smooth carnauba wax was similarly coated with beeswax as a reference surface for comparative experiments.





FIGS. 3 and 4. Wax replicas of the upper surfaces of *Festuca pratensis* and *Agropyron repens* respectively (photograph of surfaces × 70).

The Preparation of Carnauba Wax Replicas

A brass mould (5 cm. × 3 cm. × 1 cm.) consisting of two L-shaped pieces, resting on a separate brass plate generally used for section embedding, was found to be the most convenient receptacle. The brass plate was first greased with soft paraffin and a piece of fresh leaf, cut to size, placed on the surface. The mould was then put into position and

a natural rubber latex* poured over the leaf surface until it was covered to a depth of $\frac{1}{2}$ cm. and a square of linen placed on the surface of the liquid, to render the preparation stiffer when dry. After 24 hours the mould was taken apart and the leaf stripped from the negative replica. From this a positive replica on carnauba wax was prepared by pouring molten wax over the negative placed in the brass mould to a depth of $\frac{1}{2}$ cm. After 24 hours the mould was taken apart and the negative stripped from the wax replica.

Results

The wax replicas gave good facsimiles of the surface contours, especially the prominence of veins (Figs. 1 and 2) as well as the more detailed structure of the epidermis (Figs. 3 and 4). Carnauba wax gave better preparations than beeswax or hard paraffin and despite the brittle nature of the

Table I

Results for leaves and uncoated replicas*

	Fresh leaves. Apparent contact angles		Ether washed leaves. Reduction in contact angle. Per cent of fresh leaves		Wax replica Reduction in contact angle. Per cent of smooth wax	
	upper surface	lower surface	upper surface	lower surface	upper surface	lower surface
Agropyron repens Ridges slight and trichomes on both surfaces	135°	138°	50	35	34	38
Festuca pratensis Ridges prominent only on upper surface, trichomes only on upper surface	110°	66°	30	Nil	58	16
Papaver somniferum Both surfaces glaucous (waxy)	149°	155°	51	65	23	30
Papaver orientale Only lower surface glaucous (waxy). Trichomes on both surfaces	30°	138°	Nil	50	35	16

* Each figure represents a mean of five determinations and no individual determination differs from the mean by more than 8 per cent.

replicas, the surfaces were hard, and therefore not easily damaged by scratching. The natural rubber latex was satisfactory for the preparation of negative replicas as these could be made without damaging the leaf surface and were sufficiently pliable to be stripped from the final wax positive replica. At least two wax replicas could be made from a single negative without loss of detail, but the negatives deteriorated on storage.

The contact angle for water droplets on smooth carnauba wax was found to be 84° and on carnauba wax coated with white beeswax 90°. The beeswax coated wax replica of *Festuca* (upper surface) gave a value of 154°, while the beeswax coated wax replicas of other leaves (different surfaces) gave values of about 90°. The only beeswax coated replica from which droplet reflection occurred was of the upper surface of *Festuca*. Other numerical information is tabulated (Table I).

* Natural rubber latex (H807) obtainable from S. Jones & Co., New Bridge Street, London, E.C.4.

Discussion

Leaves and Uncoated Replicas

For *Agropyron repens* the largest reduction in contact angle was observed after washing the leaf upper surface with ether. This suggests that the dominant factors affecting wetting of the upper surface are surface chemicals and their sub-microscopic roughness, these factors appear to be less effective on the lower surface. The largest reduction in contact angle for *Festuca pratensis* was obtained on wax replicas prepared from the upper surface and therefore it may be concluded that surface roughness, arising from ridges or trichomes or both, is the chief factor governing wettability. For the lower surface only a small reduction in contact angle was obtained by the wax replica method, and the ether washing method made no change in the contact angle. The lower surface is therefore more easily wetted than the upper; lack of roughness and surface wax being the main reasons.

For both *Papaver* species the largest reduction in contact angle was observed after washing leaves with ether; this occurred on the lower surfaces and would suggest that the main anti-wetting factors are the ether-removable surface chemicals and perhaps sub-microscopic roughness. The apparent contact angle for the upper surface of *P. orientale* is low and is not influenced by ether washing.

The limit of accuracy of the method does not permit further conclusions to be made about differences between other surfaces. The replicas do not record roughness contributed by trichomes and therefore their effect on water repellancy cannot be determined.

