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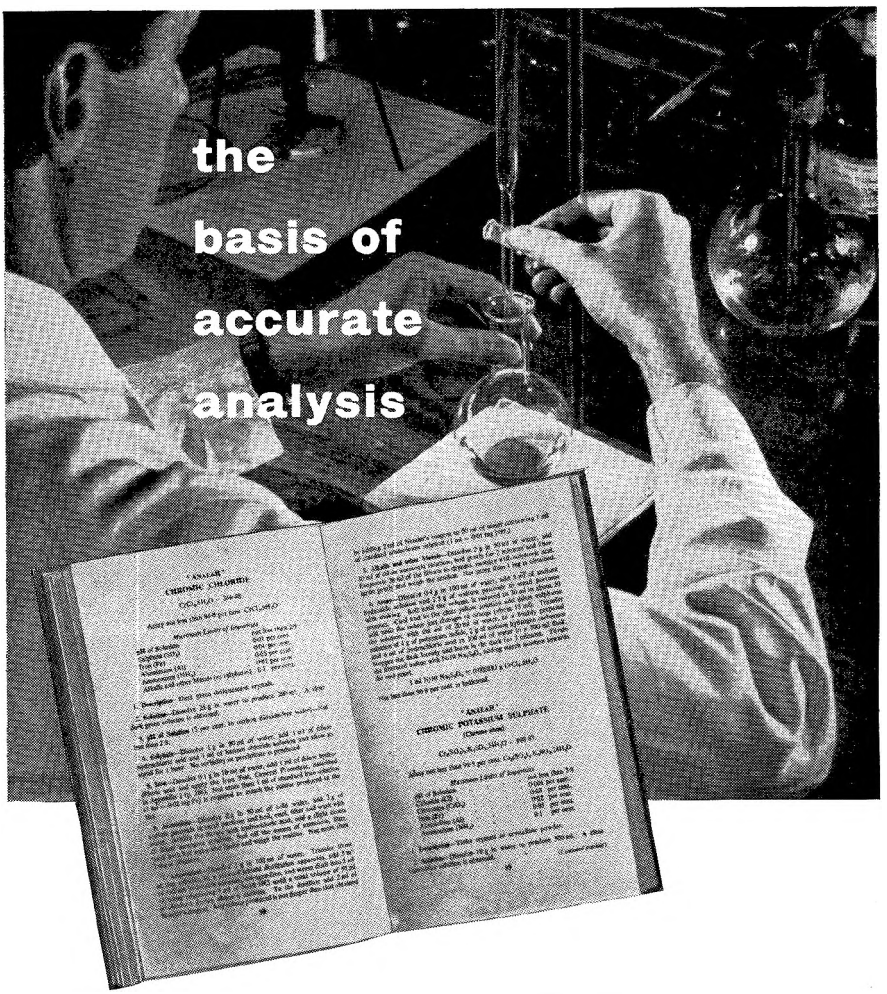

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REVIEW ARTICLE

THE MECHANISM OF HISTAMINE LIBERATION

BY BÖRJE UVNÄS, M.D.

Professor of Pharmacology at the Karolinska Institute, Stockholm

THEORIES have to be modified from time to time as fresh experimental data emerge. Recent years have brought new observations as to the localisation and the chemical binding of histamine in the tissues. Synthetic and biologically occurring histamine liberators constitute a new group of more or less specific substances and compounds, the histamine releasing properties of which were, until only a few years ago, completely unknown. The data available today necessitate reconsideration of the validity of existing theories on the mechanism of histamine liberation.

Localisation of Histamine

Histamine is present in virtually all mammalian tissues, but it is very unevenly distributed. There are also extreme variations between different species. For instance, in some species the liver is very rich in histamine (rabbit, dog, horse); in some, histamine is found in high concentrations in the lungs (guinea pig, cattle, horse); other animals have high contents of histamine in the skin (rat, cat), and so on. (For further details of the histamine distribution in the body, see Feldberg[1].)

Riley and West[2] reported a close quantitative correlation of the histamine content of a tissue and the density of its mast cell population. Mast cell tumours (in dogs) were observed to have an extremely high content of histamine (up to 1290 μg./g. of tissue[3]). The conclusion of Riley and West that mast cells store histamine has since been confirmed in various ways and is now generally accepted. In recent experiments Schayer[4] was able to demonstrate the accumulation of radioactive histamine in rat mast cells when radioactive histidine was added to the cells *in vitro*, thus indicating the intracellular formation of histamine.

But a satisfactory quantitative correlation of the histamine content and the mast cell population does not exist in all tissues. The gastrointestinal tract, not least the stomach mucosa, has a rather high histamine content but relatively few mast cells. As regards the skin, histamine is reported to occur in the corium, a layer which is said to contain no mast cells. The tissue histamine situated outside the mast cells does not seem to be released by the common histamine liberators.

Even though, for reasons which are quite obscure, histamine may occur outside the mast cells, most of it is localised in them. Of special interest in this context, is the fact that the readily mobilisable histamine in the body is localised in the mast cells. The present discussion will therefore be confined to the liberation of histamine from these cells.

The intracellular distribution of histamine has been studied by differential centrifugation of mast cells. In such experiments the bulk of the

histamine has been recovered from the granular fractions[5]. According to Blaschko[6] the enzymes which produce amines—among them histamine—occur in the cell sap. Histamine should accordingly be formed in the cell sap and then stored in the cell granules. This means that the formed histamine has to pass in through the granular membrane. Whether, under physiological conditions, histamine also passes from the granules into the cell sap, and further through the mast cell membrane into the surrounding tissues, is not known and, in my view, is by no means certain. The matter will be discussed further later in this review.

The mast cell histamine occurs, so to speak, in a double parcel, a large one—the mast cell—containing many small ones—the granules. Both of the parcels are enveloped by membranes with permeability properties of which our knowledge is very hazy and fragmentary. An acceptable theory for the mechanism of histamine release has to explain the passage of histamine through two membranes which seem normally to be impermeable to free histamine.

How is Histamine Chemically Bound in the Cell?

It has been suggested that histamine occurs in the tissue rather firmly bound to proteins or lipoproteins[7]. When these compounds are broken down by proteases or other enzymes the histamine should be set free. However, today there are grounds for believing that this is not the case. How the histamine which is found outside the mast cells is anchored to the tissue is completely unknown, but about the mast cell histamine we have at least some suggestive evidence. McIntire, White and Sproull[8] showed that histamine was readily released from the tissue by treatment with ice-cold trichloroacetic acid, distilled water, cold acetone, acid alcohol, repeated freezing and thawing, and so on. In other words histamine can be removed from a tissue by relatively mild procedures which do not suffice to break firm chemical bonds. Disruption of the anatomical structures or of the permeability barriers of the mast cell apparently suffices to cause a release of the intracellular histamine. Some of these procedures mentioned seem to rule out activation of enzyme mechanism as the cause of histamine release. It seems more reasonable to conclude, as did McIntire, that histamine is held in the mast cell by fairly weak ionic linkages. Such linkages would break rather easily, for example even with shifts in the ionic equilibrium within the granules.

The mast cell granules contain heparin, which is a mucopolysacharide with acid groups. Presumably there are, in the granules, many other compounds with acid groups; hence it is not difficult to conceive of histamine as occurring in the cell in loose ionic linkage with various acid groups.

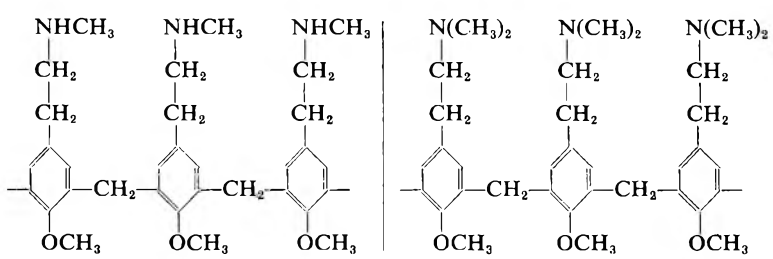
When is Histamine Liberated?

To Sir Henry Dale the credit is due for having drawn attention to the possible role of histamine as an active agent in anaphylactic reactions. The release of histamine with antigen-antibody reactions in sensitised

tissues has since been studied by Dale, by Feldberg, by Dragstedt and their associates as well as numerous others.

Many histamine liberators have been discovered in the last ten to fifteen years. MacIntosh and Paton[9] described a number of amines, amidines, guanines, guanidines and other organic bases, all of which were more or less potent histamine liberators. Since then the list of substances and compounds capable of histamine release has increased continually. To the group belong many drugs, and several others have been isolated from biological sources, as for instance sea anemone, jellyfish, Ascaris organisms, caterpillars, etc. An extensive list of known histamine liberators is given in Paton's excellent review[10].

Two synthetic liberators require further mention for the purposes of the discussion below. Compound 48/80 is a polymer amine, a condensation product between formaldehyde and *p*-methoxy-phenethyl-methylamine. It is a very potent histamine liberator in some animals like the rat, cat and dog, but fairly inactive in the guinea pig. For reasons mentioned later on, a polymer amine was synthesised that differed from compound 48/80 in that it had two methyl groups linked to the nitrogen. This polymer tertiary amine was also a potent histamine liberator. The two polymers are shown in Figure 1.



Compound 48/80 Compound PK3010*aa*

FIG. 1. Polymer histamine liberators.

Mast Cell Changes on Histamine Release

When histamine is released from a tissue as the result either of an antigen-antibody reaction or of the action of a histamine liberator, the mast cells in the tissue undergo profound morphological changes. The cells become degranulated and more or less lose their staining characteristics for basic dyes.

The mast cell changes have mostly been studied in rat tissues. In rats treated repeatedly with 48/80 the cutaneous mast cells disappear parallel with a decline of the cutaneous histamine content to extremely low levels. For a period of some weeks there is then a slow continuous increase of the cutaneous histamine, and at the same time the mast cells return[11].

When mast cells of rat mesentery are incubated *in vitro* with compound 48/80, their granules are seen to be spread around the cells, which have an "exploded" appearance. However, it is not yet clear whether this degranulation means destruction of the cell membrane and devitalisation

of the mast cell, or merely a temporary increase in the membrane permeability consistent with survival of the cell. Recent observations rather suggest that the histamine-containing granules may be discharged from the mast cells, leaving the cell capable of renewing its histamine store. Possibly all degrees may occur, from irreversible destruction to slight permeability changes in the cell membrane, according to the intensity of the liberation process. The matter will be further discussed later.

Theories of the Histamine Liberation Mechanism

Knowing a little about the localisation and chemical status of histamine in the tissue, and the circumstances under which it may be released from its storage therein, we can now proceed to discuss some of the theories propounded to explain the mechanism by which it is liberated.

Various enzyme theories have been suggested.

Protease Theories

Various snake venoms release histamine from perfused tissues. Since such venoms are observed to contain trypsin and the latter produced, in animals, a shock-like syndrome on intravenous administration, Rocha e Silva[7] advanced a protease theory to explain the histamine release in anaphylactic shock. He showed that the proteolytic enzymes papain and trypsin were able to release histamine from perfused tissues, and from leucocytes. Further, Ungar[12] reported that antigen-antibody reactions caused a rise in the fibrinolysin activity in the blood serum of guinea pigs concomitantly with the histamine release. Both processes were claimed to be inhibited by soy bean trypsin inhibitor. Increased protease activity was also induced by compound 48/80 and other histamine liberators. The histamine release was consignificantly attributed to the activation of proteases which released histamine by splitting it from the polypeptides. Ungar's latest modification of his theory is somewhat complicated; it involves kinases in the blood and cells that transform proactivators to activators, which in turn transform protease precursors to active proteases, the latter then attacking polypeptides to which histamine is supposed to be attached.

The protease theories have several weak points. The histamine releasing activity of trypsin is weak, and fibrinolysin—even in high concentrations—is not able to release histamine[13,14]. There is no satisfactory quantitative or temporal relation between the protease activity and the histamine release[15]. Lastly we have no evidence whatsoever that histamine occurs in the mast cells—the locus from which it is released—linked to proteins by bonds which require proteases for their dissolution. On the contrary, experimental evidence militates against the existence of a firm binding of histamine.

It has to be remembered that the protease theories were propounded before the localisation of histamine in the mast cells had been discovered. The survival of a modified protease theory therefore seems to depend on the ability of proteolytic enzymes to degranulate the mast cells. As will

be seen from Table I, both trypsin and fibrinolysin, even in high concentrations, lack this ability when tested on rat mast cells *in vitro*. In my view, this is an additional argument against the validity of the protease theories.

The Lysolecithin Theory

The strongly haemolytic snake venoms from, for example, *Naja naja* and *Denisonia superba* possess histamine liberating properties. The haemolytic action is due to the presence of lecithinase A in the venoms. Lecithinase A attacks lecithin causing the formation of lysolecithin, which has a haemolytic action. Besides causing haemolysis, lysolecithin is

TABLE I

THE EFFECT OF VARIOUS ENZYMES ON THE MAST CELL MEMBRANE

Enzyme	Max. conc. used μg. or units/ml.	Disruption per cent
Acetylcholinesterase	400	0
α and β Amylases	500	0
Pectinesterase	500	0
Hyaluronidase	20 I.U.	0
β-Glucuronidase	1680 Fishnam units	0
Hexokinase	500	0
Cozymase	400	0
Lipase (pancreas)	400	0
Lecithinase A (bee venom)	200	100
Malic dehydrogenase	200 U.	0
Trypsin	2500	10
Carboxypeptidase	400	0
Thrombin (1)	19 U.	2
Fibrinogen (2)	600	5
1 + 2	—	0
Plasminogen (3)	1 ml.	0
1 + 2 + 3	—	20
Streptokinase (4)	1000 U.	10
1 + 2 + 3 + 4	—	10
4 + rat serum	+ 0·4 ml.	0
Pancreatin	1000	0
Urease	500	0
Ribonuclease	1000	0
Desoxyribonuclease	1000	0
Uricase	500	0
Phosphatase, alkaline	500	10
Phosphodiesterase (snake venom)	500	0
ATP-ase	100	0
Cytochrome C	500	0
Carbonic anhydrase	500	0

capable of releasing histamine. It was therefore suggested that histamine might be linked to lipoproteins, from which it could be released by the lytic action of lysolecithin[15].

The lysolecithin theory has not aroused any great enthusiasm, presumably because no experimental evidence was presented to support the necessary assumption that histamine occurred in the tissue bound to lipoproteins sensitive to the action of lysolecithin. However, since the discovery of the histamine-bearing role of the mast cells, the validity of a cytolytic theory warrants attention. As will be seen in Table I, lecithinase A is the only enzyme among 25 tested which is capable of disrupting mast cells in the rat mesentery[16]. Lecithinase A prepared from bee venom or various snake venoms is highly active, only a few micrograms being required to cause total disruption of the mast cells.

The histamine release produced by some snake venoms might therefore

be ascribed to the lecithinase activity of the venoms. Yet, when tested on perfused cat paw, even very active lecithinase preparations were able to release only negligible amounts of histamine (unpublished observations). The reason for this discrepancy between the action of lecithinase A on rat mast cells *in vitro* and on perfused cat paw is not clear. The inability of lecithinase A to release histamine in the cat paw might depend on inadequate experimental conditions, e.g. the lecithinase not reaching its target, the mast cells. After injection of lecithinase the paws become very oedematous, and the perfusion tends to decrease and cease. Possibly the lecithinase does not pass in through the capillary walls. As will be discussed later, the ineffectiveness of lecithinase administered into the circulation does not invalidate a modified lecithinase theory.

Other Enzyme Theories

The histamine release in guinea pig tissues as a result of antigen-antibody reactions has been ascribed to an energy-requiring enzymatic process, since the histamine release is reduced by oxygen lack, and by the presence of iodo-acetic acid and some other metabolic inhibitors[17]. It was also observed to be inhibited by previous heating of the sensitised tissue to 43 to 44°[18]. All of these observations are consistent with the idea of an enzymatic release of histamine. The action of 48/80 and octylamine, on the other hand, was attributed to a different mechanism. The release of histamine produced by these agents in guinea pig tissue was found to be enhanced by oxygen lack and by iodoacetic acid.

On the other hand, Junqueira and Beiguelman[19] claimed that various enzyme inhibitors blocked the disrupting action of 48/80 on *rat mesentery mast cells*. Special attention was given to the inhibitory action or SH-blocking compounds (*p*-Cl-Hg-benzoate, *O*-iodosobenzoate, iodoacetate). There is no doubt from their figures that these enzyme inhibitors prevent degranulation of the mast cells, the implication being that the action of 48/80 is mediated by an enzymatic mechanism. However, in my view the concentrations of the inhibitors used are too high to guarantee specific blocking of SH-groups, and the experiments therefore do not yield much information about the types of enzymes involved.

The Displacement Theory

Histamine, as mentioned above, is a base that is thought to be loosely linked to acid groups in the intracellular granules; and most synthetic histamine liberators are organic bases, more or less lipid soluble. It seemed plausible to assume that such substances liberated histamine by penetrating the cell and granular membranes and, once inside the granules, simply replaced the histamine[9]. This simple, and hence attractive, hypothesis might explain the histamine release produced experimentally by some organic bases, especially *in vitro* but also *in vivo* when high doses are used or required to produce a release, as is the case with monoamines and also with compound 48/80 in guinea pigs. It is doubtful, however, whether the histamine liberation observed clinically or experimentally even after minute doses of various substances can be explained simply on

an ion exchange basis. Several arguments can in fact be advanced against the displacement theory to which Paton[10] recently subscribed, as being valid for the action of 48/80.

The displacement theory postulates that the liberating agent penetrates the mast cell membrane and enters the granules. As far as I am aware, no such passage of, for example, 48/80 has yet been demonstrated. To me, the well-known fact that one molecule of 48/80 releases several molecules of histamine appears to be a serious obstacle to a displacement theory. In a perfused cat paw, for instance, 10 μg. 48/80 is able to release up to 75 μg. of histamine. Since 48/80 injected into the tibial artery must become widely spread throughout the tissues of the paw, the concentration

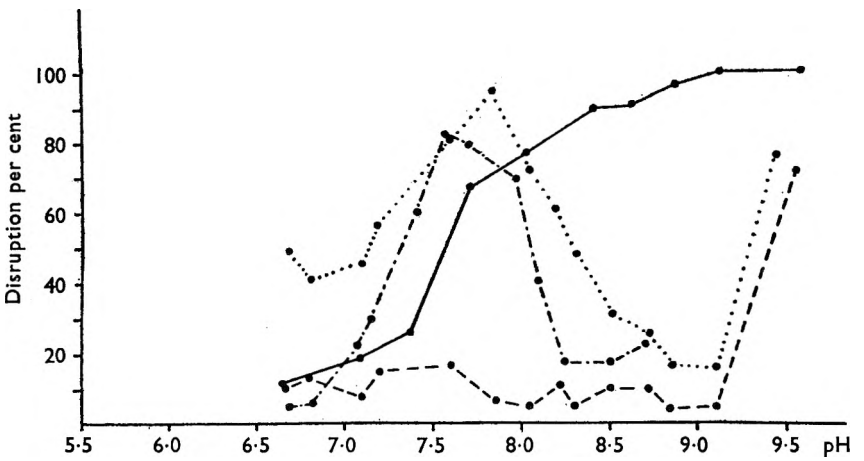


FIG. 2. The influence of pH on the disruptive action of 48/80 on rat mesentery mast cells. Barbitone buffer. · · · 48/80 0·5 μg./ml., – · – Pk3010*aa* 1 μg./ml., —— decylamine 10 μg./ml., – – – control.

of 48/80 around the mast cells will of necessity be very low. To explain the release of histamine as a simple ion exchange between 48/80 and histamine seems rather difficult.

There are other counter-arguments. Compound 48/80 is an amine and its lipid solubility, and hence its ability to penetrate the cell membrane, should increase with decreasing ionisation. In other words, the more alkaline the medium the higher should be the disrupting action of 48/80 on the mast cells. However, as shown in Figure 2 (Högberg and Uvnäs, 1957, unpublished observations), this is not the case. Pieces of rat mesentery were incubated with 48/80 at various pH values. The solution was buffered with either 10 per cent phosphate or 10 per cent barbitone buffer. The disruptive action of 48/80 on the mast cells showed a peak around pH 7·8 and reached low values on both sides of the optimal value. The shape of the pH curve is reminiscent more of an enzymatic process than of an ionic exchange mechanism. The tertiary amine Pk 3010*aa* also shows a similar pH curve.

At a pH higher than 9·2 the disruption again increases, probably due to the alkalisation of the milieu, since the control values show a similar increased spontaneous disruption when the pH surpasses this value.

The disruptive action of decylamine, on the other hand, shows no pH peak but increases continuously with rising pH. This observation is consistent with the assumption that decylamine might act according to the displacement theory. The more alkaline the medium, the less ionised does decylamine become. Its lipid solubility increases and thereby its ability to penetrate the mast cell membranes.

The histamine-releasing action of 48/80 on intact tissue is described as "explosive," most of the histamine being released in the first few minutes after administration of the compound. From isolated mast cell granules, on the other hand, 48/80 releases histamine somewhat slowly, the amounts of histamine released increasing linearly with time. These observations, too, seem to be inconsistent with a displacement theory. If this theory were correct, the speed of the histamine release ought to be as high from the granules as from the cells.

Theory of Högberg and Uvnäs

The aim of the discussion above was to show that even though some experimental evidence can be found to support each of the various theories advanced, all of them seem to have serious shortcomings. In my view, an acceptable theory has to be consonant with at least two apparently incompatible observations, namely:

(1) The liberation of histamine is due to an enzymatic mechanism; and

(2) the histamine is stored in the mast cell granules in weak (ionic) linkages which do not require enzymatic processes for their dissolution.

As will be seen from the experiments to be described, it might be possible to combine the two observations in one theory.

Högberg, Thufvesson and Uvnäs[20] isolated highly active histamine-liberating fractions from *Ascaris lumbricoides* (from swine) and *Cyanea capillata* (jellyfish). These compounds as well as compound 48/80 disrupted mast cells of rat mesentery *in vitro* and released histamine from the perfused cat paw. Both processes were blocked by a polysaccharide fraction isolated from hip seeds[21]. Polyanions of high molecular weight reversibly inhibit different enzymes[22]—an inhibition believed to be due to blocking of free amino groups of the enzymes.

Attempts were therefore made to find experimental support for the hypothesis that 48/80 disrupts mast cells by activating an enzymatic mechanism[18]. Mast cells from rat mesentery were incubated with 48/80 in the presence of various metal ions and other enzyme inhibitors. The action of 48/80 was inhibited by some heavy metal ions, Zn^{++} ions, some enzyme inhibitors such as phenylhydrazine, iodoacetic acid and γ-tocopherylphosphate, and some high molecular weight polyanions such as polyphloretin phosphate, polyestradiolphosphate and polysaccharides. Although suggestive of an enzymatic mechanism, the observations did not warrant any conclusion about the more intimate nature of a possible enzyme.