Coated Replicas

Provided there are no large differences in sub-microscopic wax roughness between coated replicas and the reference surface, the high contact angle obtained with *Festuca pratensis* can be attributed to macroscopic roughness already suggested by the results for uncoated replicas. This roughness is caused by the prominent parallel ridges of the leaf and allows air films to be trapped below water droplets and explains the reflection of droplets from the surface. This phenomenon cannot occur on the surface of uncoated replicas as the true contact angle is less than 90° when the liquid penetrates the hollows.

As the values obtained for coated replicas of *Agropyron* and *Papaver* were the same as for the reference surface and as droplet reflection did not occur; the high contact angles found for the fresh leaves may be attributed to sub-microscopic roughness of the surface chemicals; an examination of the waxy bloom of the leaf, by electron microscopy might confirm this. It was impossible to assess the effects of microscopic roughness with the beeswax coating technique since the detailed structure was impaired by the coating.

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TOXIC ACTIONS OF OESTROGENS ON THE LIVER

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Received October 19, 1959

The endogenous oestrogen level rises after hepatic damage. With its rise, the condition of the damaged liver deteriorates. This is supported by experiments in which mature ovariectomised animals were loaded with oestrogen. The introduction of oestrogen increases the strain on the damaged liver because of its reduced inactivating capacity. On comparing the results with clinical data in the literature, it is concluded that oestrogen therapy is contra-indicated when hepatic damage is present.

The oestrogens may be grouped into three categories: (i) human, natural oestrogens, (ii) other natural oestrogens, (iii) synthetic oestrogens.

Oestrogens in man are steroids which have an important role in ensuring the periodicity of hypophyseal function, especially in developing and secreting trophic hormones produced by the basophil cells of the anterior lobe. Oestrogens have become more and more widely used in therapy, and such popular use calls for the elucidation of previously unknown interrelations of these hormones.

The liver has been considered as the principal site for oestrogen inactivation. Talbot[1] produced severe lesions in female rats with carbon tetrachloride and ethanol. At death the uteri of these animals were removed and weighed, and were found to be double that of the uteri of control, untreated animals. In contrast, there were no increases of weight in previously ovariectomised rats after carbon tetrachloride poisoning. This excludes a direct effect of this substance, and supports the view that the inactivation of endogenous oestrogen is a function of the liver.

Experimenting in dogs, Israel and others[2] investigated the effects of transfused oestrogen in heart-lung preparations. They found no oestrogen inactivation in the blood *in vitro*, nor in the heart-lung preparation, but it was rapid in a heart-lung-liver perfusion. Riegel[3] mixed homogenised rat-liver fractions with oestrogen and observed that the inactivating enzymic system is in the microsomatic fraction.

The part played by the liver in oestrogen inactivation as investigated *in vivo* in man was conflicting, especially when a liver lesion was involved. While testing for the oestrogen activity of human urine in rats, Beretervide[4] did not observe any decrease of oestrogen activity, whereas Enzinger[5] recorded a significant and regular rise in the quantity of oestrogens. In *in vitro* examinations of tissues of human liver by a colorimetric method, Tagnon[6] did not observe that oestradiol breakdown was affected by a basic lesion of the liver, even when structural changes pointed to hepatic damage.

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As is evident, there are not only details but fundamental problems to be solved about the liver-oestrogen relation. The purpose of our investigations was to examine the intricate function of inactivation and particularly to look for possible changes in the liver loaded with exogenous oestrogen.

Experimental and Results

In our experiments, liver lesions were produced by Kaufmann's[7] method, using carbon tetrachloride. Most of the deaths were found to occur after 3 to 6 doses of carbon tetrachloride (0·05 ml./100 g.). The lighter the animal, the sooner it was affected. On dissection, even after a few doses of carbon tetrachloride, macroscopic changes were visible in the liver, the surface of which was dotted with yellow globules, some as large as 3–5 mm. in diameter, and striped with yellow bands. Animals weighing

TABLE I

Development of the endogenous oestrogen level following liver lesion

Phase	Average duration in	
	treated animals	controls
Oestrus	32·00 hr.	32·40 hr.
Metoestrus	23·06 ,,	30·48 ,,
Dioestrus	28·04 ,,	29·52 ,,
1(*a*)	21·00 ,,	33·80 ,,
Prooestrus	10·00 ,,	23·08 ,,
2(*a*)	9·30 ,,	24·00 ,,
Total	123·40 hr. 5·14 days	173·28 hr. 7·22 days

180–220 g. gave better results, and the death rate disappeared when the treatment was maintained for a longer period but with smaller doses (0·03 ml./100 g.). Of the 89 test rats on the 0·05 ml./100 g. dosage, 12 normal and 8 ovariectomised ones died.