The mast cells were incubated with about thirty various enzymes. Only one, lecithinase A, prepared from bee venom and from various haemolytic snake venoms, had the ability to disrupt mast cells. The lytic action on the mast cells was blocked by many of the inhibitors which blocked the effect of 48/80. Of special interest was the finding that trypsin and fibrinolysin were among those enzymes which, even in very high concentrations, were unable to disrupt the mast cells (see Table I).

The action of 48/80 is temperature dependent. The disruptive action decreases with falling temperature and disappears at temperatures below 10°. This blocking effect is reversible, the disruptive action being restored if the incubation fluid is reheated to 37°. Heating the mast cells above 45 to 50° also abolished the action of 48/80. This blocking was irreversible, however, the disruptive action of 48/80 failing to reappear on cooling of the incubation fluid to 37°. Such heated mast cells were still vulnerable to the lytic action of lecithinase A prepared from bee venom or to snake venoms as reported by Högberg and Uvnäs[16].

The following theory was propounded to explain our results (Fig. 3). A lytic enzyme is situated at the mast cell surface. The enzyme is normally inactive, since the active group is blocked by an inhibitor I. When

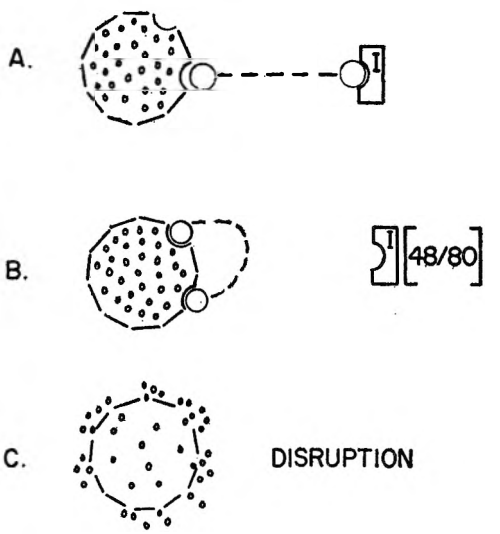


FIG. 3. Enzyme theory to explain the disruptive action of 48/80 on rat mesentery mast cells (for explanation see above).

this is removed by the liberator (48/80 in Fig. 3) the enzyme becomes active and attacks the cell membrane.

Recently Rathlev and Rosenberg[23] described a method for the phosphorylation of amines in aqueous neutral solution with a new compound, 1:3-diphosphoimidazole (DPI). Especially primary aliphatic amines seem to be easily phosphorylated with DPI. Högberg (1957, unpublished observations) reported that DPI inactivated enzymes with essential amino groups such as hyaluronidase and lecithinase A. DPI was found to cause an instantaneous inactivation of these enzymes.

It was observed by Högberg and Uvnäs that on simultaneous incubation of mast cells with DPI and the tertiary amine Pk 3010*aa*, no disruption of the mast cells occurred. In fact the mast cells become resistant to subsequent exposure to 48/80.

The reason for using compound 3010*aa* instead of 48/80 when treating the cells with DPI was that 48/80 is a secondary amine, i.e., has the group $N\begin{matrix}CH_3\\H\end{matrix}$ and thus could have been phosphorylated by DPI to $N\begin{matrix}CH_3\\P\end{matrix}$ — and thereby possibly loose its liberating properties. Pk 3010*aa* is the corresponding tertiary amine $\left(N\begin{matrix}CH_3\\CH_3\end{matrix}\right)$ of 48/80 and cannot therefore be phosphorylated.

The blocking action of DPI might be explained as follows (see Figure 4). Pk 3010*aa* removes the inhibitor I from the NH_2 group of the lytic mast

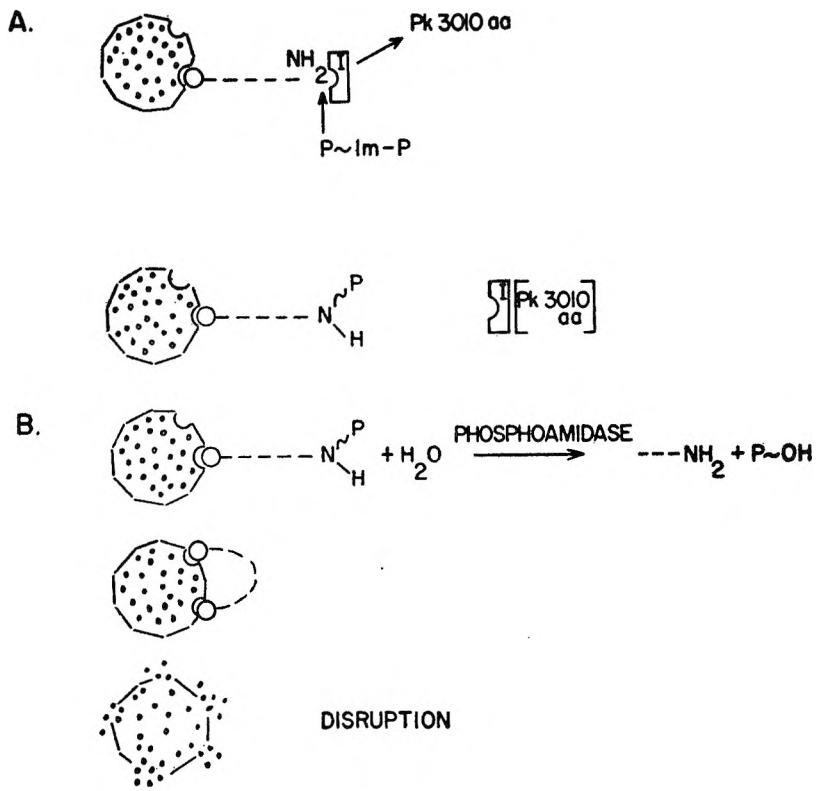


FIG. 4. Tentative explanation of the action of a phosphorylating agent (1:3-diphospholmidazole) or the mast cell enzyme (for explanation see above).

cell enzyme. The enzyme is thereby activated and the mast cell would be disrupted if the NH_2 group were not immediately phosphorylated due to the presence of DPI.

A test of the assumption that the resistance of the DPI-treated mast

cells to 48/80 was due to phosphorylation of a membrane enzyme would be to dephosphorylate, and thereby reactivate, the enzyme. Indeed, incubation of the "phosphorylated" mast cells in phosphoamidase caused a disruption of the mast cells (Fig. 4). Phosphoamidase did not disrupt "normal" mast cells. It is also of interest to note that the DPI treated cell membrane remains sensitive to the action of lecithinase A added from the outside.

Acetylation and several other processes that are thought to block NH_2 groups also inhibited the action of 48/80.

Taken together, the experiments lend strong support to the hypothesis that 48/80 disrupts mast cells by activating a lytic enzyme on the cell surface. The term "disruption," as mentioned previously, must not be taken too literally, however, since it is not known whether the mast cell membrane really is demolished or if the degranulation merely implies transient permeability changes in the membrane.

Comments upon the Theory of Högberg and Unväs

Our theory obviously has at least one weak point. It can easily explain, I think, the passage of histamine from the cell sap through a more or less damaged cell membrane. But how are we to explain the passage of histamine out of the intracellular granules, which seem to have a membrane not freely permeable to histamine? Several hypotheses can be advanced, however.

The activation of a lytic enzyme on the mast cell surface presumably results in the splitting of the phospholipid membrane into fatty acids and lysolecithins. The lysolecithins are strongly lytic, and it is easy to envisage a lysis of the granular membranes when they come into contact with the lysolecithins. The subsequent changes in the intragranular chemical milieu will result in a release of the histamine from its loose ionic linkage.

Another possibility is that the intracellular granules containing histamine are attacked by the same enzyme which breaks down the mast cell membrane.

The discharge of intracellular granules from the mast cell has been ascribed to an energy-requiring process. Junquiera and Beiguelman[19] observed in phase contrast microscopy the rapid formation of vacuoles, swelling of the cells and extrusion of the granules when 48/80 was added to living mesentery spreads from rats. The degranulation was believed to be due to metabolic activity inside the cell since it could be blocked by SH-blocking compounds (*p*-Cl-Hg benzoate, *O*-iodosobenzoate, iodoacetate), by substances blocking phosphorylative processes (2:4-dinitrophenol, arsenite and urethane), and by uranil nitrate, a metabolic inhibitor described as acting on cell membrane enzymes.

In my opinion the concentrations of enzyme inhibitors used by Junqueira and Beiguelman are such that specific inhibitory actions cannot be expected.

Is it really necessary to postulate the involvement of an enzymatic process in the transport of intracellular granules to the cell surface? The

granules are lipid structures floating in the cell sap just as oil drops float in water, and as such they are presumably very sensitive to changes in surface tension. It is not difficult to conceive changes in the membrane permeability giving rise to disturbances in the lipid-water interface with displacement, and changes in form and size of the granules. The contact with the activated membrane enzyme and formed lysolecithins might accelerate the release of histamine from the granules floating in the cell sap.

Although it may be tempting to attribute the release of histamine by 48/80 merely to activation of a lytic enzyme, the possibility cannot yet be ruled out that 48/80 triggers not only one but several enzyme mechanisms such as Junqueira and Beiguelman[19] have suggested.

The question arises, of course, whether a lytic cell enzyme may be assumed to take part in the mast cell disruption and histamine release produced by agents other than 48/80. Our observations that polysaccharides block not only the effect of 48/80 but also the action of the histamine liberating fractions from ascaris and cyanea as well as the histamine liberation caused by antigen-antibody reactions in guinea pig tissue, might indicate at least one common link in the chain of events which eventually lead to histamine liberation under these circumstances. The common link might be the activation of a lytic mast cell enzyme.

One argument against a lypolysis theory has been that little or no haemolysis occurs in anaphylactic shock, peptone shock, intoxication with ascaris products, and so on. The absence of haemolysis is not, in my opinion, a valid objection to a lypolysis theory. If a lytic enzyme (phospholipase) is anchored to the mast cell surface, activation of the enzyme will not necessarily bring about a spread of active enzyme in the blood. An enzyme attached to the mast cell membrane or to membrane fragments is unlikely on any major scale to come into contact, in active form, with circulating erythrocytes. In fact, a lytic mast cell enzyme might be very specific and lack the ability to attack the red cell membrane.

As pointed out by Mongar[14], the activity of histamine releasers varies considerably according to the species, the tissue and the methods used for determining their activity. The discrepancy might be a thousandfold. For instance, the concentrations of 48/80 required for the release of histamine from perfused[24] or chopped[25] guinea pig tissue, or from isolated intracellular granules from dog liver[26], are rather high and of about the same magnitude as those required for corresponding histamine release by simple amines such as octylamine. Under such conditions the histamine releasing action of 48/80 might be ascribed rather to a direct lytic action on the extra- and intracellular membranes than to activation of specific enzymatic mechanism.

In this discussion I have not touched upon the histamine release observed to occur in blood, especially in the rabbit and guinea pig. Our knowledge of the histamine release from thrombocytes and other formed elements of the blood, and of the role and nature of anaphylatoxin, is still too scanty to allow any fruitful discussion.

Recent discoveries concerning the role of the mast cells as histamine

bearers, and the possibility of denuding them of their histamine, have invigorated the discussions on the physiological role of histamine. Almost all authors take it for granted that histamine has important biological functions, the main arguments, of course, being the well-known pharmacological actions on smooth muscle and glands. Yet do these arguments carry any real weight? It might be that histamine for some reason is formed and stored in the mast cell, but that physiologically it does not pass through the cell membrane to the surrounding tissue. The presence of mast cells in vessel walls has led to the hypothesis that the mast cell histamine plays a role in the regulation of vascular tone. However, so far as I am aware, there is no experimental support for such an hypothesis. The presence of mast cells around the vessels may indicate transport of histamine from or towards the blood stream.

I have no suggestion to offer about physiological role of histamine and I think it would be unduly bold to dispute the physiological significance of this pet of so many laboratories. But the fact that histamine may, under pathological and experimental conditions, be released from the mast cell does not necessarily mean, I would stress, that such a release reflects a physiological mechanism.

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RESEARCH PAPERS

A NEW SERIES OF POTENT ANALGESICS:

Dextro 2:2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine and related basic amides

PART II

COMPARATIVE ANALGESIC ACTIVITY, ACUTE TOXICITY AND TOLERANCE DEVELOPMENT IN RATS FOR R875, MORPHINE, PETHIDINE AND METHADONE

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The analgesic activity, acute toxicity and development of tolerance of R875, morphine, pethidine and methadone after subcutaneous administration in rats have been measured. In this animal, R875 is shown to be several times more active than the three other compounds. R875 has a faster onset of action, higher therapeutic ratios and a slower rate of development of tolerance.

SOME pharmacological properties of a new series of secondary and tertiary basic amides[1] were described in part I of this series[2]. The dextrorotatory isomer of 2:2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine, serial number R875, was shown to be the most active analgesic tested, and therefore was selected for further study.

This paper describes some comparative pharmacological and toxicological investigations in rats with R875, morphine, methadone and pethidine.

The following substances were administered subcutaneously to rats of an inbred Wistar strain:

R875: the dextrorotatory isomer of 2:2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine was used as its (+)-tartrate; all doses are expressed in terms of the base; morphine hydrochloride; pethidine hydrochloride; (±) methadone hydrochloride; nalorphine base.

METHOD

Acute Toxicity

Male rats, weighing from 200 to 400 g. were used. At 10 a.m., groups of 10 animals were injected with 1 ml./kg. of an aqueous solution, containing R875, morphine, methadone or pethidine. They were kept in individual containers at room temperature 18°–22°, and observed at 1, 6, 12, 24 hours, 2 and 3 days after injection. Food and water was freely available. A minimum of 5 groups of 10 rats per dose, and 6 dose levels per compound were used.

Analgesic Activity

The analgesic activity was determined using the "hot plate" method, described in part I[2].

Tolerance

Groups of 10 male rats, weighing from 125 to 175 g. were used. Every day at 10 a.m. (except Sunday) the animals were injected with 1 ml./kg. of an aqueous solution containing R875, morphine or pethidine. Five and 10 minutes before, and 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes after each dose, the reaction time of the animals was measured using the "hot plate" method[2].

The rats were kept on a liberal diet in individual containers at room temperature 18°–22°. The definition of "positive response" is given in part I of this series.

Results

Acute Toxicity

The results obtained by using 660, 400, 300 and 310 animals and 10, 8, 6 and 7 dose levels respectively are summarized in Table I and Figure 1.

TABLE I

Acute toxicity in rats

(Subcutaneous injection; observation period: 3 days)

	R875			Methadone			Morphine			Pethidine		
	mg./kg.	Per cent*	*n*†	mg./kg.	Per cent*	*n*†	mg./kg.	Per cent*	*n*†	mg./kg.	Per cent*	*n*†
	0·78	0	60	6·25	0	50	12·5	0	50	50	0	50
	1·56	6·7	60	12·5	2	50	25	2	50	100	2	50
	3·13	15·6	90	17·7	12	50	50	16	50	200	10	50
	6·25	25·6	90	25	30	50	100	4	50	250	12	50
	12·5	24·6	110	50	46	50	200	6	50	300	50	50
	25	20	90	100	96	50	400	34	50	400	80	50
	50	40	70			—	800	62	50	800	100	10
	100	65	40			300	1600	84	50			—
	150	66·7	30						—			310
	200	90	20						400			
			660									
LD10		2			16·5			37			210	
LD50		74			54			590			315	
LD50: LD10		37			3·3			16			1·5	
ED50		0·38			5·14			14·3			41·0	
ED99		0·802			14·5			38·0			108	
LD50: ED50		195			10·5			41			7·7	
LD10: ED50		5·3			3·2			2·6			5·1	
LD10: ED99		2·4			1·1			1·0			1·9	

* Per cent mortality. † Number of rats injected.

Inspection of the figure shows that each "log dose-probit mortality" curve has its own particular shape. For R875 there is no detectable relation between dose and mortality within the 3 to 30 mg./kg. dose range, all four doses giving 15 to 25 per cent mortality. Mortality figures of 4 to 16 per cent are obtained with 30 to 300 mg./kg. morphine, 50 mg./kg. morphine being more toxic (16 per cent) than 100 to 200 mg./kg. (4 to 6 per cent). The pethidine- and methadone-curves are characterized by an inflexion around 40 and 7 per cent mortality respectively. Above 50 per cent mortality all curves are fairly linear, but not parallel. The acute subcutaneous toxicity of these compounds therefore cannot be fully described by one single mathematical symbol.

We propose to use the ratio LD50: LD10 for this purpose, the LD50 value symbolizing the mortality at high dose levels and LD10 the mortality at low dose levels.

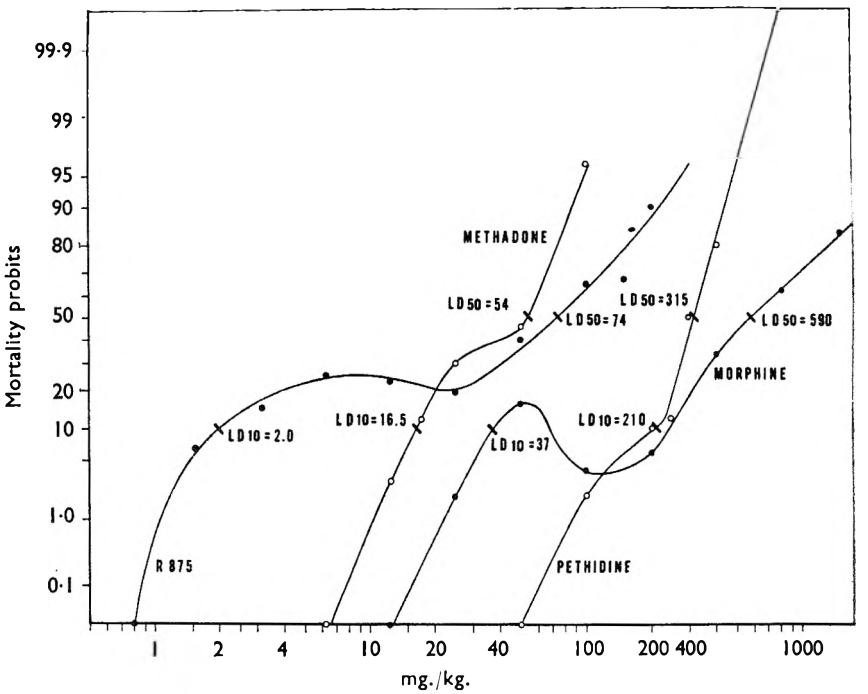

FIG. 1. Acute subcutaneous toxicity in rats (observation period: three days).

For R875 we thus obtain a value of 74:2 = 37, for morphine 590:37 = 16, for methadone 54:16·5 = 3·3 and for pethidine 315:210 = 1·5. A subcutaneous dose of 10 mg./kg. nalorphine produces notable protection against death occuring after simultaneously injected low doses of R875, morphine and methadone (Table II), but not against high doses. In all instances the LD50:LD10 ratios are sharply reduced. Death occuring after low doses thus seems to be caused by a mechanism, which is antagonised by nalorphine, for example respiratory depression.

TABLE II

ACUTE SUBCUTANEOUS TOXICITY IN RATS OF R875, MORPHINE, PETHIDINE AND METHADONE WITH AND WITHOUT NALORPHINE

Substance	mg./kg.	Without nalorphine		With 10 mg./kg. nalorphine	
		Mortality per cent	*n**	Mortality per cent	*n**
R875	6·25	25·6	90	0	50
	50	40	70	4	50
	200	90	20	100	50
Morphine ..	50	16	50	0	50
	550	50	50	2	50
	1600	84	50	82	50
Pethidine ..	300	50	50	60	50
Methadone ..	50	46	50	10	50
	100	96	50	88	50
Nalorphine ..	10	0	50	—	—

* Number of rats injected.

Analgesic Activity

Using the all-or-none criterion of effect, described in the first part of this series, the "total analgesic activity" of R875, morphine, methadone and pethidine was evaluated using a total of 1037 rats.

According to this criterion, R875 was found to be 94 to 124 times more active than pethidine as an "analgesic" in rats, 34 to 42 times more active than morphine, and 12 to 16 times more active than methadone (Table III).

Onset, peak and duration of action are graphically represented in Figure 2, showing the percentage of rats with a reaction time of 30 or more

TABLE III

ANALGESIC ACTIVITY IN RATS

(Subcutaneous injection)

	R875			Methadone			Morphine			Pethidine		
	mg./kg.	Per cent*	n†	mg./kg.	Per cent*	n†	mg./kg.	Per cent*	n†	mg./kg.	Per cent*	n†
	0·25	12	50	3·5	20	40	1	0	10	25	12	50
	0·35	33·3	30	5	47·5	40	2·5	0	10	37·5	41·7	48
	0·50	80	60	7·5	80	30	5	2·5	40	50	66·1	59
	1·0	100	99			—	10	20	50	75	93·2	44
	2·0	100	56			110	15	46·6	58			—
	4·0	100	54				20	89·3	56			201
	5·0	100	9				25	98·8	80			
			—				50	100	54			
			358				100	100	10			
									—			
									368			
ED50	0·38 (0·35–0·42)			5·14 (4·59–5·76)			14·3 (13·0–15·7)			41·0 (36·6–45·9)		
fED50	1·10			1·12			1·10			1·12		
S	1·39 (1·32–1·46)			1·56 (1·30–1·87)			1·54 (1·48–1·60)			1·51 (1·34–1·71)		
fS	1·05			1·20			1·04			1·13		
PR	1·0			0·074 (0·064–0·085)			0·027 (0·024–0·030)			0·0093 (0·0081–0·011)		
PR	13·5 (11·8–15·6)			1·0			0·36 (0·31–0·41)			0·125 (0·11–0·15)		
PR	37·6 (33·6–42·1)			2·78 (2·42–3·20)			1·0			0·35 (0·30–0·40)		
PR	107·9 (93·8–124·1)			7·98 (6·82–9·33)			2·87 (2·49–3·30)			1·0		

* Rats, per cent, showing a "positive analgesic response". † Number of rats surviving the experiment.

seconds ("complete analgesia") at 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes after subcutaneous injection of R875, methadone, morphine and pethidine.

Maximal effects are observed 20 minutes after R875, about 45 minutes after pethidine and about 60 minutes after methadone and morphine. Using atoxic doses, only R875 and, to a lesser extent, pethidine are active 10 minutes after injection.

The duration of action of these analgesics increases with increasing dosage. Two hours after injection, half of the rats showed reaction times of 30 seconds or more at approximately the following dose-levels: 2 mg./kg. R875, 10 mg./kg. methadone, 20 mg./kg. morphine and 100 mg./kg. pethidine. In view of the different shapes of the time-effect curves (Fig. 2), the duration of action of the substances studied, cannot be adequately expressed using simple mathematical symbols. It would

be desirable to make a comparative study of the rates of increase of ED50 values determined at increasing time intervals after injection[6].

Therapeutic Ratios

Using the ED50, ED99, LD50 and LD10 values, obtained as described above, the relative toxicity of R875, morphine, methadone and

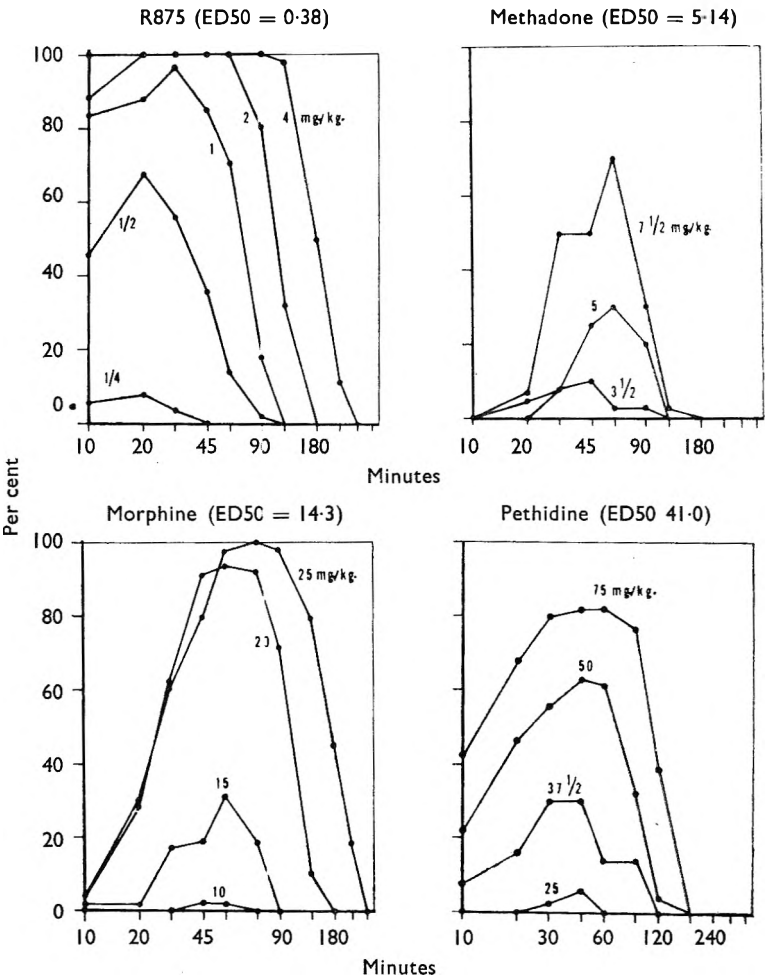

FIG. 2. Duration of "complete" analgesia in rats. (The animals per cent, showing a reaction time greater than 30 seconds at 10, 20, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes after subcutaneous injection of R875, morphine, methadone and pethidine.)

pethidine may be expressed by the following "therapeutic" ratios: LD50:ED50, LD10:ED50 and LD10:ED99.

These ratios, tabulated in Table I, show R875 to possess a lower relative toxicity than morphine, methadone and pethidine, regardless of the criterion used.

In order of increasing relative toxicity, these four analgesics may be ranked as follows:

	LD50:ED50	LD10:ED50	LD10:ED99
R875	1	1	1
Morphine ..	2	4	4
Methadone ..	3	3	3
Pethidine ..	4	2	2

Tolerance

The rate of development of tolerance in rats was studied using the following doses, which are approximately equipotent:

R875	.. 1 mg./kg.	(ED99·9 : LD0·4)
Morphine	.. 25 mg./kg.	(ED98·8 : LD2·0)
Pethidine	..100 mg./kg.	(ED98·5 : LD2·0)

In the first series of experiments a first group of 20 rats received daily injections of R875 (26 days), a second group of 40 rats were daily injected

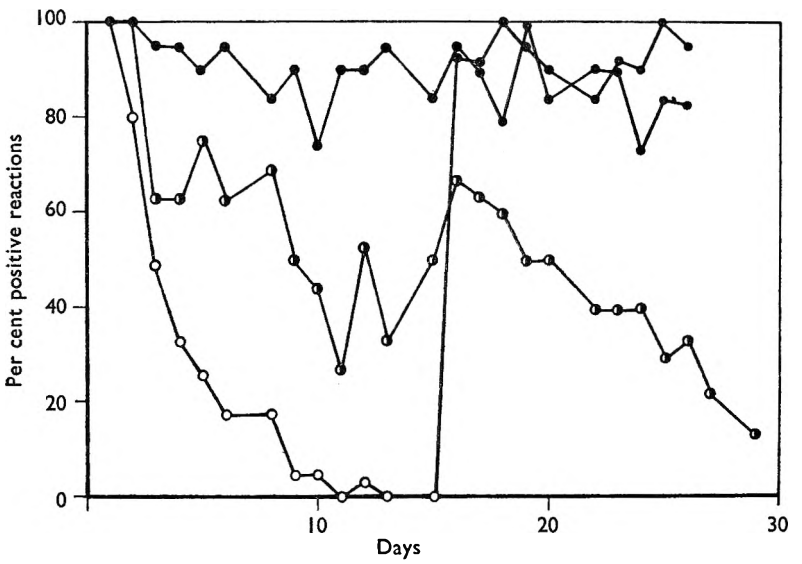

FIG. 3. Tolerance in rats. Daily subcutaneous injections of:
1 mg./kg. R875 (●—●),
25 mg./kg. morphine (○—○), or
100 mg./kg. pethidine (◑—◑).

with morphine (15 days) and a third group with pethidine (20 rats; 29 days).

From the 16th until the 26th day the rats of the morphine-group received daily injections of R875.

As shown in Figure 3, R875 induces much less tolerance than pethidi,ne and pethidine much less than morphine. After 10 days of treatment all animals of the morphine group were completely tolerant, as compared with 50 to 60 per cent tolerance in the pethidine-group and about 10 per cent in the R875 group.

There was little or no cross-tolerance between R875 and morphine, the morphine-tolerant rats responding normally to R875.

With R875, 19 rats out of 20 survived the experiment after 26 days of treatment; after 15 days of treatment with morphine, 38 out of 40 animals survived. Considerable mortality occurred however in the pethidine group, 80 per cent of the rats surviving after 10 days of treatment, 50 per cent after 20 days and only 35 per cent after 29 days. In contrast with R875 and morphine, pethidine caused pronounced local irritation, making it very difficult to inject the animals after a few days of treatment.

The "average weight"-figures of these three groups were computed as follows:

	R875	Morphine	Pethidine
1st day ..	145 g.	165 g.	173 g.
5th day ..	159 g. (+14 g.)	172 g. (+ 7 g.)	170 g. (− 3 g.)
10th day ..	173 g. (+28 g.)	176 g. (+11 g.)	182 g. (+ 9 g.)
15th day ..	184 g. (+39 g.)	184 g. (+19 g.)	195 g. (+22 g.)
20th day ..	190 g. (+45 g.)	—	199 g. (+26 g.)
26th day ..	200 g. (+55 g.)	—	202 g. (+29 g.)

The growth of the animals was obviously much less retarded with R875 than with morphine or pethidine.

The fact that R875-tolerance develops at a much slower rate than morphine-tolerance, is further demonstrated in Figure 4. Sixty rats

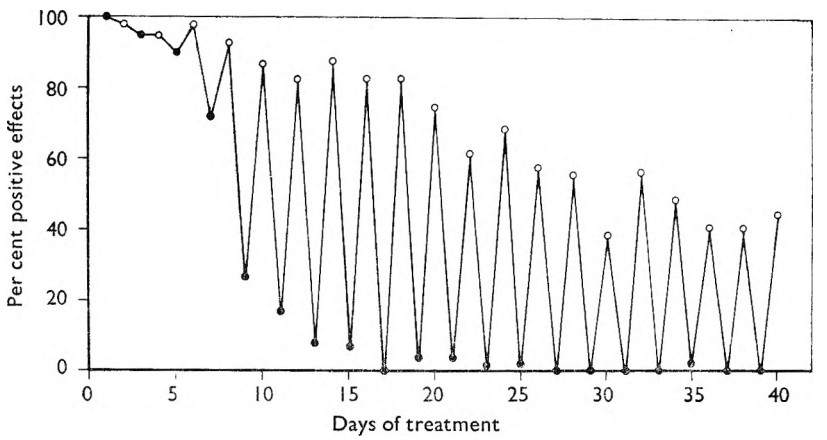

FIG. 4. Tolerance in rats: alternative subcutaneous injections of morphine and R875 in 60 rats.

○—○ R875, 1 mg./kg.
●—● Morphine, 25 mg./kg.

were given subcutaneous doses of morphine (odd days) and of R875 (even days). 49 rats survived after 40 days of treatment. At the end of two weeks all animals were completely tolerant to morphine, whereas 80–90 per cent of the animals still responded to R875.

At the end of 40 days of treatment, about half of the animals were still fully sensitive to R875. Comparing Figures 3 and 4, the impression is gained that tolerance to R875 develops at a faster rate when morphine is

administered on alternate days. It should be noted that R875 does not seem to induce tolerance in man, daily treated for prolonged periods of time[4,5].

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MORPHINE AND HISTAMINE RELEASE

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A wide range of drugs exert analeptic action in dogs treated with morphine; cyclizine, diphenhydramine, chlorcyclizine, Avil, tripelennamine and succinic acid. Benactyzine and Pacatal are without effect. Histamine and an antihistaminase (isoniazid) exert some alerting action in a morphine-treated dog. Morphine induces the signs of histamine release in some dogs. Histamine itself reproduces some of the side effects of morphine. Histamine-treated dogs appear to be analgesic. Rabbits and dogs, behaved similary but cats were different and variable when given antihistamine drugs after morphine. Some of the side actions of morphine are mediated by histamine release.

ONE serious side action of morphine, in addition to respiratory depression, is the production of nausea and vomiting. A recent survey[1] found the incidence in healthy volunteers to be as high as 30 per cent. Emesis is much less frequent in the presence of pain. The incidence of vomiting is not reduced by the use of nalorphine nor by amiphenazole[1].

In an attempt to evaluate the stimulant effect of amiphenazole on the mental depression brought about by morphine in man, the authors endeavoured to suppress the nausea, which interfered with the psychological testing, by the use of an anti-emetic, cyclizine chloride (Marzine). This weak antihistamine drug successfully prevented the nausea and vomiting but unexpectedly antagonised some of the actions of morphine. A description of the clinical observations will be published elsewhere[1]. As a result we investigated the action of certain antihistamine substances and other drugs on morphine-induced narcosis in animals.

The fact that morphine and pethidine could bring about the liberation of histamine under certain conditions had been shown previously by Feldberg and Paton[2], Nasmyth and Stewart[3], and Finer and Partington[4]. Three drugs have been used clinically to antagonise the narcotic effects of morphine, nalorphine[5], *N*-allyl-norcodeine[5] and amiphenazole[6]. There are in addition a number of compounds which can overcome the narcotic action in dogs[7,8]. To the above compounds, there must now be added a new series, of which the main members have antihistamine activity.

We employed three groups of drugs.

Antihistamine Drugs: Cyclizine hydrochloride, diphenhydramine hydrochloride, chlorcyclizine hydrochloride, *p*-amino salicylate of 1-phenyl-1-pyridyl-(2)-3-dimethyl-amino-propane (Avil) and tripelennamine.

Tranquilising Drugs: Benactyzine hydrochloride, and *n*-methyl piperidyl-(3)-methyl phenothiazine (Pacatal).

Stimulants: Methylphenidate and succinic acid in 10 per cent solution.

METHOD

Healthy mongrel dogs of both sexes were given morphine, 10 mg./kg., and sometimes hyoscine (0·5 mg./kg.) intramuscularly. This caused

deep narcosis with complete analgesia so that minor surgery could be performed; the animals did not respond to marked stimuli, such as pricking with a needle or pressure on a limb. Onset was within approximately 20 minutes and lasted for at least 8 hours. Resistance to morphine was occasionally encountered, usually in the absence of hyoscine. No animal was used unless a deep sleep was obtained. It was observed that further doses of the narcotic seldom increased the depth of sleep. The phenomenon of resistance was investigated further in an attempt to correlate our observations and histamine-release; and in these experiments histamine was administered in a dose of 1 mg./kg.

The analeptic substances under test were given at the height of the morphine effect, 1–1¼ hours after the first morphine injection. These were usually given intravenously; some were intramuscular and a few intraperitonealy.

The animals were observed for signs of arousal for approximately one hour, and if these appeared they were watched for four hours or more in case there was a relapse to unconsciousness. Since the narcosis produced by the morphine or morphine with hyoscine was not always of a uniform depth, assessment of the effect of the compounds under investigation must of necessity be subjective. The effects were recorded as *good arousal* when the animal became alert, sat up and walked voluntarily, as *definite arousal*, when the animal sat up of its own accord, but walked only when called or gently moved, and *inactive* when there was no change in behaviour.

Additional experiments were made on rabbits, cats, and rats.

Results

Analeptic Action in Dogs. The results with cyclizine, chlorcyclizine and Avil have been previously recorded[8], and are included for completeness.

Chlorcyclizine, 10 to 30 mg./kg., caused *definite arousal* in 9 of 10 dogs and was *inactive* in one. The animals were alert, but unable to stand. A minimal or absence of effect was seen on respiration. The dogs remained analgesic and did not withdraw their hind limbs when a painful stimulus was applied. They relapsed to their previous level of narcosis within about one hour.

Diphenhydramine, 20 to 25 mg./kg., was given i.v. in two and i.p. in one dog. There was a return to consciousness with minimal effect on respiration. The dose of 25 mg./kg. produced convulsions 5 minutes after injection. The dogs still showed muscular hypotonia and some uncoordinated movements, and could get up and move clumsily in response to stimulus. The animals remained analgesic and relapsed to the previous level after 1 to 2 hours.

Chlorcyclizine, 10 to 30 mg./kg., was given to nine dogs, i.m. in one. The response to injection was an immediate return to consciousness with an improvement in respiration in some. The muscles were still hypotonic. Doses of 25 and 30 mg./kg. produced prolonged and severe convulsions in three morphinised animals, ending fatally in one. Relapse occurred within ½ to 1½ hours, when the animals appeared quite anaesthetised, reverting to the pre-chlorcyclizine level.

Pyribenzamine, 10 to 20 mg./kg., was given to three dogs. After injection there was a return to a normal level of consciousness followed in all cases by gross extension and flexion spasms which lasted 20 minutes. One animal given 20 mg./kg. developed major convulsions and required the administration of barbiturate to counteract this effect. No effect was observed on respiration or analgesia and all relapsed to their previous level of narcosis within one hour.

Avil, 20 to 30 mg./kg., was given to 5 dogs. There was an immediate response with a return to full consciousness in all. Respiration and general muscle tone improved only slightly and they relapsed to their previous level after one hour.

Benactyzine, 0·33 mg./kg., was given to two dogs without effect.

Pacatal, 9 mg./kg., was given to one dog. The injection was followed by a deepening of coma and the animal remained narcotised for a longer period than the controls.

Methylphenidate, 2 mg./kg., was given to two dogs. There was an immediate return to a normal level of consciousness and there was some improvement in muscle tone. In one, respiration appeared to be impaired and in the other it was further depressed. They remained fully awake for about one hour after the injection.

Succinic acid 10 per cent was given to three different groups of animals.

1. Seven dogs were given 5 to 20 ml. i.v. There was a slight improvement in the level of consciousness in all. This consisted of an increased ability to recognise their surroundings and respond to the observer, but in most the animal was unable to get up and walk about, and remained analgesic. The respiration was markedly and consistently improved in all. No other analeptic tested was as active as succinate on respiration.

After about one hour the condition of the animals began to relapse, but they remained in a better condition than the controls.

2. A group of three dogs to which succinate was given as a resuscitative measure, one of which is briefly described. The dog was given morphine then 15 mg./kg. of cyclizine; after this the animal relapsed completely after one hour. Six ml. of 10 per cent succinic acid was then given intravenously and immediately produced a marked improvement in respiration and to a lesser degree in the level of consciousness.

3. Two dogs to which the succinate was given after the dose of morphine, but before the onset of narcosis. A dose of 10 ml. of succinic acid solution was given intraperitoneally 15 minutes after the morphine injection. The onset of morphine effects was not delayed, but the level of consciousness was appreciably better than in the control animals, also respiration was never depressed to the same degree as in the controls.

Effect of Histamine in Dogs

In these experiments 1 mg./kg. of histamine was administered i.v. to six morphinised dogs at various stages of the resultant narcosis. We also attempted to depress histaminase (diamine oxidase) action by injections of isoniazid[9].

1. A group of four dogs was given morphine and hyoscine mixture intramuscularly, and 1 mg./kg. histamine intravenously at the same time.

In all an extremely rapid onset of drowsiness and impairment of sense of balance was seen within two minutes after injection; this was followed within 10 to 20 minutes by complete loss of consciousness and muscle tone. All dogs showed signs of distress and "anxiety" during the injection of histamine; they also vomited, urinated and defecated. In one a short period of violent hyperactivity was observed, followed by a subdued, and finally inert condition.

The duration of narcosis was observed in two animals only, the others being given 30 mg./kg. Avil intravenously. This effected an improvement in the level of consciousness and respiration, which was very markedly depressed. The simultaneous treatment with histamine and morphine causes no significant changes in the duration of morphine narcosis. The onset of narcosis was much more rapid than usual, and the initial depth of narcosis was significantly deeper in all.

2. Two dogs were morphinised, and 1 mg./kg. histamine was injected intravenously one hour later. In both an immediate marked improvement in level of consciousness and muscle tone was observed, in addition general oedema and asthmatic respiration was seen.

This improvement was noticeable only for about five minutes after injection, but the dogs never relapsed completely to the level of consciousness of the controls, and were alert and normally active before the controls, that is at $4\frac{1}{2}$ to 6 hours after morphinisation. No effect was noted on respiration, apart from the transient "asthma".

3. Four dogs were treated with isoniazid.

(*a*) Two animals were given isoniazid alone, in doses of 30 and 45 mg./kg. intraperitoneally. The larger dose causes slight hyperactivity lasting about $1\frac{1}{2}$ hours, the smaller showed no apparent effect on the animal.

(*b*) One dog was given 30 mg./kg. isoniazid intraperitoneally, followed at once by normal morphinisation. For about $\frac{1}{4}$ hour, the dog was normally active, and was able to eat, but it collapsed suddenly and dramatically to a condition resembling the control, and one hour later was even further depressed in its general level of consciousness.

(*c*) One dog was morphinised and 30 mg./kg. isoniazid was injected intraperitoneally about $\frac{1}{4}$ hour later, before onset of morphine narcosis—the level of consciousness appeared similar to that of the control, being initially better, then becoming depressed.

Some transient oedema was noted soon after the injection of isoniazid; the dog recovered at the normal time after the initial morphinisation.

4. Two dogs were given 10 mg./kg. morphine and 0·5 mg./kg. hyoscine twice, intramuscularly.

(*a*) One animal was morphinised, and allowed to return to normal level of consciousness, about 6 hours later, when a second dose of morphine was injected. Slight drowsiness ensued, but the dog was always easily roused. Within ten minutes of the second treatment with morphine, general oedema of the tissues was noted, which lasted more than two hours.

(*b*) A second dog was morphinised, and was given a second dose of morphine one hour later. No apparent change in consciousness, muscle tone or respiration was observed.

These results may be summarised.

1. Histamine given before morphine narcosis appears to increase the rapidity of onset without affecting time of recovery. If injected during this narcosis histamine appears to improve the level of consciousness, and shorten the duration of effect of morphine.

2. Repeated morphinisation after short periods of 1 to 6 hours shows a decrease in effects of oedema and depression of consciousness which are more noticeable as the period between the doses decreases. This corresponds to the known effect on the blood pressure. This tachyphylaxis has been commented upon by Shaw and Bentley[10].

3. Isoniazid injected at same time as, or after morphine, causes an initial improvement in level of consciousness over that in the control, followed by a sudden and dramatic collapse, almost complete loss of consciousness and muscle tone occurring within 2 to 5 minutes. The recovery time is not affected.

4. Histamine in a dose of 1 mg./kg. i.v. causes disturbances in behaviour similar to those caused by morphine. These are, vomiting, defecation, loss of balance, and finally in some animals, drowsiness, in the initial stage of which the dogs were rousable. The animals appeared to be analgesic. Respiratory embarrassment was a common feature.

The Effects of Histamine in Cats

Morphine, 10 to 25 mg./kg., with hyoscine 0·5 to 1·2 mg./kg., was given i.v. (once i.m.) to 5 cats. Two were given Avil, 6 and 20 mg./kg., i.p. about one hour after morphine and hyoscine.

(i) *Morphine and Hyoscine without Avil.* Two cats were given 10 mg./kg. morphine intramuscularly and intraperitoneally respectively, and one cat 20 mg./kg. morphine injected intraperitoneally.

All showed bewilderment. One showed hyperactivity, which occurred about one hour after intramuscular injections of morphine, and lasted about one hour. There was minimal respiratory depression.

(ii) *Morphine and Hyoscine with Avil.* (*a*) Morphine, 20 mg./kg., with hyoscine injected intraperitoneally, no hyperactivity noted. Avil, 20 mg./kg., injected intraperitoneally one hour after morphine caused respiratory depression and fatal muscular tremors, without affecting activity. (*b*) Morphine, 25 mg./kg., with hyoscine injected intraperitoneally, no hyperactivity noted. One hour later Avil, 6 mg./kg., injected intraperitoneally produced no effect on the general level of consciousness, but slight muscular spasms were noted.

(iii) *Histamine.* Two cats were given histamine 5 mg., intravenously, and 9 mg./kg. respectively. The dosage of 5 mg./kg. produced severe shock and asthma, while that of 9 mg. caused some disturbance of the behaviour, but no shock.

The results may be summarised. The effect of morphine on cats is a variable bewilderment response, with hyperactivity in some. The only

antihistamine used as treatment showed no apparent effect on the morphine syndrome, but had fatal side-effects at doses found to be safe in dogs. It is possible that the results with the antihistamine drugs obtained with cats were at variance with those found in dogs because morphine affects cats differently.

The Effect of Morphine on Rabbits

Morphine, 8·3 mg./kg., was given 5 to rabbits intravenously; when drowsy an antihistamine drug was injected intravenously; in one rabbit the antihistamine drug was given before, and again after the morphine.

Avil, 5 mg./kg., was given intravenously to two rabbits; they became more active and moved about freely in the cage, but after an hour reverted to their previous condition, but were not analgesic. In one, Avil, 5 mg./kg., was given i.v. first, then morphine, 16·7 mg./kg. The animal became drowsy; a second injection of Avil, 5 mg./kg., was then given and the animal began to move about and explore its cage.

Cyclizine, 4 mg./kg., was given i.v. to two animals. They became active and started to walk about their cage but the drug was not analgesic. These animals remained active for about $1\frac{1}{2}$ hours.