Our basic observations were on the modification of the oestrus cycle of the group with liver lesions compared with the untreated control group. These tests were made in 28 fully grown animals treated with carbon tetrachloride, and 10 controls. On comparing the cycle of treated and control animals, it can be seen (Table I) that in the treated animals this averaged 2·08 days less than the control value. This is a significant difference. It is striking that the shortening of the cycle occurred in phase 1(*a*), in prooestrus and the following 2(*a*) transitional phase, while oestrus and dioestrus remained unchanged.

In addition to the data in Table I, the following changes were observed. Under normal circumstances, epithelial plugs occur in the 2(*a*), oestrus and metoestrus phases only. After liver lesions epithelial plugs were also atypically found in the vaginal smear in 82 per cent of the examined cycles. It was also atypical in that the phases did not always follow each other regularly.

Consideration of the atypical and frequently appearing epithelial plugs and the fact that oestrus always sets in sooner in the group with liver

lesions than in the control group suggests the probability that the endogenous oestrogen level rose after liver lesions.

We then investigated how normal and ovariectomised animals with loaded livers reacted to the introduction of oestrogen. If mature animals are given oestrogen the oestrus cycle of the animals with normal livers is prolonged. The question then arises: how can a liver with decreased

TABLE II

EFFECT OF OESTROGEN LOADING OF MATURE ANIMALS AFTER LIVER LESION ON PROLONGATION OF OESTRUS

Treatment	Oestrone dose	Average duration of oestrus	Prolongation
CCl_4	4 U	67 hr.	39 hr.
Control	4 U	28 ,,	
CCl_4	8 U	132 ,,	95 ,,
Control	8 U	37 ,,	
CCl_4	12 U	194 ,,	138 ,,
Control	12 U	56 ,,	

function assimilate introduced oestrogen, and how will this loading influence liver function? To study this, 28 animals with liver lesions and 10 control animals were used. The animals received a single injection of oestrone acetate, 4, 8 or 12 U/100 g. (0·4, 0·8, 1·2 μg./100 g.), during dioestrus. Oestrus started 28 hours later in the group with liver lesions, and lasted 67 hours. In control animals with normal livers the oestrus appeared also after 28 hours, but lasted only 28 hours. The difference

TABLE III

EFFECT OF OESTROGEN LOADING OF OVARIECTOMISED ANIMALS AFTER LIVER LESION ON PROLONGATION OF OESTRUS

Treatment	Oestrone dose	Average duration of Oestrus	Prolongation
CCl_4	1 U	not measurable	
Control	1 U		
CCl_4	2 U	24 hr.	18 hr.
Control	2 U	6 ,,	
CCl_4	4 U	48 ,,	21·5 ,,
Control	4 U	26·5 hr.	
CCl_4	8 U	94 hr.	52 ,,
Control	8 U	42 ,,	

between the two groups therefore was 39 hours. In fact, 15 animals in the group with liver lesion attained the criterion of "lasting oestrus," a reaction of 72 hours. None of the control animals did this.

With 8 U/100 g. of oestrone, the reaction in both groups occurred in 28 hours; in the group with liver lesions it lasted 132 hours, and in the control group 37 hours, a difference of 95 hours. This difference is even more striking if we consider that in the controls the double dose of oestrogen prolonged the oestrus by 9 hours, while in the treated animals it was prolonged seven times as much. This suggests that not only the rise in

the dose of oestrogen but some other influence of the hormone had played a part.

Using 12 U of oestrone, oestrus in the group with liver lesions began 30 hours later, and lasted 194 hours. In the control group the oestrus also began 30 hours later, but lasted only 56 hours. Thus the oestrus cycle effected by a triple dose of oestrogen was nearly four times as long as the control group. The data are presented in Table II.

Other tests were made in 31 ovariectomised rats, of which 21 were given 1, 2, 4 and 8 U of oestrone acetate per 100 g. and 10 were used as controls. 1 U of oestrogen per 100 g. produced no measurable results, 2 U brought about a 24 hour reaction in the group with liver lesions, while in the control group this lasted 6 hours. With 4 U the duration of oestrus in the group with liver lesion was 48 hours, while in the control group it was 26·5 hours, and 8 U/100 g. produced a reaction lasting 94 hours in the loaded animals and 42 hours in the controls.