The effect of antihistamine drugs on the morphine-induced narcosis in rabbits is a temporary recession, similar to that in dogs.

The only other compound which calls for comment is succinic acid.

Succinate was given to a total of 18 dogs that were previously given morphine. In each, the respiratory depression was markedly and consistently improved. This response to succinate was more marked than with any other agents. The effect on respiration was mainly an increase in depth and to a lesser extent in rate. There was also a slight improvement in the level of consciousness, consisting mainly of an increased ability to recognise their surroundings and respond to the observer, but most of the animals were unable to get up and walk about. A similar response has been observed in barbiturised animals[14]. Barrett[15] found that succinate was an effective analeptic in the treatment of morphine poisoning in man. This has also been observed in this laboratory by Gershon and reported by Martin[16]. The analeptic effect of succinate in man has been found to be directly related to the depth of narcosis and the dose required is entirely dependent on the individual and the depth of depression. The succinate may be given with complete safety in the treatment of supposed or assumed morphine or barbiturate poisoning or a combination of the two.

Discussion

Numerous drugs of differing chemical structure can alleviate the narcotic action of morphine in dogs[7,10–12]. The same drugs have a less positive effect on narcosis and respiratory effect in man[1]. Both in man and animals these drugs do not affect the analgesia, as do the specific morphine antagonists like nalorphine. In addition the members of the miscellaneous group have very little effect themselves on the animals in doses which bring about the antagonism.

Without exception the antihistamine drugs tested restore consciousness to dogs given morphine; they effect a slight improvement in respiration, but do not restore normal tone to the limb muscles. In this latter respect they differ from amiphenazole and tetrahydroaminacrin[10]. The administration of histamine before morphine results in an increase in the rapidity of the onset of narcosis and the action of an antihistaminase is consistent with this result as is actions of the antihistamine drugs. Paradoxically histamine injected during narcosis transiently improves the level of consciousness. It would thus appear that at least part of the pharmacology of morphine is mediated by the release of histamine. It should be pointed out however, that the improvement brought about in a morphine-treated dog by the antihistamine drugs is brief and requires large doses. It is possible that the beneficial effect is not due to the antihistaminic activity but to an additional central action.

In many of the animals gross effects of histamine-release were seen, such as redness and oedema of the eyelids, and also of the areas above the eyes; there was also general swelling of a limb around the site of injection. In man, an extensive wheal is often produced around the site of intravenous injection, and a red streaking extending along the draining vein. The production of an urticarial response by morphine when applied to an area of scarification of the skin was described by Sollman and Pilcher[13]. Nasmyth and Stewart[3] found that morphine and codeine elicit a triple response. Similar phenomena can be produced in the human skin with diamorphine, papaverine and pethidine injected intradermally, and these responses are reduced by antihistamines. In cats intravenous morphine produces a sudden fall in blood pressure of 30 minutes duration. This response is similar to that obtained with the injection of histamine. Nasmyth and Stewart[3] also showed that opium alkaloids produced a release of histamine from a rat diaphragm preparation. Feldberg and Paton[2] in experiments on cats confirmed the above findings. They found that morphine and codeine given intravenously caused a fall in arterial blood pressure, and once fallen the blood pressure stays low. This prolonged action is unlike any of the other histamine liberators.

The release of histamine is probably the cause of the idiosyncracy to morphine and pethidine in some people as well as the hypersensitivity observed in those with bronchial asthma. The clinical aspects of histamine release are discussed in another paper in which the anti-emetic effect of cyclizine on morphine-induced emesis is well demonstrated[1].

The compounds investigated did not grossly affect the analgesic state and it seems probable that the pain aleviation mechanism differs from the histamine releasing syndrome.

The analeptic action of succinic acid merits discussion. Succinate is outstanding amongst the other intermediates of the tricarboxylic acid cycle, because unlike others, its dehydrogenation to fumarate proceeds directly to the cytochrome system over an intermediate barbiturate-sensitive flavine system[17]. It is therefore not surprising that it is equally readily oxidised in the presence or absence of barbiturate. It has been shown that certain drugs other than the barbiturates such as chloretone, scopolamine

and diphenyloxazolidinedione are also specific depressants of oxidation of glucose and pyruvate at levels of concentration that do not effect succinate. Quastel and Wheatley[18] also demonstrated that the addition of 0·12 per cent morphine caused an inhibition of oxygen consumption by cerebral tissues. Seevers and Shideman[19] reported the blockade by morphine of the activity of lactic, citric and glucose dehydrogenases, and that succinic and ethanol dehydrogenase were not affected. It has been shown that succinate alone remains oxidisable both in anaesthesia and oxygen poisoning, and thus it may be that this simple aliphatic drug might be a valuable physiological antidote in various conditions of central nervous system depression.

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WATER-SOLUBLE CELLULOSE DERIVATIVES

Factors affecting the Viscosity of Aqueous Dispersions. Part II

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A study has been made of the effects of salts, alcohol and surface-active agents, of contamination by micro-organisms, and of storage at various temperatures on the viscosities of aqueous dispersions of methyl-, methylethyl-, and sodium carboxymethylcellulose. Small concentrations of salts decreased the viscosity of sodium carboxymethylcellulose sols but had no effect on methyl- and methylethylcellulose sols. Larger amounts caused the viscosities of methyl- and methylethylcellulose sols to increase. Industrial methylated spirit, propylene glycol and glycerol increased the viscosities of all dispersions. The effect of sodium lauryl sulphate on sodium carboxymethylcellulose sols was similar to that of salts, and cetrimide precipitated the derivative. Methyl- and methylethylcellulose dispersions increased and then decreased in viscosity with increasing concentrations of sodium lauryl sulphate; cetrimide increased the viscosity. All derivatives were degraded by micro-organisms. The viscosity of preserved dispersions of methyl- and methylethylcellulose altered little over a year. The greatest decreases occurred in the highest viscosity grade samples stored at the highest temperature (30°). All dispersions of sodium carboxymethylcellulose decreased in viscosity over the storage period; storage in light producing the most unstable sols. The pH change of any dispersion on storage was negligible.

In an earlier paper[1] we reported the viscosity variations in aqueous dispersions of three cellulose derivatives under the influence of heat and in the presence of acid and alkali. Considerable changes were observed under those conditions and there were distinct differences in the behaviour of methyl- and methylethylcellulose sols and sodium carboxymethylcellulose sols. The effects on these systems of various added substances, of contamination by micro-organisms and of storage have now been examined.

Materials and Apparatus

The cellulose derivatives used and the method of preparing the dispersions were those previously described.[1] Reagents were of A.R. quality unless otherwise described. Purified water B.P. was used. The viscometer was the Redwood No. 1 and viscosity measurements were made at 25°. pH determinations were made with a Cambridge bench pH meter.

Experimental and Results

Effect of Added Substances

Dispersions of methyl- (medium), methylethyl-, and sodium carboxymethylcellulose (medium) were prepared in suitable concentrations and the additive, dissolved or mixed with the calculated volume of water, incorporated.

Salts. With methylcellulose the presence of sodium chloride in concentrations below 6 per cent w/v was without effect. As the salt concentration was increased, however, the dispersion thickened, becoming turbid and thixotropic, eventually forming a gel. Similar results were obtained with methylethylcellulose (Table I). In contrast, the addition

TABLE I

EFFECT OF ADDED SODIUM CHLORIDE ON THE EFFLUX TIMES OF METHYL- AND METHYLETHYLCELLULOSE SOLS AT 25°

NaCl content per cent w/v	Methylcellulose (medium, approx. 0·8 per cent w/v)		Methylethylcellulose (approx. 3 per cent w/v)	
	Efflux time (sec.)	Appearance	Efflux time (sec.)	Appearance
0	180	Opalescent mucilage	147	Turbid mucilage
4·5	—	—	157	As above
5·25	—	—	177	As above
6·0	181	As above	265	As above
7·5	190	Turbid, tending to separate into two layers—upper thicker than lower. Dispersible on shaking	765	Semi-solid mucilage
9·0	330, 277, 208	Turbid and thixotropic. (See decreases in consecutive efflux times)	—	Soft gel. Syneresis on standing
12·0	—	Gelation. Considerable syneresis on standing	—	Soft gel with syneresis. Milky appearance
15·0	—	As above	—	Gel with considerable syneresis

of small quantities of sodium chloride to sodium carboxymethylcellulose dispersions produced decreases in viscosity, and, as the salt concentration was further increased, the viscosity fell to a minimum (Table II). An

TABLE II

EFFECT OF ADDED SODIUM CHLORIDE ON THE EFFLUX TIME OF SODIUM CARBOXYMETHYLCELLULOSE SOLS AT 25°

NaCl content per cent. w/v	Sodium carboxymethylcellulose (medium, approx. 0·9 per cent w/v)	
	Efflux time (sec.)	Appearance
0	176	Clear mucilage
0·01875	169	As above
0·0375	155	As above
0·075	137	As above
0·15	118	As above
0·3	103	As above
0·75	83	As above
1·5	73	Slight opalescence
7·5	62	Opalescent mucilage
15·0	63	As above

extension of this study to other salts indicated that the efflux time reductions caused by cations of the same valency were approximately equal and that the magnitude of the reduction varied with the valency (Table III).

Alcohol (*I.M.S.* 74 o.p.). Dispersions of all three derivatives behaved similarly on the addition of alcohol, their efflux times increasing with increased alcohol content (Table IV). Further, there was a linear relation between efflux times and alcohol content. Excess of alcohol precipitated the ether without gel formation.

Alcohol and Salts. Of two 20-ml. quantities of a methylcellulose dispersion, one was diluted with an equal volume of water and the other with industrial methylated spirit. A saturated solution of sodium chloride was added slowly from a burette, stirring continuously. Neither sample precipitated after 20 ml. of salt solution had been added. When the experiment was repeated with a sodium carboxymethylcellulose

TABLE III

EFFECTS OF ADDED SALTS ON THE EFFLUX TIME OF SODIUM CARBOXYMETHYLCELLULOSE SOLS AT 25°

Salts in 0·2 *M* concentration	Sodium carboxymethylcellulose (medium, approx. 1 per cent w/v)	
	Efflux time (sec.)	Appearance
—	222	Clear mucilage
Potassium iodide	114	Very faint opalescence
Potassium chloride	117	Very faint opalescence
Potassium thiocyanate ..	115	Clear mucilage
Potassium nitrate	111	Clear mucilage
Potassium acetate	120	Clear mucilage
Potassium bromide	115	Very faint opalescence
Sodium chloride	119	Clear mucilage
Ammonium chloride ..	111	Very faint opalescence
Magnesium chloride	89	Very faint opalescence
Calcium chloride	85	Slight turbidity
Ferric chloride..	—	Gel—insoluble salt

TABLE IV

EFFECT OF ADDED INDUSTRIAL METHYLATED SPIRIT ON THE EFFLUX TIMES OF METHYL-, METHYLETHYL- AND SODIUM CARBOXYMETHYLCELLULOSE SOLS AT 25°

Constitution of sample (ml.)			Efflux times (sec.)		
Dispersion	Water	I.M.S. (74 o.p.)	Methyl- (medium, approx. 0·8 per cent w/v)	Methylethyl- (approx. 3·25 per cent w/v)	Sodium carboxymethyl- (medium, approx. 0·9 per cent w/v)
100	100	—	179	201	184
100	90	10	216	—	212
100	80	20	257	261	—
100	75	25	—	—	266*
100	60	40	334	328	310*
100	50	50	—	—	353*
100	40	60	411	392	—
100	25	75	—	—	420*
100	20	80	472	435	—
100	—	100	548*	528	487†

* Slight initial turbidity, clearing almost immediately.
† The ether at first partly precipitates with milky floccules and gelatinous threads. On shaking and allowing to stand for a few minutes, the dispersion clears.

dispersion, the sample diluted with water remained no more than faintly opalescent when 20 ml. of salt solution had been added, whereas that containing alcohol gave a copious precipitate with less than one ml. of the salt solution.

Propylene glycol and Glycerol. The inclusion of increasing concentrations of propylene glycol (Laboratory Reagent grade) or glycerol caused a progressive thickening of dispersions of all three derivatives.

Surface-active Agents. The effect of adding sodium lauryl sulphate B.P. to sodium carboxymethylcellulose sols was comparable with that of salt addition. With methyl- and methylethylcellulose dispersions, the

reaction was more complex. At first, with increasing quantities of sodium lauryl sulphate, the viscosity rose, as on the addition of sodium chloride. As the concentration of sodium lauryl sulphate was further increased, however, the system began to decrease in viscosity. In the extreme case, the efflux time of a methylcellulose sol was reduced to little more than that of water. The ether appeared to have settled out as minute fibres to give a perfectly clear supernatant layer which, since it showed no turbidity on heating, contained no hydrated methylcellulose. With intermediate concentrations of sodium lauryl sulphate, sols which had undergone a diminution in viscosity tended to thicken again on standing. (Table V.)

TABLE V

EFFECT OF ADDING SODIUM LAURYL SULPHATE TO METHYLCELLULOSE SOLS

Constitution of sample (ml.)			Efflux time (sec.)*	Appearance	Behaviour on heating
Methylcellulose sol (medium, approx. 1·6 per cent w/v)	Water	Sodium lauryl sulphate soln. (10 per cent w/v)			
100	100	—	180	Opalescent mucilage	Gel. Slight syneresis
100	99·5	0·5	230	Turbid mucilage	As above
100	97·5	2·5	315	As above	As above
100	90	10	865	As above	Gel. Considerable syneresis
100	75	25	Initial: 573 After 5 mins.: 685 After 8 hrs.: 762	Less turbid	No gel, but tendency to coagulate
100	50	50	Initial: 54, 60, 66, 74, 79, 85, 90 After 15 mins.: 101, 105, 107 After 16 hrs.: 162 After 24 hrs.: 162	Less turbid	Slight increase in turbidity
100	25	75	46	Almost clear	No change
100	—	100	47	Clear; fibrous sediment	No change

* Single samples.

When an equal volume of a 10 per cent solution of the cationic substance cetrimide B.P. was added to the anionic sodium carboxymethylcellulose sols there was an immediate precipitate. With methyl- and methylethylcellulose dispersions, the addition of increasing concentrations (maximum 5 per cent) of cetrimide led to progressive increases in viscosity.

Effect of Contamination by Micro-organisms

A suspension of micro-organisms isolated from laboratory dust was used to inoculate 6 samples each of methyl-, methylethyl- and sodium carboxymethylcellulose dispersions. One pair of samples consisted of the derivatives in water; another pair contained in addition 0·001 per cent phenylmercuric nitrate; the third pair consisted of the derivatives in water to which 0·012 per cent of thiourea had been added—it having been noted that dispersions containing thiourea were particularly prone

to what was assumed to be bacterial degradation. The efflux times were determined initially and at intervals after incubation at 37°.

Of the six samples of methylcellulose, only two—those containing thiourea—underwent gross viscosity decreases. In these dispersions the percentage decreases in efflux time after two months were 63 and 86 per cent respectively. The most rapid decrease occurred during the first two weeks. All other dispersions retained from 85 to 93 per cent of their original efflux times after storage for two months. Apart from mycelia in one of the thiourea-containing samples, there was no change in the appearance of the dispersions.

Of the six samples of methylethylcellulose, only one showed a gross decrease in efflux time (38 per cent) after storage for two months; this sample consisted of the derivative in water alone. The viscosity dropped most rapidly during the third week after inoculation. The remaining dispersions retained between 83 and 96 per cent of their original efflux times after two months. There was no change in the appearance of any of the dispersions at the end of this period.

All the dispersions of sodium carboxymethylcellulose decreased in efflux time on storage. The two samples containing phenylmercuric nitrate retained 60 and 65 per cent respectively, of their original efflux times after eight weeks, compared with 15 to 27 per cent in the non-preserved dispersions. The appearance of the preserved dispersions was unchanged. Of the remainder, two contained traces of mycelia, and one, a bulky diffusible sediment. In the non-preserved samples the most rapid decrease in efflux time occurred during the first two weeks of storage.

Effect of Prolonged Storage

Dispersions of low, medium and high viscosity grades of methyl- and sodium carboxymethylcellulose and of one grade of methylethylcellulose were prepared in 0·001 per cent phenylmercuric nitrate solution. The concentrations were chosen so as to give an efflux time of about 200 seconds. The containers were clear-glass 8-oz. jars with cardboard-lined screw caps. These were made air-tight by dipping in paraffin wax. Duplicate samples were stored at room temperature, in a refrigerator (4°) and in an oven (30°). Of the two samples stored at room temperature, one was kept on a shelf exposed to daylight and the other in a cupboard in the dark. Efflux times and pH values were determined initially and, thereafter, at periods of 2 weeks, 1, 2, 3, 4, 8 and 12 months. The results may be summarised as follows:

Methylcellulose. The efflux times of dispersions of the low viscosity grade remained constant over a year's storage. The efflux times of the medium viscosity grade increased slightly, mainly during the first two weeks of storage. Only very small increases occurred thereafter, the efflux times at the end of the year being from 10 to 15 per cent above the initial value. With the high viscosity grade, efflux times declined in all samples during the storage period; the efflux times of dispersions stored at room temperature and at 4° were 79 per cent of the original at the end

of the year. Those of dispersions stored at 30° were lower, at 66 per cent of the original. The pH values of the dispersions remained substantially unchanged.

Methylethylcellulose. The efflux times of dispersions remained constant for a year. The pH values of the dispersions remained substantially unchanged.

Sodium carboxymethylcellulose. Except for a darkening in colour in low viscosity grade dispersions, there was no difference between the behaviour of the three grades. All dispersions decreased in efflux time over the storage period. The most stable samples were those stored at 4° which retained 69 to 79 per cent of their original efflux times after a year. The most unstable samples were those stored at room temperature in the light, having final efflux times equivalent to only 39 to 45 per cent of the original, compared with 62 to 74 per cent for similar dispersions stored in the dark. With the exception of the highest viscosity grade, samples stored at 30° showed greater decreases in efflux time than samples stored at room temperature in the dark, the final values being 48 to 52 per cent of the original. The pH values of the dispersions remained substantially unchanged.

A subsequent experiment showed that dispersions of sodium carboxymethylcellulose could decrease in efflux time by over 50 per cent in one month when stored in positions in which they received the maximum amount of direct daylight. The maximum decrease in control samples stored in the dark for the same period was 10 per cent. These losses were augmented rather than diminished by the inclusion of 0·1 per cent sodium metabisulphite, presumably as a result of the lowered pH. It did, however, prevent the darkening of low viscosity grade dispersions on storage.

Discussion and Conclusions

Effect of Added Substances

The viscosity of dispersions of hydrophilic colloids has been variously attributed to the film of solvent which envelopes each particle, to the existence of ramifying micellar aggregates which hinder the flow of the sol, and to the electric charge on the particle (electro-viscous effect). Similarly, the stability of such sols is ascribed both to the electric charge which makes the particles mutually repellent, and to the surrounding hydration sheaths which prevent them approaching each other closely. Since these two mechanisms can operate independently, there are three possible types of disperse system, stabilised by (*a*) an electrical double layer only, (*b*) an hydrated sheath and (*c*) both mechanisms, the last of these three being in general the most stable.[2] Further, it is well known that in order to flocculate systems of the last-mentioned kind both discharge and dehydration are necessary. Thus, the addition of small quantities of electrolyte generally tend to decrease the viscosity of a hydrophilic sol considerably, the particle losing all or part of its charge. The addition of further electrolyte causes no appreciable difference until it is present in a concentration sufficient to exert a dehydrating action,

which leads eventually to coagulation. Alternatively, a sol discharged by a small quantity of electrolyte may be dehydrated and coagulated by adding alcohol, and vice versa.

Sodium carboxymethylcellulose dispersions, although having a high resistance to dehydration by electrolytes (no precipitation occurring even in the presence of an equal volume of a saturated solution of sodium chloride), conform in other respects to the general pattern of behaviour of sols stabilised by both charge and hydration sheath. With methyl- and methylethylcellulose sols, however, the charge (which must be ascribed to the preferential adsorption of hydroxyl ions, as opposed to ionisation of the colloid as in the case of sodium carboxymethylcellulose) does not appear to be of first importance as a stabilising factor. Indeed, it has been suggested that methylcellulose in dispersion is substantially uncharged.[3] This view receives not a little support from the present finding that methyl- and methylethylcellulose sols, unlike those of sodium carboxymethylcellulose, are not "sensitised" to dehydrating agents by the addition of electrolyte in very low concentration.

The addition of alcohol to a hydrophilic sol will partially or completely dehydrate the particles, depending on the quantity added. From one point of view it might be expected that dehydration of sols of the cellulose derivatives would be accompanied by decreases in viscosity, since the effective size of the particles is reduced by removal of their hydration layers. The results show, however, that addition of alcohol leads to an increase in viscosity with all three derivatives. Indeed, with methyl- and methylethylcellulose sols, this increase in viscosity is a feature of dehydration whether it be accomplished by adding salts or alcohol, or (when the concentration is high enough) by heating.[1] But although the effects of alcohol may be interpreted solely in terms of dehydration as far as these two derivatives are concerned, with sodium carboxymethylcellulose sols account has also to be taken of the stabilising effect of the charge. Thus the addition of alcohol must tend not only to dehydrate the particle, but also, probably by adsorption on to its surface, lower the ζ-potential[4].

Whereas sodium carboxymethylcellulose sols behave in a predictable manner towards wetting agents, the reactions of methyl- and methylethylcellulose dispersions are more complex. It seems reasonable to postulate that the increase in viscosity which occurs with these two derivatives in the presence of cetrimide and, at first, with sodium lauryl sulphate, results from a progressive removal of some of the bound water. But the reason for the subsequent decrease in viscosity when the proportion of sodium lauryl sulphate is further raised is not immediately apparent. However, it is well known that soaps are readily adsorbed at interfaces, that compounds containing hydroxyl groups adsorb them very strongly[4], and that adsorbtion can occur with orientation in the reverse sense (the hydrophilic groups being bound to the surface of the particle and the hydrophobic groups being orientated towards the aqueous phase)[2]. The assumption of such an interaction occurring in the present instance would satisfy all the observed results; the cellulose ether particles would be

completely dehydrated, the incipient gel structure destroyed, and the viscosity of the system would become minimal.

Effect of Contamination with Micro-organisms

A survey of the literature reveals several contradictory statements regarding the stability of dispersions of the cellulose derivatives to bacterial attack. One manufacturer of methylcellulose has stated that if contamination occurs some loss of viscosity will result[5]. Osborn and DeKay[6] reported that mucilages of methylcellulose "are stable over a long period of time and require no preservative." The effect of bacterial contamination on the viscosity of dispersions of sodium carboxymethylcellulose and methylethylcellulose has been investigated by Freeman and colleagues[7]. They found that both derivatives suffered degradation through the growth of bacteria widespread in soil and normally present in manufactured soluble cellulose derivatives. Pollok[8] studied the stability of sodium carboxymethylcellulose in detergent solutions and concluded that, in combination with anionic commercial detergents, the derivative was "not likely to cause trouble from bacterial decomposition". Two manufacturers[9,10] of sodium carboxymethylcellulose advise the addition of a preservative when contamination is possible or when dispersions are to be stored for any length of time, but a third states that "only rarely is it desirable to add some form of antiseptic"[11]. The present results show that all three derivatives are susceptible to microbiological attack, while the frequency and extent of the viscosity decreases in sodium carboxymethylcellulose dispersions suggest that they support growth much more readily than do those of the two other ethers. The viscosity losses in preserved samples are evidently the result of an ageing process and have been ascribed to an irreversible chemical breakdown of the derivative[7]. It may be of interest to record that whereas Freeman and colleagues observed that viscosity decreases in contaminated dispersions were most marked between 32 and 61 days after inoculation, in the present work the most rapid decreases generally occurred within 21 days.

Effect of Prolonged Storage

The variations in viscosity of dispersions of the cellulose derivatives on storage is usually discussed in relation to the effects of contamination by micro-organisms. It has been stated, however, that high-viscosity types degrade more rapidly than low-viscosity types, that the changes are irreversible, that at high temperatures the reduction in viscosity continues indefinitely, but at room temperature appears to cease after a time, and that the rate of degradation in a given solvent is independent of concentration. It has further been suggested that all these factors can be explained by assuming that the drop in viscosity is due to a shortening of the average chain-length[12]—the chance of a chain breaking becoming greater as the chain becomes longer, and the effect of a given reduction in molecular weight on viscosity being much greater if the original chain is very long. The literature, generally, however, gives the impression that in the absence of contamination, the viscosity of dispersions of these ethers alters little on

storage, although Freeman and colleagues noted that the viscosity of dispersions, even when preserved, tended to decrease over a period of 61 days. The results now obtained show that the viscosities of dispersions of methyl- and methylethylcellulose remain, in general, remarkably constant over the period of a year. Some loss of viscosity occurred with the high-viscosity grade methylcellulose and illustrates the greater susceptibility to degradation of the longer molecular chain. The increase in viscosity of medium-grade samples, which is considered to be a delayed-hydration effect, was unexpected, since preliminary studies[1] had indicated that hydration was usually complete 24 hours after the dispersion had been prepared. The results with sodium carboxymethylcellulose illustrate once more its relatively high instability and recalling the effect of heat on this derivative[1] the viscosity decreases in the samples stored at 30° are not surprising. It has been reported[12] that cellulose ether dispersions are sensitive to oxygen under the influence of light. The present results show that sodium carboxymethylcellulose sols are particularly susceptible, and that gross decreases in viscosity occur under these conditions. According to Ott the action may be practically eliminated by the addition of oxygen inhibitors, although it is evident that the inhibitors must be carefully chosen to avoid creating conditions which will themselves bring about a decrease in viscosity.

General Observations

It is well known that several substances yield precipitates with cellulose ether dispersions and very many reactions of the kind have been recorded[13–18]. What has seemed an undue preoccupation with precipitates has tended to imply however that in the absence of a visible reaction product the stability and properties of these systems remain unaffected by anything that may be added to them. The present work amply demonstrates the fallacy of such an assumption. For while such purely chemical reactions as the liberation of free carboxymethylcellulose or the formation of insoluble salts can be detected visually, the cellulose derivatives, like other hydrophilic colloids, are subject to changes of a physicochemical nature which although they might not cause the slightest alteration in the appearance of a sol can radically change its properties. The electro-viscous effect of very small concentrations of salts on sodium carboxymethylcellulose dispersions is a striking example. Because many of the pharmaceutical applications of the cellulose derivatives are directly linked to the viscosity of their aqueous dispersions, these reactions—whether they result in a thinning or thickening of the mucilage—are of first importance to the formulator. To what extent such changes influence the efficiency of these ethers as suspending and emulsifying agents remains to be determined, and work is proceeding to that end.

From the results obtained on contaminated dispersions it would seem prudent to include a preservative in all dispersions of sodium carboxymethylcellulose and all dispersions of methyl- and methylethylcellulose which are to be stored for much longer than two weeks. Further, in the absence of a suitable oxygen inhibitor, sodium carboxymethylcellulose

dispersions should be protected from light as well as from elevated temperature if gross viscosity losses on storage are to be avoided.

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THE DIETHYLAMINOETHOXYETHYL ESTER OF DIETHYLPHENYLACETIC ACID. A NEW ANTITUSSIVE AGENT

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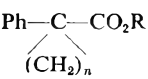
The preparation is described of some dialkylaminoethoxyethyl esters (X) of diethylphenylacetic acid and of some corresponding esters of 1-phenyl*cyclo*pentane-1-carboxylic acid and 4-phenyltetrahydropyran-4-carboxylic acid. These were required for biological study which revealed that the diethylaminoethoxyethyl ester (X; R = R′ = Et) of diethylphenylacetic acid (Oxeladin) was the most potent antitussive agent of the present series.

There is a need for a new antitussive agent resembling codeine as a cough suppressant, but differing from it in not producing constipation even at minimal dose levels. In seeking such a product we turned to derivatives of phenylacetic acid as such compounds often show antispasmodic properties and, less frequently, antitussive action.

Interest in C-substituted phenylacetic acid esters stemmed from the discovery of Trasentin (β-diethylaminoethyl diphenyl acetate) in 1936. In 1938 Halpern[1] described a series of esters of C-alkylphenyl acetic acids in which the alkyl group ranged from ethyl to heptyl. The preparation of many other compounds of related type followed in quick succession[2–5]. Diethylaminoethyl phenylethyl acetate, first described by Halpern, was introduced as an antitussive, some years ago.

In 1946 Rubin and Wishinsky[6] described the preparation of a novel series of esters of C-disubstituted acetic acids in which the α-carbon atom formed an integral part of

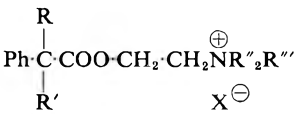


(I)

a *cyclo*hexane (I; $n = 5$) or *cyclo*hexanone nucleus and soon afterwards Weston[7] reported the synthesis of a closely related group of esters, and in particular the diethylaminoethyl esters of structure (I; $n = 2$ or 5). The series was further extended by Tilford, van Campen, Jr., and Shelton[8] who, *inter alia*, esterified 1-phenyl*cyclo*hexane-1-carboxylic acid (I; $n=5$, R = H) with dimethylaminoethoxyethanol. They failed to study the antitussive properties of the resulting ester, but reported that it was more potent than the corresponding dimethylaminoethyl ester against the acetylcholine and barium chloride induced spasms of the isolated rabbit jejunum, but less active in antagonising the effect of histamine on the isolated guinea pig intestine. The antitussive properties of the dimethylaminoethoxyethyl ester were subsequently reported by Levis, Preat and Moyersoons[9], who examined a number of derivatives of type (I; $n = 2$, 3, 4 and 5), and

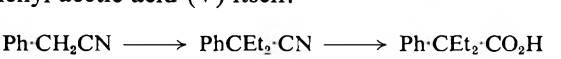
concluded that optimal activity was attained in the diethylaminoethoxyethyl ester of 1-phenyl*cyclo*pentane-1-carboxylic acid (I; $n = 4$; $R = \cdot CH_2 \cdot CH_2 \cdot O \cdot CH_2 \cdot CH_2 \cdot NEt_2$).

Our own studies in the antitussive field have dealt largely with esters of diethylphenyl acetic acid. Very little information is available on the biological properties of compounds of this type apart from a publication by Jensen, Hansen and Hammer[10] describing the spasmolytic activity of the quaternary esters (II).



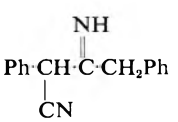
(II) (where R = R′ = Et)

We therefore prepared a series of typical esters containing the dialkyl aminoethyl moiety. Their study as antitussives by David and his colleagues[11] revealed the superiority of the dialkylaminoethoxyethyl ester type (X), which was chosen for detailed study. Before embarking on this project, however, it proved necessary to develop a preparative method for diethylphenyl acetic acid (V) itself.

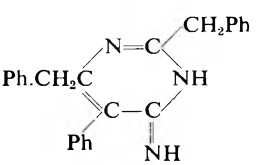


(III) (IV) (V)

αα-Diethylphenylacetonitrile (IV) was prepared after Bodroux and Taboury[12] by heating the disodium derivative of phenylacetonitrile with ethyl iodide in ether. It was converted in low yield into the acid (V) by heating with amyl alcoholic potassium hydroxide. In our hands αα-diethylphenylacetonitrile (IV) was obtained in 85 per cent yield by condensation of an excess of ethyl chloride with the disodium salt of phenylacetonitrile, prepared *in situ* using sodium in liquid ammonia. The product so formed was free from unchanged nitrile and from the monoethylated material, but was admixed with very small quantities of toluene and of 3-phenylpentane, which were readily separated by distillation. The last compound probably arises through reduction of the product (IV) by the reducing metal. Alternatively, the alkylated nitrile (IV) was obtained in 85 per cent yield by condensing phenylacetonitrile with ethyl chloride or bromide in toluene using sodamide as condensing agent. Attempts to effect the alkylation with potassium *tert.*-butoxide in *tert.*-butanol led to the formation of substantial quantities of phenylacetonitrile self-condensation products such as (VI) and (VII).



(VI)



(VII)

Hydrolysis of αα-diethylphenylacetonitrile (IV) to the required acid (V) was accomplished in 90 per cent yield employing potassium hydroxide in ethylene glycol under reflux.

Conversion of diethylphenylacetic acid (V) into the dialkylaminoethoxyethyl esters (X) was effected in several ways:

$$\underset{\text{(VIII)}}{Ph{\cdot}CEt_2{\cdot}COCl} + \underset{\text{(IX)}}{HO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2NRR'} \longrightarrow \underset{\text{(X)}}{Ph{\cdot}CEt_2{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2NRR'}$$

(i) by reaction of the acid chloride (VIII) with the appropriate amino alcohol (IX) in a solvent such as benzene, when the product (X) separated as the hydrochloride;

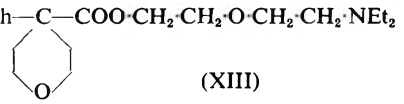
(ii) by interaction of the acid (V) with the chloroalkamine (XI) in *iso*-propanol[13]:

$$\text{(V)} + \underset{\text{(XI)}}{Cl{\cdot}CH_2CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NRR'} \rightarrow \text{(X)}$$

and (iii) by condensing the potassium salt of αα-diethylphenylacetic acid with ββ′-dichlorodiethyl ether in ethylene glycol solution to give the β-chloroethoxyethyl ester (XII), which passed smoothly into the required basic ester (X) on reaction with the appropriate amine.

$$\underset{\text{(XII)}}{Ph{\cdot}CEt_2{\cdot}CO{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2Cl}$$

By applying the above methods, and in particular method (iii), we obtained the diethylamino-, ethylmethylamino-, ethylpropylamino-, di-*n*-butylamino-, di-*n*-hexylamino-, *N*-pyrrolidino-, *N*-Δ^3-piperideino- and *N*-piperidinoethoxyethyl esters of diethylphenylacetic acid. In addition, we prepared the *N*-pyrrolidino-, *N*-Δ^3-piperideino- and *N*-piperidinoethoxyethyl esters of 1-phenyl*cyclo*pentane-1-carboxylic acid [*cf.* (I; $n = 4$)] as well as the diethylaminoethoxyethyl ester of 4-phenyltetrahydropyran-4-carboxylic acid (XIII).



(XIII)

Biological study of the above esters by David and his colleagues showed clearly that the diethylaminoethoxyethylester (X; R = R′ = Et) of diethylphenylacetic acid was a potent antitussive agent and the most active compound of the present series[11]. The free base (X; R = R′ = Et) formed a colourless, odourless oil, b.p. 140° at 0·1 mm., which was quite stable on exposure to air. Its salts with hydrochloric, carbonic, benzoic, cinnamic and other organic acids were difficult to crystallise. The citrate, in contrast, formed small needles, m.p. 90° to 91° which were readily soluble in water (> 80 per cent w/w at 25°) to give solutions of only moderate acidity. It thus proved suitable for pharmaceutical presentation.

Experimental

αα-Diethylphenylacetonitrile (IV). (*a*) To *ca.* 2 litres of liquid ammonia in a 5-litre three-necked flask fitted with stirrer and Drikold condenser was added 2 g. of ferric nitrate as catalyst followed portionwise by sodium metal (200 g. = 8·7 g. atoms). The mixture was stirred for 1 hour after addition of sodium was complete to ensure formation of sodamide. Phenylacetonitrile (468 g., 4 moles) was then run in with stirring over about 20 minutes. As soon as the addition of nitrile was complete a solution of ethyl chloride (650 ml. = *ca.* 9 mole) in toluene was added slowly. The apparatus was surrounded by a Drikold bath and the rate of addition of ethyl chloride regulated to control the exothermic reaction. The mixture was stirred for one hour after the addition of ethyl chloride was complete and the ammonia was then allowed to evaporate freely from the mixture (usually overnight).

A current of nitrogen was passed through the apparatus and water (2 litres) added slowly to the mixture with stirring.

The toluene layer was separated and the toluene removed under reduced pressure on the steambath. The residual oil was distilled at reduced pressure to yield the product as an oil (590 g.), b.p. 145° at 30 mm. or 155° at 45 mm. $n_D^{20} = 1{\cdot}5040$. Found: C, 82·9; H, 8·3; N, 8·2. Calc. for $C_{12}H_{15}N$: C, 83·2; H, 8·7; N, 8·1 per cent.

(*b*) (With Mr. A. J. Thomas, B.Sc.) To a stirred suspension of powdered sodamide (32 g.; 0·8 mole) in dry toluene (250 ml.) in a flask fitted with a Drikold condenser, was added phenylacetonitrile (46·8 g., 0·4 mole) over 5 minutes, the temperature of the mixture rising to *ca.* 45°. The mixture was then cooled to 5° and a solution of ethyl chloride (64·5 g., 1·0 mole) in toluene (100 ml.) added at such a rate that the temperature of the mixture was kept at *ca.* 5°. The addition took 45 minutes and stirring was then continued for 3 hours at 5° to 10°. The reaction was then completed by heating at 70° for 2 hours. The mixture was cooled to room-temperature, water (500 ml.) added with stirring and the aqueous layer just acidified with hydrochloric acid. The toluene layer was separated and washed with water. After removal of the toluene the residual oil was distilled under reduced pressure and the product (59 g.) collected at 144° to 146° at 30 mm. $n_D^{20} = 1{\cdot}5040$.

αα-Diethylphenylacetic acid (V). To αα-diethylphenylacetonitrile (450 g.) in a stainless steel vessel was added a solution of potassium hydroxide (320 g.) in ethylene glycol (1300 ml.). The mixture was heated to reflux and water (36 ml.) distilled off. The mixture was refluxed gently for 24 hours when evolution of ammonia had ceased. It was cooled, poured into water and the mixture extracted with a little light petroleum (b.p. 60° to 80°) to remove non-acidic material. Acidification of the aqueous layer with hydrochloric acid, followed by cooling, yielded diethylphenylacetic acid. This was collected and purified by crystallisation from 50 per cent aqueous methanol. After drying at 50° to 60° in a current of air it had m.p. 93° to 94°. As an alternative the acid could be purified by rapid distillation and had b.p. 195° at 30 mm.

2-(*β-Chloroethoxy)ethyl diethylphenylacetate* (XII). To sodium hydroxide (40 g.) in ethylene glycol (300 ml.) was added diethylphenylacetic acid (192 g.) which was dissolved by warming. 2:2′-Dichlorodiethyl ether (300 g., 2·1 moles) was added and the mixture heated under reflux for 1 hour, when it was poured into water and the oily layer separated and distilled at reduced pressure to remove unchanged 2:2′-dichlorodiethyl ether. The required *chloro-ester* (254 g.) had b.p. 130° at 0·5 mm. Found: C, 64·3; H, 7·6; Cl, 12·2. $C_{16}H_{23}O_3Cl$ requires C, 64·3; H, 7·8; Cl, 11·9 per cent.

2-(*β-Diethylaminoethoxy)ethyl diethylphenylacetate* (X; R = R′ = Et). A mixture of 2-(β-chloroethoxy) ethyl diethylphenylacetate (120 g.), diethylamine (84 g. = 2·9 mole equivs.) and *n*-hexanol (480 ml.) was heated under reflux for 6 hours.

The hexanol and excess diethylamine were removed in steam. The cooled residue was acidified with hydrochloric acid and extracted with toluene to remove non-basic impurities. It was then basified with aqueous sodium hydroxide and extracted with toluene. After removal of the toluene the residual oil was distilled at 0·5 mm. to yield the *product* (111 g.) as an oil, b.p. 150°. Found: C, 71·6; H, 9·8; N, 4·2. $C_{20}H_{23}O_3N$ requires C, 71·5; H, 9·9; N, 4·2 per cent.

The base (150 g.) was added with stirring to a hot solution of citric acid monohydrate (100 g.) in ethyl acetate (2·5 litres). The *citrate* separated in small needles on cooling. After crystallisation from ethyl acetate it had m.p. 90° to 91°. Found: C, 58·8; H, 7·8; N, 2·7. $C_{26}H_{41}O_{10}N$ requires C, 59·2; H, 7·8; N, 2·7 per cent.

2-(*β-N-Piperidinoethoxy)-ethyl diethylphenylacetate* (X; NRR′ = piperidino). A mixture of 2-(β-chloroethoxy)ethyl diethylphenylacetate (20 g.) and piperidine (30 g.) was heated gently under reflux for 1 hour. The cooled mixture was acidified with dilute hydrochloric acid and extracted with ether to remove non-basic material. The aqueous phase was basified with aqueous sodium hydroxide and extracted with ether. The ether extract was dried and the ether and excess of piperidine removed under reduced pressure. The residual oil was fractionated *in vacuo* to yield the product, b.p. 165° at 0·5 mm.

The base was converted into the *citrate*, m.p. 73° after crystallisation from ethyl acetate. Found: C, 59·6; H, 7·5; N, 2·7. $C_{27}H_{41}O_{10}N$ requires C, 60·1; H, 7·7; N, 2·6 per cent.

2-(*β-N-Δ³-Piperideino ethoxy)ethyl diethylphenylacetate* (X; NRR′ = Δ³-piperideino) was prepared as for the preceding analogue using Δ³-piperideine in place of piperidine. The *citrate* had m.p. 80° after crystallisation from ethyl acetate. Found: C, 60·3; H, 7·1; N, 2·4. $C_{27}H_{39}O_{10}N$ requires C, 60·3; H, 7·3; N, 2·6 per cent.

2-(*β-N-Pyrrolidinoethoxy)ethyl diethylphenylacetate* (X; NRR′ = pyrrolidino). A mixture of 2-(β-chloroethoxy) ethyl diethylphenylacetate (30 g.) and pyrrolidine (20 g.) was heated under reflux for 3 hours then cooled and acidified with dilute hydrochloric acid. After extraction with ether to remove non-basic impurities the aqueous layer was basified with aqueous sodium hydroxide and extracted with ether. The ether was

dried, the solvent removed and the oil distilled at 1·0 mm. to yield the *product*, b.p. 170°. Found: C, 71·4; H, 9·9; N, 4·1. $C_{20}H_{31}O_3N$ requires C, 72·0; H, 9·4; N, 4·2 per cent.

2-(*β*-N-*Ethylmethylaminoethoxy*)*ethyl diethylphenylacetate* (X; R = Et, R′ = Me). A mixture of 2-(*β*-chloroethoxy)-ethyl diethylphenylacetate (25 g.), ethylmethylamine (30 ml.) and *n*-hexanol (200 ml.) was heated under reflux for 6 hours, when hexanol and excess amine were distilled off. The cooled residue was acidified with dilute hydrochloric acid, extracted with ether to remove unchanged chloroester, then basified with aqueous sodium hydroxide. The separated oil was extracted with ether and isolated by distillation *in vacuo*. The *product* had b.p. 140° at 0·5 mm. Found: C, 70·9; H, 9·5; N, 4·3. $C_{19}H_{31}O_3N$ requires C, 71·0; H, 9·7; N, 4·4 per cent.

2-(*β*-N-*Ethylpropylaminoethoxy*)-*ethyl diethylphenylacetate* (X; R = Et, R′ = *n*-Pr) was an oil, b.p. 155° at 0·2 mm. Found: C, 72·1; H, 10·0; N, 4·4. $C_{21}H_{35}O_3N$ requires C, 72·2; H, 10·1; N, 4·0 per cent.

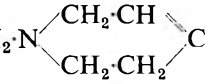
2-(*β*-N-*Di*-n-*butylaminoethoxy*)*ethyl diethylphenylacetate* (X; R = R′ = *n*-Bu). A mixture of 2-(*β*-chloroethoxy) ethyl diethylphenylacetate (20 g.) di-*n*-butylamine (30 g.) and *n*-hexanol (80 ml.) was heated under reflux for 20 hours. The hexanol and excess of di-*n*-butylamine were removed in steam. The residue was cooled and basified directly since the hydrochloride of the product is soluble in organic solvents. Extraction with ether yielded the *product*, isolated as an oil, b.p. 169° at 1·0 mm. Found: C, 73·7; H, 10·3; N, 3·4. $C_{24}H_{41}O_3N$ requires C, 73·6; H, 10·6; N, 3·6 per cent.

2-(*β*-N-*Di-n-hexylaminoethoxy*)*ethyl diethylphenylacetate* (X; R = R′ = *n*-hexyl). A mixture of 2-(*β*-chloroethoxy)ethyl diethylphenylacetate (20 g.) and di-*n*-hexylamine (35 g.) in *n*-hexanol (40 ml.) was heated under reflux for 2 hours. The *product* had b.p. 190° at 0·03 mm. Found: C,74·4, H, 10·6; N, 3·1. $C_{28}H_{49}O_3N$ requires C, 75·1; H, 11·0; N, 3·1 per cent.

2-(*β*-N-*Pyrrolidinoethoxy*)-*ethyl*-1-*phenyl*-1-cyclo*pentane carboxylate* (I; $n = 4$, R = $-CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}N\begin{matrix}CH_2-CH_2\\ \\ CH_2-CH_2\end{matrix}$).—A solution of 1-phenyl*cyclo*pentanoyl chloride (12 g.) in chloroform (12 ml.) was treated with 2-(*β*-*N*-pyrrolidinoethoxy)ethanol (12 g.) in chloroform (12 ml.) and the mixture heated under reflux for 1 hour. After removal of the chloroform the residue was acidified with dilute hydrochloric acid and extracted with ether to remove by-products. The aqueous phase was basified with aqueous sodium hydroxide and extracted with ether. The *product* was an oil, b.p. 168° to 172° at 0·4 mm. Found: C, 72·4; H, 8·7; N, 4·0. $C_{20}H_{29}NO_3$ requires C, 72·5; H, 8·8; N, 4·2 per cent.