Discussion

From our results we may claim that the role of the liver in oestrogen metabolism and inactivation is established. This process is bound up in an intricate enzyme system and closely connected with bile excretion. The latter phenomenon has also been demonstrated by Heard's[8] tests with radioactive iodo-oestradiol. It should be remembered that the liver as well as deactivating substances also activates, in so far as it reduces the oestrone to the more effective oestradiol-17β. We found that the damaged liver quickly lost its ability to inactivate oestrogen. Presumably, therefore, any kind of interference preventing oestrogen inactivation may result also in the increased lesion of the liver. We observed that the capacity of the damaged liver to inactivate oestrogen decreased disproportionately with dosage of oestrogen. This was also shown by the difference between the oestrus cycles of control and loaded animals. The evaluation of our tests in ovariectomised animals supports the other experiments, and demonstrates the harmful effect of endogenous oestrogen in any case of liver lesion.

Ovariectomised animals react to an equal loading of oestrogen by a shorter oestrus—even after liver lesion—than do normal animals with loaded livers. Thus by ovariectomy the liver is protected.

In groups possessing active ovaries, the oestrus is not longer when compared with the groups of ovariectomised animals (67 : 48 hours, or 132 : 94 hours), because the amount of non-inactivated endogenous oestrogen is additive with the introduced oestrogen, as it is well known that on the introduction of oestrogen the production of endogenous oestrogen will cease. The difference between the two arises from the fact that endogenous oestrogen will further damage a liver poisoned with carbon tetrachloride at least in its function of inactivating oestrogen.

Our animal tests are in substantial agreement with what we know of human pathology, as in Long's[9] observation in a patient in whom *coma hepaticum* was induced by oestrogen administration. The patient had jaundice in childhood, and received treatment for primary amenorrhoea.

Autopsy revealed cirrhosis of the liver. In his paper this author reported on 29 patients suffering from cyclic disturbances that were relieved by liver therapy.

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PRELIMINARY CHEMICAL INVESTIGATIONS ON SOME INDIAN SUBSTITUTES OF MALE FERN

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Received January 4, 1960

To find suitable indigenous substitutes for the official male fern, *Dryopteris filix-mas* (L.) Schott, which has to be imported into India, fourteen species of *Dryopteris* Adanson, two of *Ctenitis* C. Chr. and one each of *Hypodematium* Kuhn and *Cyrtomium* Presl, have been assayed to determine the percentage of oleoresin and crude "filicin" in each. The studies have revealed that some of the Indian species have a far higher oleoresin and crude "filicin" content than the official drug. Only some species occur in abundance in nature; their pharmacological activity has not yet been assessed.

INDIA is mainly dependent upon foreign countries for the rhizome of male fern and its extract, as the species of the crude drug official in the British Pharmacopoeia and the United States Pharmacopoeia are not found in India. The British Pharmacopoeial drug is derived from the rhizomes and frond bases of *Dryopteris filix-mas* (L.) Schott, a fern indigenous to Great Britain. In America *D. marginalis* (L.) Asa Gray, which is found in eastern and central United States and north to Prince Edward Island, forms the source of American male fern, and has been official in the United States Pharmacopoeia since 1881[1].

However, there are other taxonomically valid species of *Dryopteris* which grow wild in the temperate Himalayas and in mountainous ranges of Kashmir and Darjeeling in particular, which required evaluation for possible exploitation as a substitute for the official drugs. The literature revealed that except for two notes by Handa and others[2,3] ferns have not been investigated with this purpose. These authors determined the amount of active principle in five species of *Dryopteris* collected from Kashmir and Mussoorie Hills.

The present study was undertaken to find suitable indigenous substitutes, and for this purpose 18 members of the family Aspidiaceae have been analysed for their oleoresin and "filicin" contents.

MATERIAL

The sources of material for the present investigation are summarised in Table I.

The identification was confirmed by the late Mr. A. H. G. Alston of the British Museum of Natural History, London, and Dr. R. R. Stewart of the Gordon College, Rawalpindi, Pakistan, to whom our thanks are due.

The rhizomes were collected in their entirety, washed free of soil, and most of the fronds, roots, and dead tissues were removed. These were

* This work forms part of the thesis submitted to the Panjab University, India, for the degree of Doctor of Philosophy in Pharmacognosy, by T. C. M.

carefully dried at a temperature below 70° since the active principle is decomposed at or above 80°. The collections were made for three consecutive years in late August or September, because of the higher amount of active principle present during this season.

TABLE I

SOURCES OF MATERIAL

Species	Locality	Altitude ft.	Frequency
Dryopteris hirtipes (Bl.) O. Ktze.	Lebong forest, Darjeeling	5,500	Locally abundant
D. scotti (Bedd.) Ching	Lebong forest, Darjeeling	5,000	Growth localised and scanty
D. cochleata (Don) C.Chr.	Raiwala forests, Dehra Dun; Majitar Bridge, Darjeeling	1,500–3,000	Exceedingly common
D. barbigera (Moore) O. Ktze.	Alpine meadows, above Khillanmarg, Kashmir; on way to Rohtang Pass, Kulu hills	10,000–13,000	Fairly abundant
D. serratodentata (Bedd.) Hayata	On exposed rocks, Sandakphu, Darjeeling	11,000	Rare
D. splendens (Hk.) O. Ktze.	Along water streams, on way to Sanchal Lake and Gairabas, Distt. Darjeeling	8,500	Fair
D. fibrillosa (Clarke) Hand-Mzt.	On exposed rocks, Sandakphu, Darjeeling	11,000	Rare
D. rosthernii (Diels) C. Chr.	Forest Floor on way to Adoob, Pahlgam, Kashmir	6,000	Fair
D. blanfordii (Hope) C. Chr.	Forest floor around Gulmarg, Kashmir	9,000	Fair
D. chrysocoma (Christ) C. Chr.	All round Darjeeling. Also Tehri Road, Mussoorie	5,000–8,000	Exceedingly common
D. paleacea (Don) Hand-Mzt.	Along roadside to Tiger hill and also Sandakphu, Darjeeling; on way to Chandanbari, Pahlgam, Kashmir	7,000–9,000	Fair
D. ramosa (Hope) C. Chr.	Pahlgam, Gulmarg, plentiful above Ningli Nallah, Tungmarg, Kashmir	6,000–8,000	Fairly abundant
D. pulvinulifera (Bedd.) O. Ktze.	Along roadside to Aloobari Monastery and also on Carmechal Road, Darjeeling	6,500	Not common
D. sparsa (Don) O. Ktze.	Forest floor, near Sedrapong, Darjeeling	3,500	Fair
Ctenitis apiciflora (Wall.) Ching	Forest floor, near Tiger hill summit, near Dak Bungalow Tonglu and Sandakphu, Darjeeling	9,000–12,000	Fairly common
C. nidus (Clarke) Ching	Near Dak Bungalow, Tonglu, Sandakphu, Darjeeling	10,000–12,000	Not common
Hypodematium crenatum (Forsk.) Kuhn	Dehra Dun—Rajpura, on way to Mussoorie	2,500	Common
Cyrtomium falcatum Presl	Along water streams, Brewery Road, Mussoorie	5,000	Common

METHODS AND RESULTS

Each of the species was assayed 6–10 times (within one year after collection) for their oleoresin and "filicin" contents according to the methods given in the British Pharmacopoeia 1953. The results are summarised in Table II.

For determination of ether extractive it was found that soxhlet extraction with ether was quicker, and yet gave results which were comparable to the percolation method of the B.P. 1953. The rhizomes, divested of their adhering roots and dead tissues, were reduced to a moderately coarse powder (22/60) and exhausted by percolation or soxhlet extraction with ether. The ether extract was evaporated on a water bath to a thick oily consistency till it no longer gave any smell of ether. The residue was

weighed and from it the percentage of ether extractive or the oleoresin calculated. The proportion of crude filicin in the extract was determined by the B.P. 1953 method.

TABLE II

OLEORESIN AND "FILICIN" BY B.P. 1953 METHOD

Species	Oleoresin per cent	Crude "filicin" in the oleoresin per cent	Crude "filicin" in the drug per cent
Dryopteris hirtipes	8–10	20–25	2·0
D. scotti	9–11	25–30	2·4
D. cochleata	7–9	20–25	1·8
D. barbigera	7–9	25–30	2·2
D. serratodentata	8–11	26–36	2·9
D. splendens	12–14	30–37	4·1
D. fibrillosa	9–11	20–26	2·3
D. rosthernii	8–11	24–32	2·6
D. blanfordii	8–10	26–32	2·6
D. paleacea	7–10	28–40	2·8
D. chrysocoma	14–17	25–30	4·3
D. ramosa	12–15	25–30	3·8
D. pulvinulifera	11–15	25–30	3·5
D. sparsa	In traces	Nil	Nil
Ctenitis apiciflora	7–9	32–40	2·9
C. nidus	12–16	18–24	2·8
Hypodematium crenatum	Nil	Nil	Nil
Cyrtomium falcatum	In traces	Nil	Nil

B.P. and U.S.P. require that the drug, "male fern" should contain not less than 1·5 per cent of crude filicin as determined by the official assay method.