2-(*β*-N-*Piperidinoethoxy*)-*ethyl*-1-*phenyl*-1-cyclo*pentane carboxylate* (I; $n = 4$, R = $CH_2CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}N\begin{matrix}CH_2-CH_2\\ \diagdown\quad\diagup\\ CH_2-CH_2\end{matrix}CH_2$).—Condensation of 1-phenyl *cyclo*pentanoyl chloride with 2-(*β*-*N*-piperidinoethoxy)-ethanol

was carried out as described in the previous example. The base was obtained as an oil b.p. 180° at 0·6 mm. It was converted to its *hydrobromide* which separated from ethanol-ether in needles, m.p. 89°. Found: C, 59·5; H, 7·6; N, 3·2. $C_{21}H_{32}O_3NBr$ requires C, 59·1; H, 7·6; N, 3·3 per cent.

2-(β-N-Δ³-*Piperideinoethoxy*)-*ethyl*-1-*phenyl*-1-cyclo*pentane carboxylate* (I; $n = 4$, R = $-CH_2CH_2\cdot O\cdot CH_2\cdot CH_2\cdot N$ ).—This was prepared as for the pyrrolidino analogue by condensing 1-phenyl*cyclo*pentanoyl chloride with 2-(β-*N*-Δ³-piperideinoethoxy)-ethanol. The base was converted directly into the *hydrobromide* which separated from ethanol-ether in needles, m.p. 84°. Found: C, 59·0; H, 7·2; N, 3·0; Br, 18·9. $C_{21}H_{30}O_3NBr$ requires C, 59·4; H, 7·1; N, 3·3; Br, 18·9 per cent.

2-(β-N-*Diethylaminoethoxy*)-*ethyl*-4-*phenyl-tetrahydropyran*-4-*carboxylate* (XIII).—4-Phenyltetrahydropyranoyl chloride was prepared by a slight variation of the method used by Eisleb[14].

Condensation of the acid chloride (11·5 g.) with 2-β-diethylaminoethoxy) ethanol (11·5 g.) in dry chloroform (25 ml.) yielded the base as an oil b.p. 179° at 0·2 mm.

The base was converted into the *hydrobromide* which separated from *iso*propanol/ether in needles, m.p. 101°. Found: C, 55·7; H, 7·5; N, 2·8 Br, 18·1. $C_{20}H_{32}O_4NBr$ requires C, 55·8; H, 7·5; N, 3·3; Br, 18·6 per cent.

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ANALGESICS. PART I. SOME ARYLOXYPROPANOLAMINES

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The synthesis is described of some 3-aryloxy-2-hydroxypropylamines and *N*-(ω-aryloxyalkyl)-piperidines, which were required for study as analgesics.

Our object was the preparation of an orally active generally useful analgesic agent free from the limitations of acetylsalicylic acid. To this end we selected the aryloxypropanolamine type (II) for initial study. Some compounds of this class had previously been examined in these Laboratories for local anaesthetic activity[1] (cf. Ing and Ormerod[2]), when the marked analgesic properties of certain members had become apparent (cf. Fourneau[7,8]). The series was therefore extended as indicated in the present communication. Some miscellaneous types bearing a formal resemblance to the aryloxypropanolamines (II) were also prepared and form the subject of Part II. At this stage a publication appeared by Way and others[3] describing the analgesic activity of some substituted salicylamides, which led us to extend our studies to the salicylic acid derivatives described in Part III. Finally, the preparation of aryloxypropanolamines from $\triangle^3$-piperideine was undertaken. These proved superior in analgesic activity to the earlier compounds and one of them, 1-$\triangle^3$-piperideino-3-*o*-toloxypropan-2-ol hydrochloride ("Tolpronine") was selected for fuller evaluation. Biological studies were conducted by Dr. A. David and his colleagues, who kindly provided the analgesic data.

Work on the aryloxypropanolamines began with a study of 3-*o*-chlorophenoxy-2-hydroxypropylamine as a model compound. Mono-alkyl and alkaryl derivatives (II; Ar = *o*-Cl·C_6H_4–, R = H, R′ = alkyl or alkaryl) were first prepared and the series thereafter extended to the dialkyl derivatives (R and R′ = alkyl) and to compounds in which the primary amino-group was replaced by a cyclic structure such as piperidine or morpholine. *o*-Methoxyphenylhydroxypropylamines were next synthesised, as well as two 3:4:5-trimethoxyphenoxypropylamines which contained the trimethoxyphenyl group, characteristic of mescaline. The series (see Table II) was completed with some *o*-toloxy- and substituted phenoxyhydroxypropylamines.

The preferred route to the foregoing compounds (II) lay in the condensation of the 3-aryloxy-1:2-epoxypropanes (I) with the appropriate amines:

$$\underset{\text{(I)}}{ArO{\cdot}CH_2{\cdot}CH\!\!<\!\!\begin{matrix}O\\ \diagdown\\ CH_2\end{matrix}} + HNRR' \longrightarrow \underset{\text{(II)}}{ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2NRR'} \qquad \text{(i)}$$

The synthesis of the intermediate 3-aryloxy-1:2-epoxypropanes (I) needed for this purpose required initial study. Though generally obtained by condensing the phenol with 2:3-epoxypropyl chloride in aqueous alkali,

their preparation is by no means as simple as is implied in the literature[4,5], in that the products obtained depend not only upon the experimental conditions employed for the condensation, but also upon the nature of the phenol. We have, therefore, studied the condensation in some detail.

Reaction between a phenol and 2:3-epoxypropyl chloride is slow and incomplete in aqueous solutions containing a catalytic quantity (0·1 mole) of sodium hydroxide. The main product in this case is the 3-aryloxy-2-hydroxypropyl chloride (III) (cf. reaction (ii)), admixed with much starting material and only minimal quantities of the required epoxide (I).

$$\mathrm{Ar{\cdot}OH + CH_2 \cdot CH{\cdot}CH_2Cl \longrightarrow ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2Cl} \quad \text{(ii)}$$

(III)

The reaction thus resembles that which obtains with basic catalysts in non-aqueous media[6] when the chlorides (III) are obtained, though in much higher yields.

Increase in the amount of alkali employed leads to increased condensation between the phenol and 2:3-epoxypropyl chloride to give the chloride (III) which then undergoes partial conversion to the epoxide (I). The yield of epoxide obtained reaches a maximum for many phenols when 1·2 moles of alkali hydroxide are present. Reaction presumably occurs between the phenolate ion and 2:3-epoxypropyl chloride in the following way:

$$\mathrm{ArO^- + CH_2{-}CH{\cdot}CH_2{-}Cl \longrightarrow ArO{\cdot}CH_2{\cdot}CH{-}CH_2 + Cl^-} \quad \text{(iii)}$$

Still higher concentrations of alkali lead to the formation of appreciable quantities of the bis-1:3-aryloxypropan-2-ols (IV), which are otherwise obtained in only small amounts.

$$\mathrm{ArO^- + CH_2 \cdot CH{\cdot}CH_2{\cdot}OAr \longrightarrow ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}OAr} \quad \text{(iv)}$$

(IV)

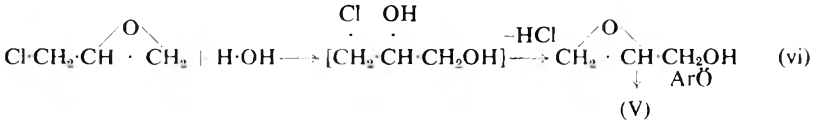
Their (IV) formation is facilitated by increase of reaction temperature.

The glyceryl ether (V) forms a further product of the condensation. Its production is unlikely to proceed *via* the epoxide (I) (reaction (i)) as we have found that the latter (I; Ar = *o*-tolyl) is hydrolysed only to the extent of about 1·5 per cent after 16 hours treatment with 0·4N aqueous sodium hydroxide.

$$\mathrm{ArO{\cdot}CH_2{\cdot}CH \cdot CH_2 + H{\cdot}OH \longrightarrow ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH{\cdot}OH} \quad \text{(v)}$$

(V)

Its (V) formation may well take place through condensation of the phenol with 2:3-epoxypropyl alcohol ("glycidol"), itself formed by hydrolysis of the 2:3-epoxypropyl chloride (reaction (vi)).



(vi)

The rates at which the foregoing transformations occur are influenced by the substituents in the aryl nucleus of the phenol and varying proportions of by-products (III, IV and V) are obtained from different phenols under the same reaction conditions. Thus for example electron-releasing substituents such as methyl, which lower the pseudo-acidic character of the phenol, depress reactivity. This is illustrated by the condensation of phenol and *o*-cresol with 2 : 3-epoxypropyl chloride in normal aqueous

TABLE I

CONDENSATION OF PHENOL (2·0 MOLES) WITH 2 : 3-EPOXYPROPYL CHLORIDE (2·2 MOLES) IN ALKALINE SOLUTION. YIELDS OF PRODUCTS IN G.

Mole equiv. of potassium hydroxide	3-Phenoxy-1 : 2-epoxypropane b.p. 80° at 1·0 mm.	3-Phenoxy-2-hydroxypropyl chloride b.p. 112° at 1·0 mm.	1 : 3-Bis-phenoxy propane-2-ol m.p. 81°
0·1	103 b.p. 90 to 115° at 1·0 mm.		0
0·5	230 b.p. 96 to 112° at 1·0 mm.		8
1·0	162	58	9
1·2	195	3	25
2·0	113·5	0	42·5

sodium carbonate solution at room temperature for 48 hours. The former gives a 30 per cent and the latter only a 6 per cent yield of the corresponding 3-aryloxy-2-hydroxypropyl chloride. Here however the retarding steric effect of the *ortho* methyl group must also be taken into consideration.

The 3-aryloxy-1 : 2-epoxypropanes (I), prepared as described above, condense readily with amines (see reaction (i)) to give the required aryloxyhydroxypropylamines[7–11]. Primary amines additionally give low yields of tertiary bases of type (VI) as by-products.

$$(Ar{\cdot}O{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2)_2N\,R$$

(VI)

Secondary bases such as piperidine may give complex products of unknown constitution if excess of epoxide (I) is present in the mixture. In addition, quaternary salts of type (VII) may be isolated. These result from reaction between the product (II) and the 3-aryloxy-2-hydroxypropyl chloride (III). The last compound is generally present to the extent of about 5 per cent in samples of the epoxide (I) unless these have been specially purified by further distillation. This additional purification step, however, is not regarded as necessary.

TABLE II

ARYLOXYPROPANOLAMINES

$Ar{\cdot}O{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}NR\,R'$

Ar	R	R′	Base (B) Hydrochloride (H)	m.p. or b.p. °C.	Formula	Found per cent				Required per cent			
						C	H	N	Cl	C	H	N	Cl
Phenyl	Et	Ph	B	156/0·1 mm.	$C_{19}H_{25}O_3N$	74·8	7·9			75·2	7·8		
	Piperidine		B	55	$C_{24}H_{41}O_3N$	71·6	8·9	6·0		71·4	9·0	6·0	
		O-Acetyl	H	124/0·1 mm.	$C_{18}H_{23}O_3N$	69·7	8·3	4·9		69·3	8·4	5·1	
	Pyrrolidine		B	124/0·4 mm.	$C_{13}H_{19}O_2N$			5·9				6·3	
			H	121–122	$C_{13}H_{20}O_2NCl$	60·1	7·7	5·4		60·5	7·8	5·4	
o-Tolyl	H	*iso* Bu	H	132	$C_{14}H_{24}O_2NCl$	61·4	8·5	5·4		61·4	8·8	5·1	
	H	*sec.*-Octyl	H	98	$C_{18}H_{32}O_2NCl$	65·7	9·5	4·4		65·5	9·8	4·2	
	H	Allyl	B	68	$C_{13}H_{19}O_2N$	70·0	8·5	6·3		70·5	8·7	6·3	
	H	Allyl	H	93	$C_{13}H_{20}O_2NCl$	60·6	7·8			60·5	7·8		
	H	Benzyl	B	84	$C_{17}H_{21}O_2N$	75·2	7·9			75·2	7·8		
	H	β-Phenylethyl	H	130	$C_{18}H_{24}O_2NCl$	67·7	7·1	4·5		67·2	7·5	4·4	
	H	*cyclo*Hexyl	H	166	$C_{16}H_{26}O_2NCl$	64·6	8·7			64·1	8·4		
	H	2-Pyridyl	B	138	$C_{15}H_{18}O_2N_2$	69·9	7·2	11·4		69·8	7·0	10·9	
	Benzyl	Benzyl	B	206/0·4 mm.	$C_{31}H_{31}O_2N$	80·2	7·5	3·7		79·7	7·5	3·9	
	β-Hydroxyethyl	β-Hydroxyethyl	B	230/0·1 mm.	$C_{14}H_{23}O_4N$	63·2	8·6	5·6		62·4	8·6	5·2	
	Piperidine		B	56–58	$C_{15}H_{29}O_2N$			5·5				5·6	
			H	128–130	$C_{15}H_{30}O_2NCl$	63·1	8·5	4·9	12·2	63·0	8·5	4·9	12·4
	2-Methylpiperidine		B	80–82	$C_{16}H_{25}O_2N$	73·5	9·4	5·2		73·0	9·6	5·3	
			H	162–164	$C_{16}H_{26}O_2NCl$	64·2	8·5	4·5	11·9	64·1	8·7	4·7	11·8
	4-Methylpiperidine		B	54–55	$C_{16}H_{25}O_2N$	73·3	9·5	5·1		73·0	9·6	5·3	
	3-Ethoxycarbonylpiperidine		H	139–140	$C_{18}H_{28}O_4NCl$	60·4	7·9	3·6		60·4	7·9	3·9	
	Morpholine		B	68–69	$C_{14}H_{21}O_3N$	67·0	8·5	5·6		66·9	8·4	5·6	
	Pyrrolidine		B	126/0·4 mm. solidified	$C_{14}H_{21}O_2N$	71·2	9·2	5·8		71·4	9·0	6·0	
			H	108–110	$C_{14}H_{22}O_2NCl$	61·8	8·1	5·4		61·8	8·2	5·2	
(β-Methyl)	Pyrrolidine		B	120/0·6 mm.	$C_{15}H_{23}O_2N$	72·2	9·2	5·6		72·2	9·3	5·6	
			H	161–163	$C_{15}H_{24}O_2NCl$	62·9	8·4	4·9	12·2	63·0	8·5	4·9	12·4
	H	3:4:5-Trimethoxy	H	154–156	$C_{19}H_{32}O_5NCl$	59·2	6·9			59·4	6·8		
p-Tolyl	Et	Et	B	132/0·5 mm.	$C_{14}H_{23}O_2N$	71·4	9·3	5·8		70·8	9·8	5·9	
2:4-Xylyl	H	Et	B	103	$C_{13}H_{21}O_2N$	70·1	9·7	6·2		69·9	9·5	6·3	
			H	118	$C_{13}H_{22}O_2NCl$	60·3	8·6	5·4	13·2	60·1	8·5	5·4	13·7
	H	*n*-Pr	B	102	$C_{14}H_{23}O_2N$	70·4	9·7	5·8		70·8	9·8	5·9	
			H	111	$C_{14}H_{24}O_2NCl$				13·0				13·0
	H	*n*-Bu	B	75	$C_{15}H_{25}O_2N$	71·7	9·7	5·6		71·7	10·0	5·6	
			H	134	$C_{15}H_{26}O_2NCl$	62·8	8·9		12·4	62·6	9·1		12·3

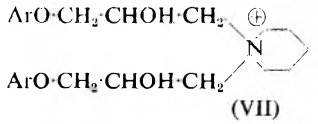
TABLE II—*continued*

Ar	R	R′	Base (B) Hydrochloride (H)	m.p. or b.p. °C.	Formula	Found per cent				Required per cent			
						C	H	N	Cl	C	H	N	Cl
o-Chorophenyl	H	Me	B	85–88									
			Picrate	139	$C_{19}H_{17}O_9N_4Cl$	43·6	4·0	12·2	7·7	43·2	3·9	12·6	8·0
	H	Et	B	85–88	$C_{13}H_{18}O_2NCl$			5·7	15·3			6·1	15·5
	H	*n*-Pr	B	93–95	$C_{15}H_{19}O_2NCl$			5·8				5·8	
	H	*iso*Pr	B	88–90									
			Picrate	170–171	$C_{18}H_{21}O_9N_4Cl$	45·8	4·3	11·5	7·6	45·7	4·5	11·9	7·5
	H	*n*-Bu	B	64–68	$C_{15}H_{20}O_2NCl$	60·2	7·8	5·4	13·4	60·5	7·8	5·4	13·8
			H	100–102	$C_{15}H_{21}O_2NCl_2$				24·2				24·1
	H	*sec.*-Bu	B	140/0·5 mm.									
			H	150–152	$C_{15}H_{21}O_2NCl_2$	53·5	7·6	4·6	24·1	53·0	7·2	4·8	24·1
	H	*n*-Amyl	B	64–68	$C_{16}H_{22}O_2NCl$	62·2	8·0	5·2	13·0	61·8	8·2	5·2	13·1
	H	*iso*Amyl	B	137/0·2 mm.									
			H	119–121	$C_{16}H_{23}O_2NCl_2$	54·6	7·4	4·5	23·2	54·5	7·5	4·5	23·0
	H	Allyl	B	62–65	$C_{13}H_{16}O_2NCl$			6·0				5·8	
	H	Phenyl	B	184/0·1 mm.	$C_{17}H_{16}O_2NCl$			5·2				5·0	
	H	*p*-Tolyl	B	75–78									
			Picrate	130–133	$C_{25}H_{21}O_9N_4Cl$	50·5	4·2	10·5	6·6	50·7	4·1	10·8	6·8
	H	Benzyl	B	85–87	$C_{19}H_{18}O_2NCl$	65·8	6·1	4·6	12·2	65·9	6·2	4·8	12·2
	H	β-Phenyl *iso*Propyl	B	184–188/0·3 mm.	$C_{18}H_{22}O_2NCl$			4·4	11·5			4·4	11·1
	H	*cyclo*Hexyl	B	81–84									
			Picrate	204–205	$C_{24}H_{25}O_9N_4Cl$	49·2	4·7	10·8	7·0	49·2	4·9	10·9	6·9
			H	178–179	$C_{15}H_{23}O_2NCl_2$			4·4	21·5			4·4	22·2
	H	$-CONH_2$	B	113–115	$C_{10}H_{13}O_2N_2Cl$	49·4	5·2	11·2	14·5	49·1	5·4	11·5	14·5
	Me	Me	B	110–114/0·4 mm.	$C_{21}H_{18}O_2NCl$			5·9	15·1			6·1	15·5
	n-Bu	*n*-Bu	B	136–140/0·1 mm.	$C_{17}H_{28}O_2NCl$			4·4				4·5	
	n-Hexyl	*n*-Hexyl	B	192–194/0·5 mm.	$C_{21}H_{36}O_2NCl$			4·2	10·1			3·8	9·6
	n-Octyl	*n*-Octyl	B	206/0·3 mm.	$C_{25}H_{44}O_2NCl$			3·4	7·9			3·3	8·3
	Piperidine		B	71–73	$C_{14}H_{20}O_2NCl$			5·0	13·2			5·2	13·2
								5·1	12·9				
			H	145–147	$C_{14}H_{21}O_2NCl_2$	54·6	6·9	4·5	23·7	54·9	6·9	4·6	23·2
			Aspirin Salt	114–116	$C_{23}H_{26}O_6NCl$	61·8	6·1	3·2		61·4	6·3	3·1	
			Salicylate	119	$C_{21}H_{26}O_5NCl$			3·4	9·2			3·4	8·7
			"O" acetyl	136/0·1 mm.	$C_{16}H_{22}O_4NCl$			4·6				4·5	
	Morpholine		B	66–69	$C_{13}H_{18}O_2NCl$	57·3	6·4	4·8		57·4	6·7	5·2	
	Pyrrolidine		B	132/0·5 mm.; 60–61	$C_{13}H_{18}O_2NCl$	60·8	6·8	5·4		61·0	7·1	5·5	
p-Chlorophenyl	H	*n*-Bu	B	74	$C_{13}H_{20}O_2NCl$	60·8	7·8	5·4	13·9	60·5	7·8	5·4	13·8
	H	*iso*Amyl	H	182	$C_{14}H_{22}O_2NCl_2$	54·9	7·4	4·1	23·0	54·5	7·5	4·5	23·0
	H	Benzyl	B	100	$C_{16}H_{18}O_2NCl$	66·4	6·2	4·7		65·9	6·2	4·8	
	H	β-Phenyl *iso*Propyl	H	163	$C_{18}H_{23}O_2NCl_2$	61·1	6·5	3·5		60·7	6·5	3·9	
	H	*Cyclo*Hexyl	B	109	$C_{15}H_{28}O_2NCl$	63·0	7·8	4·5		63·5	7·8	4·9	
	Me	Phenyl	B	180/0·3 mm.	$C_{14}H_{18}O_2NCl$	66·2	6·3	4·9		65·9	6·2	4·8	
	Piperidine		B	73–75	$C_{14}H_{28}O_2NCl$	62·1	7·5	5·1	13·3	62·3	7·5	5·2	13·2
	Morpholine		B	68	$C_{13}H_{18}O_3NCl$	56·9	6·6	4·8		57·4	6·7	5·2	