DISCUSSION AND CONCLUSIONS

It is evident from Table II that all the species of *Ctenitis* and *Dryopteris*, with the exception of *D. sparsa*, form excellent substitutes of the official drug with respect to their ether extractive value and the crude "filicin" content. *Cyrtomium falcatum* and *Hypodematium crenatum* may contain oleoresin in traces but are totally devoid of "filicin". Furthermore, Table II shows that while *D. cochleata* just meets the official requirement, the crude "filicin" content is higher than the official requirement in the other species. The highest amount is contained in *D. chrysocoma*, *D. splendens*, *D. ramosa* and *D. pulvinulifera*.

As seen in Table I, *D. chrysocoma*, *D. ramosa*, *D. barbigera* and *D. cochleata* grow in abundance in nature and can thus be exploited to advantage commercially. *D. cochleata*, though not very rich in active principle compared to the other investigated species, can still be of a substantial commercial value because of its luxuriant growth in Dehra Dun forests where it is easy to collect. *D. splendens* and *D. pulvinulifera* and many other species though relatively richer in crude "filicin" content are not so abundant, and thus may not be a profitable proposition unless cultivated. However, further exploration in the Himalayas may show their abundance in certain areas.

The pharmacological activity of the extracts was not assessed, and therefore the true value of these substitutes has yet to be determined. A comparison with the official male fern preparations is in hand.

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BOOK REVIEW

DETOXICATION MECHANISMS. Second Edition. By R. Tecwyn Williams Pp. x + 796 (including Index). Chapman and Hall, Ltd., London, 1959. £6 6s.

Readers unfamiliar with Professor Williams first edition should not be misled by the title. The sub-title "The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds" reveals much more the scope of the book. It should be added that under the term "Metabolism" the author includes work with many species of animal, some work with insects, and also experiments on isolated organs, tissue preparations, cell components and enzyme systems; microbial metabolism is excluded. The second edition coming twelve years after the first is two and a half times as big and contains about 2,600 references. This increase in size demonstrates the rapidity with which this branch of knowledge is expanding.

In plan the book is based on chemical structure. Separate chapters deal with such topics as "The Metabolism of Phenols," and "The Metabolism of Dyestuffs and Other Colouring Matters." The reviewer feels that Professor Williams uses the term "Mechanism" as a description of the chemical changes undergone rather than the biochemical processes by which these are brought about. The biochemistry is dealt with, but somewhat incidentally to the chemistry. Thus mercapturic acid formation occupies a section in the chapter on the metabolism of halogenated aromatic hydrocarbons. Since this form of conjugation also occurs with hydrocarbons the arrangement involves some fragmentation of information on related processes.

The most impressive features of this book are its meticulous completeness and the masterly way in which a most intractable mass of heterogeneous and sometimes conflicting information has been tamed and reduced to order. Yet it remains easily readable. Errors are surprisingly few and the book reflects a great deal of care for detail and accuracy. Each compound receives separate consideration and the author allows himself reasonable latitude to mention its uses and special features of its toxicity or pharmacological effects. It is useful to find that for most of the acids mentioned pKa values are given; these are often difficult to find in the literature; there is no systematic quoting of pK values for bases. In discussing drugs the author has used proprietary names, and free names indiscriminately sometimes with capitals and sometimes without. Since nomenclature of drugs is already very confusing it would be preferable to adopt the convention of using free names without capitals wherever possible; where proprietary names are unavoidable they should have a capital and inverted commas. Some factors such as the time course of metabolic processes, individual variations between animals and the influence of protein binding on the course of metabolism receive little mention.

The use of this book will be essentially practical. It will provide a guide to anyone who wishes to know how a chemical compound can be expected to behave in the animal body. It is unique in its scope and is an indispensable reference work for those interested in foodstuff and pharmaceutical chemistry and in chemical toxicology.

G. A. SNOW.