TABLE II—*continued*

Ar	R	R′	Base (B) hydrochloride (H)	m.p. or b.p. ° C.	Formula	Found per cent				Required per cent			
						C	H	N	Cl	C	H	N	Cl
2:4-Dichlorophenyl		Piperidine	B	94–96	$C_{14}H_{19}O_2NCl_2$	55·8	5·7	4·8	23·2	55·3	6·3	4·6	23·4
2:4:6-Trichlorophenyl	H	*n*-Bu	B	109	$C_{13}H_{18}O_2NCl_3$	48·3	5·7	3·9	33·3	47·8	5·6	4·3	32·6
o-Bromophenyl		Piperidine	B	72	$C_{14}H_{20}O_2NBr$	53·1	6·3	4·4	25·1[1]	53·2	6·4	4·5	25·5[1]
			H	146–149	$C_{14}H_{21}O_2NClBr$	48·3	6·1	3·9	32·7[2]	47·9	6·1	4·0	32·9[2]
			Acetyl Salicylate	119–121	$C_{23}H_{26}O_6NBr$	55·8	5·9	3·0		55·9	5·7	2·8	
o-Iodophenyl		Piperidine	H	163–165	$C_{14}H_{21}O_2NCII$	42·2	5·3			42·3	5·3		
2-Chloro-4-ethoxycarbonylphenyl		Piperidine	H	158–160	$C_{17}H_{23}O_4NCl_2$			3·6	18·6			3·7	18·8
2:6-Dichloro-4-ethoxycarbonylphenyl		Piperidine	B	74–76	$C_{17}H_{23}O_4NCl_2$			4·0				3·7	
			H	143–145	$C_{17}H_{21}O_4NCl_2$	48·8	5·7	3·4		48·3	5·9	3·3	
p-Methoxycarbonylphenyl	H	Benzyl	B	121	$C_{18}H_{21}O_4N$	68·8	6·8	4·4		68·5	6·7	4·4	
		Piperidine	H	179	$C_{16}H_{22}O_4NCl$	58·0	7·0	4·7		58·2	7·3	4·3	
o-Hydroxyphenyl		Piperidine	B	140/0·1 mm.	$C_{14}H_{21}O_2N$	67·6	8·6	6·0		66·9	8·4	5·6	
			H	188	$C_{14}H_{22}O_2NCl$	58·0	7·4	4·9	12·3	58·4	7·7	4·9	12·5
p-Acetamidophenyl		Piperidine	H	174–176	$C_{16}H_{23}O_3N_2Cl$	58·5	7·4	8·3	10·6	58·4	7·7	8·5	10·8
2-Naphthyl	H	Et	B	100	$C_{18}H_{19}O_2N$	73·5	7·7	5·7		73·5	7·8	5·7	
			H	187	$C_{18}H_{20}O_2NCl$	63·4	7·2		12·5	64·0	7·1		12·6
2-(1-Chloronaphthyl)-*cyclo*Hexyl		Piperidine	H	174–175	$C_{18}H_{23}O_2NCl_2$	60·5	5·8	3·9	20·3	60·7	6·5	3·9	19·9
		Piperidine	B	110/0·3 mm.	$C_{24}H_{27}O_2N$	69·0	11·2	5·9		69·7	11·3	5·8	
Benzyl		Piperidine	H	138–140	$C_{19}H_{22}O_2NCl$	63·2	8·2	5·1	12·4	63·0	8·5	4·9	12·4
o-Methoxyphenyl	H	Et	B	71	$C_{20}H_{19}O_3N$	64·1	8·5	6·3		64·0	8·5	6·2	
			H	94	$C_{12}H_{20}O_3NCl$	55·2	7·4		13·5	55·0	7·7		13·6
	H	*n*-Pr	B	86	$C_{13}H_{21}O_3N$	65·0	8·8	5·8		65·2	8·9	5·9	
			H	100	$C_{13}H_{22}O_3NCl$	56·6	8·1		12·9	56·6	8·0		12·9
	H	*n*-Bu	B	67–70	$C_{14}H_{23}O_2N$	66·2	9·0	5·6		66·4	9·2	5·5	
	H	*iso*-Bu	B	135/0·3 mm.	$C_{14}H_{23}O_2N$	66·3	9·2	5·5		66·4	9·2	5·5	
	H	Benzyl	B	94	$C_{17}H_{21}O_2N$	71·1	7·3	4·7		71·0	7·4	4·9	
		Piperidine	B	134–136/0·1 mm.	$C_{19}H_{29}O_2N$	67·5	8·6			67·9	8·7		
	Et	Et	B	122/0·1 mm.	$C_{14}H_{29}O_2N$	66·2	9·2	5·7		66·4	9·2	5·5	
	n-Bu	*n*-Bu	B	140/0·05 mm.	$C_{20}H_{41}O_2N$	70·2	10·0	4·2		69·9	10·1	4·5	
p-Methoxyphenyl	H	Et	B	75	$C_{20}H_{19}O_3N$	64·1	8·1			64·0	8·5		
			H	167	$C_{12}H_{20}O_3NCl$	54·9	7·7	5·4	13·6	55·0	7·7	5·4	13·6
	H	*n*-Bu	B	78	$C_{14}H_{21}O_2N$	66·2	8·8	5·1		66·4	9·2	5·5	
			H	175	$C_{14}H_{24}O_3NCl$	58·0	8·5		12·1	58·0	8·4		12·3
	H	Benzyl	B	101	$C_{17}H_{21}O_3N$			4·7				4·9	
		Piperidine	B	50	$C_{13}H_{23}O_3N$	68·1	8·5			67·9	8·7		
			H	164	$C_{15}H_{24}O_3NCl$	59·7	7·7			59·7	8·0		
3:4:5-Trimethoxyphenyl		Piperidine	H	143–145	$C_{17}H_{26}O_5NCl$	56·1	7·8	3·6	9·8	56·4	7·8	3·9	9·8
		Morpholine	H	192–194	$C_{16}H_{24}O_6NCl$	52·3	7·0	4·0	9·7	52·8	7·2	3·9	9·8

1 = Bromine 2 = Total halogen



(VII)

A heterocyclic base such as piperazine gives mono (VIII; R — N N—R′; R = $ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2$; R′ = H) and bis (IX; R = R′ = ArO $CH_2{\cdot}CHOH{\cdot}CH_2$) products, the former being readily separated from the latter by distillation or *via* the solid condensation products (X) obtained from (VIII) and carbon bisulphide.

Biological study of the compounds listed in Table II showed that in general monoalkylamino- and dialkylamino-derivatives (I; R and/or R′ = alkyl) were weakly analgesic only. Increase in analgesic activity occurred, however, on replacing the primary amino-group by cyclic structures such as piperidine, pyrrolidine and morpholine, but not by piperazine. Although 3-*o*-chlorophenoxy-, 3-phenoxy- and 3-*o*-toloxy-2-hydroxypropylpiperidine proved effective by the subcutaneous route, their analgesic potency fell to low levels on oral administration. This may well have been associated with the presence of secondary hydroxyl groups in the compounds which might be expected to undergo oxidation in the body. We therefore prepared the aryloxyamines (XI) (see Table III)

$ArO{\cdot}(CH_2)_n{\cdot}N$ (XI) Ar = *o*-Cl– and *p*-$Cl{\cdot}C_6H_4$. n = 3, 4 and 5

but these proved only weakly active.

Experimental

Condensation of phenols with 2:3-*epoxypropyl chloride.* Condensations were carried out at room temperature for 8 to 20 hours. 10 to 20 per cent excess of 2:3-epoxypropyl chloride was generally employed, although the yield of 3-aryloxy-1:2-epoxypropane obtained was increased by using a 50 to 100 per cent excess of the chloroepoxide. The products were normally isolated for use by fractional distillation under reduced pressure through a 12″ to 18″ Vigreux column. They were generally contaminated with about 5 per cent of the corresponding 3-aryloxy-2-hydroxypropyl chloride, which could be removed, if desired, by a second fractionation.

Condensation of o-*cresol with* 2:3-*epoxypropyl chloride.* A typical condensation is described below. The conditions used do not lead to an optimum yield of 3-*o*-toloxy-1:2-epoxypropane.

2:3-Epoxypropyl chloride (305 g., 3·3 mole) was added over 10 minutes to a stirred solution of *o*-cresol (324 g., 3·0 mole) in N potassium hydroxide (3 litres) which had been cooled to 15°. After the addition was complete the mixture was allowed to warm to 20° and stirred at this temperature for 16 hours. The oil was separated and the aqueous layer extracted with three 300 ml. portions of chloroform. The combined extracts were washed with water, neutralised by the addition of a few drops of glacial acetic acid and rewashed with water. The chloroform extract was concentrated and the residue distilled at 2 mm. to yield:

Fraction (i) b.p. 104 to 107°. 343 g. (70 per cent). (ii) b.p. 107 to 140°. 97 g. (iii) b.p. 156 to 174°. 40 g. (iv) <10 g. residue.

TABLE III

ARYLOXYALKYLAMINES

$$Ar{\cdot}O{\cdot}(CH_2)_n{\cdot}N\langle\ \rangle$$

Ar	n	Base (B) Hydrochloride (H)	m.p. or b.p. ° C.	Formula	Found per cent				Required per cent			
					C	H	N	Cl	C	H	N	Cl
o-Chlorophenyl	3	B	115/0·1 mm.									
		H	157	$C_{14}H_{21}ONCl_2$	57·9	6·8	4·8	24·6	57·9	7·3	4·8	24·5
	4	B	120/0·1 mm.									
		H	128	$C_{15}H_{23}ONCl_2$	59·6	7·6	4·4	23·2	59·2	7·6	4·6	23·3
	5	B	122/0·01 mm.	$C_{16}H_{24}ONCl$	68·2	8·4	4·9	12·8	68·2	8·6	5·0	12·6
p-Chlorophenyl	3	B	114–116/0·1 mm.	$C_{14}H_{20}ONCl$	65·9	7·7	5·4	14·2	66·2	8·0	5·5	14·0
	4	B	132–134/0·1 mm.	$C_{15}H_{22}ONCl$			4·9				5·2	
	5	B	142–144/0·1 mm. (solidified)	$C_{16}H_{24}ONCl$	67·8	8·4			68·2	8·6		
o-Methoxyphenyl	3	B	124/0·2 mm.									
		H	155–156	$C_{15}H_{21}O_2NCl$	62·3	8·5	5·1	12·6	63·0	8·5	4·9	12·4
		Picrate	157	$C_{21}H_{26}O_9N_4$	52·7	5·5	11·8		52·7	5·5	11·7	
	5	B	136–138/0·2 mm.	$C_{17}H_{27}O_2N$	73·6	9·5	5·1		73·6	9·8	5·1	
		H	140–143	$C_{17}H_{28}O_2NCl$			4·8	11·7			4·5	11·3

Fraction (i) contained 0·68 per cent of chlorine, corresponding to a chlorohydrin content of about 3·8 per cent. Fraction (ii) contained 7·2 per cent of chlorine corresponding to a chlorohydrin content of about 40·7 per cent.

Fraction (i). A sample (27 g.) was suspended in water (300 ml.) containing sodium carbonate (17·5 g.), and the mixture heated under reflux for 6 hours. The product crystallised after cooling to 0° for several hours. It was collected and purified by crystallisation from carbon tetrachloride yielding white fluffy needles of 3-*o*-toloxy-propane-1 : 2-diol (22 g.), m.p. 70 to 72°, unchanged on admixture with an authentic specimen.

Fraction (ii). 87 g. was treated with diethylamine (64 g.) and the mixture heated under reflux for 4 hours. After cooling the product was dissolved in chloroform and washed well with water. The chloroform was distilled off and the residue distilled at 1 mm. to give 1-*diethylamino*-3-o-*toloxypropan*-2-*ol*, b.p. 138° (93·5 g.). The product formed a *methiodide* which separated from a mixture of ethanol and ether in white prisms, m.p. 151 to 153°. Found: C, 47·5; H, 6·8. $C_{15}H_{26}O_2NI$ requires C, 47·5; H, 6·9 per cent.

Fraction (iii), on trituration with ether yielded 3-*o*-toloxypropane-1 : 2-diol (24 g.) which had m.p. 70 to 72° after crystallisation from carbon tetrachloride.

Fraction (iv) consisted of 1 : 3-bis-*o*-toloxy-propan-2-ol, b.p. 170° at 0·5 mm. on refractionation.

Condensation of phenol with 2 : 3-*epoxypropyl chloride in aqueous potassium hydroxide solution.* The method used is illustrated below: Phenol (188 g., 2 moles) and 2 : 3-epoxypropyl chloride (204 g., 2·2 moles) were suspended in water (2 litres) containing varying amounts of potassium hydroxide and stirred for 16 hours at 20 to 23°. After extraction with chloroform, unchanged material was distilled off and the residue distilled at reduced pressure. The results obtained are summarised in Table I.

Condensation of phenol with 2 : 3-*epoxypropyl chloride in aqueous sodium carbonate solution.* A mixture of phenol (188 g., 2 moles), 2 : 3-epoxypropyl chloride (204 g., 2·2 moles) and sodium carbonate (106 g., 1 mole) in water (2 litres) was stirred for 20 hours at 22°. After extraction with chloroform and washing, the residue was distilled at 0·5 mm. to yield 3-*phenoxy*-2-*hydroxypropyl* chloride (116·5 g., 31 per cent) b.p. 106°. Found: Cl, 18·6. $C_9H_{11}O_2Cl$ requires Cl, 19·0 per cent. No other product was isolated apart from unchanged phenol).

When the reaction was repeated and stirring continued for 70 hours, the yield of chlorohydrin was 35 per cent (b.p. 118 to 120° at 1·3 mm.).

Condensation of o-*cresol with* 2 : 3-*epoxypropyl chloride in aqueous sodium carbonate solution.* A mixture of *o*-cresol (216 g., 2 moles), 2 : 3-epoxypropyl chloride (204 g., 2·2 moles) and sodium carbonate (116·6 g., 1·1 mole) in water (1 litre) was stirred at 20° for 46 hours. After removal of unchanged material, distillation at 0·5 mm. yielded only 23 g. (6 per cent) of 3-*o*-toloxy-2-hydroxypropyl chloride, b.p. 110°.

Condensation of 3-*aryloxy*-1 : 2-*epoxypropane with amines.* (*a*) Pressure apparatus, as recommended in earlier publications, proved unnecessary and low boiling amines such as methylamine were condensed in ethanolic or benzene solution for about 16 hours at room temperature. Propylamine and higher amines were condensed in the absence of solvent in a reflux apparatus. The reaction mixture was maintained at 20° by water cooling, when the product usually crystallised after several hours.

(*b*) Cyclic amines such as piperidine reacted so vigorously that it was necessary to employ a diluent such as light petroleum or ethanol.

Condensation of 3-o-*toloxy*-1 : 2-*epoxypropane with ethylamine.* 3-*o*-Toloxy-1 : 2-epoxypropane (164 g., 1 mole) was added to a solution of ethylamine (100 g., 2·2 moles) in benzene (250 ml.) cooled in ice-water. The mixture was left overnight under reflux and surrounded by cold water (15 to 20°). The mixture was heated for 2 hours to remove excess of ethylamine and on cooling deposited 1-*ethylamino*-3-o-*toloxypropane*-2-*ol*, m.p. 87° (150 g.). Found: N, 6·8. $C_{12}H_{19}O_2N$ requires N, 6·9 per cent.

The mother liquors were concentrated and the residue distilled at 0·2 mm. to yield N-*ethyl*-*bis*-(2-*hydroxy*-3-o-*toloxypropyl*)-*amine* (42 g.) as a clear viscous oil, b.p. 210° after refractionation. Found: C, 70·8; H, 8·3; N, 3·7. $C_{22}H_{31}O_4N$ requires C, 70·8; H, 8·4; N, 3·8 per cent.

N-(2-*Hydroxy*-3-o-*chlorophenoxy*)-*propylpiperazine* and NN′-*bis*-(2-*Hydroxy*-3-o-*chlorophenoxy*)-*propylpiperazine.* To a solution of piperazine hexahydrate (155·2 g., 2 moles equivs.) in ethanol (200 ml.) was added 3-*o*-chlorophenoxy-1 : 2-epoxypropane and the mixture heated under reflux for 30 minutes. It was diluted with water to near turbidity and allowed to stand overnight. The solid which separated (23 g.) was NN′-*bis*-(2-*hydroxy*-3-o-*chlorophenoxy*)-*propylpiperazine* which had m.p. 182° after washing with boiling ethanol. Found: C, 58·0; H, 6·0; N, 6·0; Cl, 15·7. $C_{22}H_{28}O_4N_2Cl_2$ requires C, 58·0; H, 6·2; N, 6·2; Cl, 15·6 per cent. Its *dihydrochloride* had m.p. 230 to 233° after crystallisation from aqueous ethanol. Found: C, 50·1; H, 5·5; N, 5·4; Cl, 26·9. $C_{22}H_{30}O_4N_2Cl_4$ requires C, 50·0; H, 5·7; N, 5·3; Cl, 26·9 per cent.

After removal of the bis compound, the original filtrate was diluted with water and extracted with chloroform. The extract was washed with water, the chloroform distilled off and the residue distilled at 0·3 mm. to yield a viscous oil, b.p. 164 to 170°.

Part of the gum (10 g.) was dissolved in ethanol (40 ml.), stirred at room temperature and treated with carbon disulphide (5 ml.). A gum separated which solidified after standing overnight. The solid was collected, drained, suspended in ethanol and treated with hydrochloric acid gas. The solid dissolved after heating under reflux for 30 minutes. The ethanolic solution was concentrated to yield the dihydrochloride of N-(2-*hydroxy*-3-o-*chlorophenoxy*)-*propyl piperazine* which had m.p. 205 to 208° after crystallisation from ethanol. Yield 8·6 g. Found: C, 46·0; H, 6·0; N, 7·7. $C_{13}H_{21}O_2N_2Cl_3$ requires C, 45·4; H, 6·2; N, 8·1 per cent The original gummy base yielded a mono*picrate* which separated from ethyl acetate and had m.p. 195 to 197°. Found: C, 45·8; H, 4·3; N, 14·3. $C_{19}H_{22}O_9N_5Cl$ requires C, 45·6; H, 4·4; N, 14·0 per cent.

3-cyclo*Hexyloxy*-2-*hydroxy-propyl chloride.* To a mixture of *cyclo*hexanol (300 g., 3 moles) and 2:3-epoxypropyl chloride (92·5 g., 1 mole), concentrated sulphuric acid (2·3 ml.) was added dropwise with intermittent shaking over 10 minutes. The mixture was heated on the steam bath for 40 hours. It was then cooled, washed with water, dilute sodium carbonate solution and again with water and distilled at reduced pressure.

After recovery of unchanged *cyclo*hexanol (176 g.) there was obtained:

Fraction (i) 19 g., b.p. 38 to 76° at 0·25 mm.

„ (ii) 72 g., b.p. 76 to 80° at 0·25 mm.

Fraction (ii) was redistilled to give the *product* as an oil, b.p. 84° at 0·5 mm. Found: C, 55·8; H, 8·6; Cl, 18·7. $C_9H_{17}O_2Cl$ requires C, 56·1; H, 8·9; Cl, 18·4 per cent.

3-cyclo*Hexyloxy*-1-*piperidinopropan*-2-*ol.* The foregoing chlorohydrin (9·6 g.) was heated with piperidine (9·3 g., 2·2 moles) on the steam bath for 16 hours. After addition of chloroform (50 ml.) piperidine hydrochloride was removed by washing with water. The chloroform extract was concentrated and the residue distilled at reduced pressure to yield the *product* as an oil, b.p. 110° at 0·3 mm.

3-(3′:4′:5′-*Trimethoxyphenoxy*)-2-*hydroxypropyl chloride.* 3:4:5-Trimethoxyphenol was dissolved by warming in 2:3-epoxypropyl chloride (24 g. = 4·5 moles). Pyridine (5 drops) was added and the mixture heated on the steam bath. Heating was stopped when an exothermic reaction occurred and was then continued for 6 hours. Excess of 2:3-epoxypropyl chloride was removed at reduced pressure, the residual oil was dissolved in chloroform (50 ml.), shaken with concentrated hydrochloric acid[6], and then washed acid-free with water. After removal of the solvent, the residue was distilled at reduced pressure yielding an oil, b.p. 180° at 0·5 mm. which solidified on standing. It was purified by crystallisation from a mixture of ethyl acetate and light petroleum (b.p. 40 to 60°) to give white needles, m.p. 87 to 89°. Found: C, 52·0; H, 5·9; Cl, 13·4. $C_{12}H_{17}O_5Cl$ requires C, 52·1; H, 6·2; Cl, 12·8 per cent.

3-(3′:4′:5′-*Trimethoxyphenoxy*)-2-*hydroxypropylamine hydrochloride.* The foregoing chlorohydrin was dissolved in ethanol (50 ml.), concentrated ammonia (50 ml., d = 0·880) was added, the solution warmed on the steam bath for several hours, and then evaporated to dryness. The residue was treated with alcoholic hydrochloric acid, filtered, and the filtrate diluted with ether. The *product* separated on cooling and was purified by crystallisation from a mixture of ethanol and ether. It had m.p. 198 to 200°. Found: C, 49·2; H, 7·1; N, 5·1; Cl, 12·1. $C_{12}H_{20}O_5NCl$ requires C, 49·0; H, 6·9; N, 4·8; Cl, 12·1 per cent.

3:4:5-*Trimethoxyphenol and* 3:4:5-*Trimethoxyaniline.* 2:6-Dimethoxybenzoquinone and 3:4:5-trimethoxynitrobenzene were prepared as follows by a variation of the published method[12].

A solution of 1:2:3-trimethoxy benzene (42 g., 0·25 mole) in ethanol (200 ml.) was heated to boiling under reflux. Sodium nitrite (1 g.) was added, followed immediately by 60 per cent (v/v) nitric acid (100 ml.). Heating was stopped until the vigorous exothermic reaction was complete, when the mixture was heated for 10 minutes. It was cooled rapidly to

25° and the 2:6-dimethoxybenzoquinone collected. It was purified by boiling with a little ethanol and had m.p. 262 to 263°. Yield 24 g. The combined filtrate and washings by concentration and cooling yielded 3:4:5-trimethoxynitrobenzene (10·2 g., m.p. 98 to 100°). One crystallisation from methanol raised the m.p. to 104° (8·7 g.). This product was identical with that obtained by nitration of 3:4:5-trimethoxybenzoic acid[13].

3:4:5-Trimethoxyphenol was prepared *via* 2:6-dimethoxybenzoquinone by the method of Chapman, Perkin and Robinson[14].

3:4:5-Trimethoxyaniline was prepared by reduction of the nitro compound with iron powder[13] or by catalytic reduction in ethanol using Raney nickel. By the latter method it was obtained in 97 per cent yield in fawn coloured needles, m.p. 112 to 114°.

The *hydrochloride* separated from ethanol in white needles, m.p. 256° (decomp.). Found: C, 49·5; H, 6·4. $C_9H_{14}O_3NCl$ requires C, 49·2; H, 6·4 per cent.

Treatment of the hydrochloride (2·2 g.) in water (20 ml.) with sodium cyanate (0·8 g.) furnished the *urea*, which separated from water in small white needles, m.p. 178°. Found: C, 53·2; H, 6·1; N, 12·1. $C_{10}H_{14}O_4N_2$ requires C, 53·1; H, 6·2; N, 12·4 per cent.

3-*o*-*Chlorophenoxy*-1-*piperidinopropan*-2-*ol*. (*a*) A mixture of 3-*o*-chlorophenoxy-1:2-epoxypropane (184·5 g.) and piperidine (89 g., 1·05 mole) in light petroleum (500 ml., b.p. 60 to 80°) was heated on the steam bath for 2 hours, heating being controlled in the early stages when an exothermic reaction occurred. The product crystallised after cooling. Yield 233·5 g. m.p. 72 to 73° after crystallisation from light petroleum (b.p. 60 to 80°).

Alternatively, after cooling, the solution was washed with water, the solvent removed and the base purified by distillation at reduced pressure. (*b*) A mixture of *o*-chlorophenol (12·85 g.) and 3-piperidino-1:2-epoxypropane (14·1 g.) was warmed to 90° when an exothermic reaction occurred. Heating was discontinued to keep the reaction temperature below 105°. The mixture was warmed for a further 2 hours and was then distilled directly at reduced pressure. The product was obtained as an oil, b.p. 128° at 0·1 mm. which solidified and was purified by crystallisation from light petroleum (b.p. 60 to 80°). It had m.p. 72 to 73° and was identical with the material obtained under (*a*).

A solution of the foregoing base (2·7 g.) in ethanol (5 ml.) was treated with a solution of acetylsalicylic acid (1·8 g.) in ethanol (5 ml.). The mixture was warmed for a few minutes and then diluted with light petroleum (b.p. 40 to 60°). The solid obtained was crystallised from light petroleum (b.p. 60 to 80°) containing a trace of ethanol. The *acetylsalicylate* formed white needles, m.p. 114 to 116°.

3-(o-*Methylcarbonylphenoxy*)-1:2-*epoxypropane* was prepared by condensation of 2:3-epoxypropyl chloride with *o*-hydroxyacetophenone in aqueous alkaline solution. It was obtained as an oil, b.p. 120° at 0·3 mm. which solidified. The *product* separated from a mixture of ether and light petroleum (b.p. 40 to 60°) in white needles, m.p. 46 to 48°. Found: C, 68·5; H, 6·3. $C_{11}H_{12}O_3$ requires C, 68·7; H, 6·3 per cent.

1-(o-*Methylcarbonylphenoxy*)-3-*piperidinopropane*-2-*ol* was prepared by condensation of the foregoing epoxide with piperidine. It was isolated as its *hydrochloride* which separated from a mixture of ethanol and ether in pink tinged crystals, m.p. 126 to 128°. Found: C, 60·6; H, 7·4; N, 4·3; Cl, 11·2. $C_{16}H_{24}O_3NCl$ requires C, 61·2; H, 7·7; N, 4·5; Cl, 11·3 per cent.

NN'-*Bis*-(2-*hydroxy*-3-o-*toloxy*)-*propyl*-4:4'-*dipiperidyl* was prepared by condensation of 3-*o*-toloxy-1:2-epoxypropane (2 moles equiv.) with 4:4'-dipiperidyl in benzene containing a trace of ethanol. The *base* crystallised from ethanol in small white needles, m.p. 153 to 156°. Found: C, 72·9; H, 8·7; N, 5·7. $C_{30}H_{44}O_4N_2$ requires C, 72·5; H, 8·9; N, 5·6 per cent.

The *dihydrochloride* separated from aqueous ethanol in shining microcrystals, m.p. 293° (decomp.). Found: C, 63·3; H, 8·1; N, 5·0; Cl,12·5. $C_{30}H_{46}O_4N_2Cl_2$ requires C, 63·2; H, 8·2; N, 4·9; Cl, 12·5 per cent.

N:N'-*Bis*-(3-o-*allylphenoxy*-2-*hydroxy*)-*propyl*-4:4'-*dipiperidyl* crystallised from a mixture of ethyl acetate and light petroleum (b.p. 60 to 80°) in small cream coloured needles m.p. 106 to 107°. Found: C, 74·1; H, 8·7; N, 4·7. $C_{34}H_{48}O_4N_2$ requires C, 74·4; H, 8·8; N, 5·1 per cent. The *dihydrochloride* crystallised from a mixture of ethanol and ether in white needles, m.p. 244 to 246°. Found: C, 65·4; H, 8·3; N, 4·6. $C_{34}H_{50}O_4N_2Cl_4$ requires C, 65·7; H, 8·1; N, 4·5 per cent.

5-o-*Chlorophenoxypentyl bromide*, b.p. 200 to 204° at 20 mm., was prepared by condensation of *o*-chlorophenol with pentamethylene dibromide (3 mole equivs.) in ethanol containing 1 equivalent of sodium ethoxide. 3-p-*Chlorophenoxypropyl bromide* had b.p. 185 to 190° at 85 to 90 mm. 4-p-*Chlorophenoxybutyl bromide* had b.p. 198 to 200° at 20 mm. Found: Total halogen, 43·8. $C_{10}H_{12}OCl\,Br$ requires total halogen 43·8 per cent. 5-p-*Chlorophenoxypentyl bromide* had b.p. 226 to 228° at 30 mm. 3-o-*Methoxyphenoxypropyl bromide* had b.p. 98° at 0·25 mm. Found: C, 49·4; H, 5·3; Br, 32·2. $C_{10}H_{13}O_2$ requires C, 49·0; H, 5·4; Br, 32·6 per cent. It formed a low-melting solid. 5-o-*Methoxyphenoxypropyl bromide* b.p. 118° at 0·25 mm.

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ANALGESIC AND OTHER PHARMACOLOGICAL PROPERTIES OF 1-$\triangle^3$-PIPERIDEINO-3-*O*-TOLOXY PROPAN-2-OL HYDROCHLORIDE (TOLPRONINE)

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1-Δ^3-Piperideino-3-*o*-toloxypropan-2-ol hydrochloride, Tolpronine, possesses marked analgesic activity, being approximately 20 times as active as metamizolum subcutaneously and approximately 11 times as active orally. The induction time after subcutaneous injection is shorter than with metamizolum and the duration similar. Excessively large amounts constipate normal mice; a weak spasmolytic effect is seen in the isolated guinea pig ileum and rabbit duodenum. The jejunum of the anaesthetised cat shows a spasmogenic effect. Chronic administration to rats and rabbits caused a reduction in the growth rate of rats.

The local anaesthetic activity of some 1-amino-3-aryloxy-propan-2-ols was described by Petrow, Stephenson and Thomas[1]. Certain of these compounds were also found to possess analgesic properties (*cf.* Fourneau[2]) and a comprehensive series of 1-amino-3-aryloxypropan-2-ols (I)

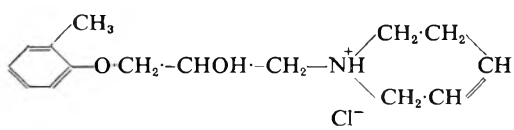
$$ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2NRR'$$

(I)

was prepared by Dr. V. Petrow and his colleagues[3], and evaluated for analgesic activity. The results proved encouraging and the work was extended to the formally related types indicated below.

$ArO{\cdot}(CH_2)_n{\cdot}NRR'$
$ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}OCH_2{\cdot}CH_2{\cdot}NRR'$
$ArO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}O{\cdot}CH_2CHOH{\cdot}CH_2{\cdot}NRR'$
$ArO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2NRR'$
$ArO{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}NRR'$
$(o)\text{–}HO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}O{\cdot}C_6H_4{\cdot}CONRR'$

1-Δ^3-Piperideino-3-*o*-toloxypropan-2-ol hydrochloride, Tolpronine (II) was the most promising of more than 50 compounds examined and was selected for further studies.



(II)

It is a white crystalline substance, molecular weight 283·8, with a melting point of 136 to 137·5°. It has a bitter taste and is readily soluble in water, a 10 per cent solution having a pH of approximately 5·8. Aqueous solutions are stable to heat but unstable to acids and alkalies.

METHODS

Acute Toxicity

This was estimated in male albino mice by the oral, intraperitoneal and subcutaneous routes. Mice weighing approximately 20 g., were fasted overnight before oral or intraperitoneal administration. The compounds were given in aqueous solution adjusted to 0·5 ml./20 g. body weight. Oral and subcutaneous toxicities were estimated by giving the compound at four, five or six dose levels, increasing in geometrical progression by a factor of 4/3. Ten animals were used at each dose. Intraperitoneal toxicities were estimated by giving the compounds at five dose levels increasing by a factor of 1·5, five animals being used at each dose level. The LD50s were calculated by Litchfield and Wilcoxon's[4] method or Kärber's[5] formula from the mortalities in seven days.

Acute oral and subcutaneous toxicities were also estimated in adult male albino rats. The animals were fasted overnight before oral administration and the compounds were given at four, five or six dose levels, with a dose ratio of 1·5. The volumes were adjusted to 25 ml./kg. for the oral route and to 10 ml./kg. for the subcutaneous route. Ten animals were used at each dose level. LD50s were calculated from the mortalities in seven days using Kärber's[5] formula.

Chronic Oral Toxicity

Immature male albino rats in two groups of ten animals were given an aqueous solution of the compound by stomach tube five days a week or water only. The daily dose of 100 mg./kg. was adjusted to the individual body weights at the beginning of each week and all volumes were adjusted to 25 ml./kg. The animals were kept under identical conditions and were allowed diet 41 and water *ad lib.* At the end of the six weeks experimental period five animals from each group were sacrificed and the spleen, liver, kidney, heart and lung were examined histologically. The remaining animals were kept under observation for a further period of seven weeks.

Five immature rabbits were given 50 mg./kg. in aqueous solution by stomach tube five days a week for eight weeks. Five controls were given water, 5 ml./kg. daily. The animals were kept under identical conditions and were allowed diet 18 and water *ad lib.* Red, white and differential cell counts, haemoglobin values, and weights were determined weekly.

Analgesic Activity

Analgesic acitivity after oral, intraperitoneal and subcutaneous administration was estimated in male albino mice, by the method of Bianchi and Franceschini[6]. The animals weighed 15 to 20 g. and were fasted overnight for the oral and intraperitoneal tests. A bulldog artery clip covered with catheter tubing was applied to the base of the tail; only those making continuous attempts to remove the clip within 15 seconds were regarded as sufficiently sensitive for use.

The compounds were given in aqueous solution, in volumes adjusted to 0·5 ml./20 g. body weight. They were given at four or five dose levels,

with dose ratios of 1·5 for the oral and 2·0 for the intraperitoneal and subcutaneous routes. Five animals were used initially at each dose level, the doses being injected in a random order. Thirty minutes after administration, the clip was applied to each mouse in turn. If no attempt was made to remove the clip within 30 seconds an analgesic state was assumed to be present.

In certain experiments the clip was also applied at 60 and 90 minutes after administration. The ED50 was calculated, using Litchfield and Wilcoxon's[4] method or Kärber's[5] formula.

Duration of Analgesic Action

The compounds were given subcutaneously to groups of 20 mice at the ED80 dose level. The clip was then applied at 30 minute intervals after injection. Onset of analgesia was taken to be 15 minutes before the first failure to respond and the time of recovery to be 15 minutes before the normal response reappeared. The difference between the two provided an estimate of the duration of analgesia to the nearest half-hour.

Local Anaesthetic Activity

The intracutaneous wheal test of Bülbring and Wajda[7] was employed. On the day before the test, the hair on the posterior half of each guinea pig's back was clipped and shaved. The animals were divided into three groups of six, the groups being allocated to high, medium or low concentrations. Three animals from each group were injected in the anterior half of the shaved area with the test compound and with the reference drug in the posterior half, the positions being reversed in the other three animals. The order of injection was unknown to the observer. Each dose was injected intracutaneously in the mid-line in 0·2 ml. of normal saline and the resultant wheal was outlined in ink. The response to six pin pricks applied at various points inside each wheal area was determined 5 minutes after injection and at 5 minute intervals for 30 minutes. The number of negative responses to the total of 36 stimuli was recorded. The mean of the six results for each concentration was calculated and plotted against the log of the concentration.

Anticonvulsant Activity

The leptazol seizure test described by Goodman and others[8] was used. Aqueous solutions or suspensions in 5 per cent acacia mucilage were given orally to groups of five fasted male albino mice at four dose levels, in volumes adjusted to 0·5 ml./20 g. body weight. The doses increased by a factor of 2·0. Two hours after administration the animals were given a rapid intravenous injection of a convulsant dose (50 mg./kg.) of leptazol. Those animals not developing the hindleg tonic extensor component of the convulsion were counted. The dose required to give protection to 50 per cent of the animals was calculated.

Action on the Gastrointestinal Tract

Isolated rabbit duodenum. Segments of rabbit duodenum were suspended in Ringer solution in a 70 ml. bath at 35°. A mixture of 95 per

cent oxygen and 5 per cent carbon dioxide was bubbled through the solution. When rhythmic contractions started, varying volumes of 0·1, 1·0 and 10 per cent aqueous solutions of the compound were added at suitable intervals and usually allowed to act for 2 minutes before washing out.

Isolated guinea pig ileum. Spasmolytic activity was estimated on the isolated guinea pig ileum. A 4 cm. segment was suspended in Tyrode's solution in a 25 ml. bath at 35°. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled through the solution. Submaximal doses (0·8 μg.) of acetylcholine were added at 3 minute intervals and allowed to act for 30 seconds. Varying amounts of the compounds were added 30 seconds before the addition of acetylcholine. The response to acetylcholine was allowed to return to normal between doses of the spasmolytics. The heights of the contractions immediately before and after the addition of the spasmolytic were measured. The inhibition per cent was plotted against log dose and the amount causing 50 per cent inhibition determined.

The effect on the peristaltic reflex was investigated on a 5 cm. segment suspended in oxygenated Tyrode's solution at 36° in a 70 ml. bath. Using the method of Trendelenberg, a record of peristaltic contractions was obtained by raising the pressure in the lumen by 3 cm. of Tyrode's solution for 2 minutes at 5 minute intervals. The compound was added to the bath 1 minute before raising the pressure and was washed out on reducing the pressure.

Defaecation. The method was based on that described by Lou[9] for the assay of vegetable purgatives. Male albino mice, weighing approximately 20 g., were divided into five groups of nine animals. Four groups were given varying amounts of the compound in aqueous solution by stomach tube, the volume being adjusted to 0·5 ml./20 g. body weight. The fifth group served as controls and were given a similar volume of water. Fifteen minutes after administration, the mice were placed in individual compartments over a wire grid and the faeces collected on blotting paper. The 15 minute interval was considered advisable as defaecation frequently occurred after handling. The mice were allowed free access to a paste made of Rat Diet 41 and water. The total number of faecal pellets from each animal was counted at 8 and 24 hours after administration.

Cat jejunum in situ. The cat was anaesthetised with ether followed by chloralose, 60 mg./kg. intravenously. A water filled balloon was inserted into the jejunum through an abdominal incision and contractions were recorded by means of a water/air transmission system connected to a small piston recorder. The pressure in the balloon was approximately 10 cm. of water. Aqueous solutions of the compound were injected intravenously when spontaneous rhythmic contractions had become established.

Actions on the Cardiovascular and Respiratory Systems

Cat blood pressure. The carotid blood pressure was recorded in cats anaesthetised with ether followed by chloralose, 60 to 80 mg./kg.,

ntravenously. Heparin, 500 to 1000 units, was injected intravenously to prevent clotting. All doses were given in aqueous solution through the cannulated femoral vein.

Isolated rabbit heart. A Langendorff preparation was perfused with Ringer-Locke's solution at 39° and aerated with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The amplitude of the cardiac

TABLE I

ACUTE TOXICITY IN MALE ALBINO MICE

Compound	Route	LD50 mg./kg.	Limits of error (P=0·95) mg./kg.
Tolpronine ..	Oral	330	290–380
Metamizolum ..		4030	3570–4550
*Tolpronine ..	Intraperitoneal	200	—
Metamizolum ..		3050	—
Tolpronine ..	Subcutaneous	500	430–590
Metamizolum ..		3440	2940–4020
*Tolpronine ..	Subcutaneous	390	—
Codeine phosphate		220	—
Tolpronine ..	Subcutaneous	530	460–620
Procaine hydrochloride		1210	1050–1390

*Calculated by Kärber's formula.

contractions was recorded by means of a thread attached to a heart lever. Varying volumes of an aqueous solution of the compound were administered by injection into the perfusion cannula.

Respiration of the anaesthetised cat. Respiration was recorded by Paton's[10] method.

RESULTS

Acute Toxicity

The acute toxicity of Tolpronine in mice was compared with metamizolum, codeine phosphate and procaine hydrochloride by various routes. Metamizolum was usually employed as the reference compound as it is not related chemically to the opium alkaloids and possesses moderate analgesic properties. Table I records the LD50s.

TABLE II

ACUTE TOXICITY IN MALE ALBINO RATS

Route	Compound	LD50 mg./kg.
Oral	Tolpronine	340
	Metamizolum	4250
Subcutaneous	Tolpronine	1030
	Metamizolum	2780

Tolpronine was rapidly fatal by the oral and intraperitoneal routes but death was frequently delayed for 24 hours after subcutaneous doses. Loss of the righting reflex was observed at near lethal subcutaneous doses.

Table II records LD50 values in rats. Death, as in mice, occurred somewhat more rapidly following oral than subcutaneous administration.

Loss of the righting reflex was observed with near lethal amounts by the latter route.

Chronic Oral Toxicity

The mean growth curves of control animals and rats given 100 mg./kg. of Tolpronine on five days a week are compared in Figure 1. The mean increase in weight of the treated group after six weeks was 62·9 g., standard

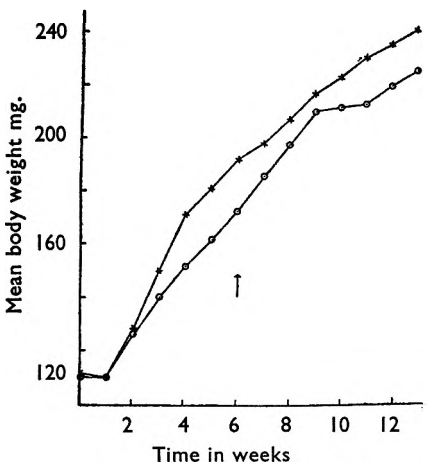


FIG. 1. Effect of Tolpronine (100 mg./kg./day) on the growth of immature male rats. ○——○ treated group, ×——× control group, ↑ indicates where treatment discontinued.

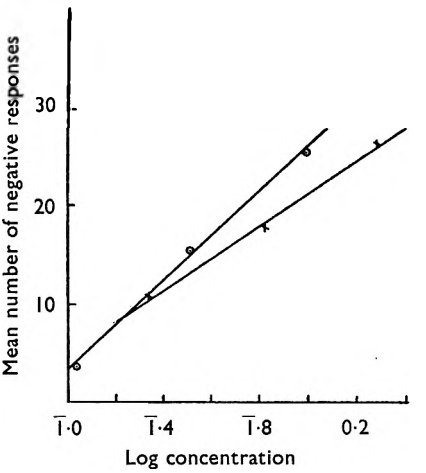


FIG. 2. Local anaesthetic activity of Tolpronine (○——○) and procaine hydrochloride (×——×). Guinea pig intracutaneous wheal test.

error 5·25 g., compared with 81·1 g., ± 5·28 g. for the controls. This difference was statistically significant ("Student's" test). No deaths occurred. Histological examination of spleen, liver, kidney, lung and heart from animals killed at the end of six weeks revealed no significant changes.

TABLE III

ANALGESIC ACTIVITIES IN MALE ALBINO MICE

Compound	Route	ED50 mg./kg.	Limits of error (P=0·95) mg./kg.
Tolpronine	Oral	130	90–180
Metamizolum		1470	1100–1976
*Tolpronine	Intraperitoneal	65	—
Metamizolum		710	—
Tolpronine	Subcutaneous	35	23–54
Metamizolum		730	495–1070
*Tolpronine	Subcutaneous	45	—
Codeine phosphate		30	—

*Calculated by Kärber's formula.

There was no significant difference between the weekly body weights and haematological values of rabbits given Tolpronine 50 mg./kg. daily by mouth for eight weeks and the control animals. Histological examination of liver, spleen, kidney and stomach also failed to demonstrate any abnormalities.

Analgesic Activity

Table III compares the analgesic activities of Tolpronine and metamizolum by various routes. For oral and subcutaneous routes, 15 animals were used at each dose level. Intraperitoneally, five animals at each dose level were tested at 30 and 60 minutes following administration. Table III gives the relative analgesic activities of Tolpronine and codeine phosphate by the subcutaneous route. Five animals at each dose level were tested at 30, 60 and 90 minutes after the injection.

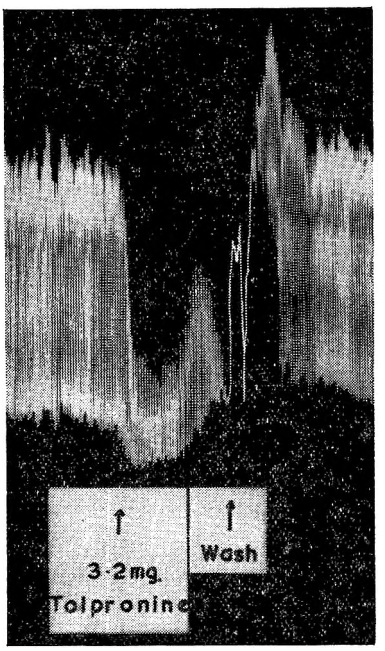


FIG. 3. Effect of Tolpronine (3·2 mg. in 70 ml. bath for two minutes) on pendular movements of isolated rabbit duodenum.

Duration of Analgesic Action

Tolpronine and metamizolum were compared for duration of analgesia at the ED80 level, i.e., 85 and 1600 mg./kg. respectively. After subcutaneous injection of Tolpronine, 17 of 20 animals showed analgesia. The mean induction time was 15 minutes and the mean duration 180 minutes (range 30 to 270 minutes). Metamizolum induced analgesia in 19 of 20 mice with a mean induction time of 30 minutes and a mean duration of 195 minutes (range 60 to 330 minutes).

Local Anaesthetic Activity

Tolpronine was injected as 0·11, 0·33 and 1·00 per cent solutions and procaine hydrochloride as 0·22, 0·67 and 2·00 per cent solutions. Figure 2 shows the mean number of negative responses plotted against log concentration. From this graph the concentration producing 18 out of a possible 36 negative responses, was determined. The value for Tolpronine was 0·45 per cent and for procaine hydrochloride 0·67 per cent. Tolpronine produced no local irritation or tissue damage.

Anticonvulsant Activity

Tolpronine had no anticonvulsant properties in mice at doses up to 200 mg./kg., i.e., approximately 60 per cent of its oral LD50. Troxidone,

TABLE IV

THE EFFECT OF TOLPRONINE AND METHANTHELINE BROMIDE ON THE ACETYLCHOLINE INDUCED CONTRACTION OF THE ISOLATED GUINEA PIG ILEUM

Compound	Dose μg.	Per cent reduction	Dose causing 50 per cent reduction μg.
Tolpronine	10	28	25
	20	40	
	40	61	
	80	77	
Methantheline bromide	0·01	22	0·03
	0·02	42	
	0·04	54	
	0·08	71	

suspended in 5 per cent acacia, had a PD50 of approximately 300 mg./kg., corresponding to approximately 10 per cent of its oral LD50.

Action on the Gastrointestinal Tract

Isolated rabbit duodenum. Tolpronine had no effect on normal rhythmic contractions in amounts below 0·4 mg. Larger amounts, up to 12·8 mg., had slight spasmolytic action. The higher concentrations cause a reduction in tone, but complete recovery occurred on washing out. Figure 3 records a typical response.

Isolated guinea pig ileum. The spasmolytic activity of Tolpronine was estimated using methantheline bromide as the reference compound. The results are recorded in Table IV.

At 0·1 mg. Tolpronine produced little or no inhibition of the peristaltic reflex, while 0·2 mg. caused a partial or complete inhibition. The addition of 0·4 mg. or of larger amounts caused complete inhibition. Figure 4 records typical responses.

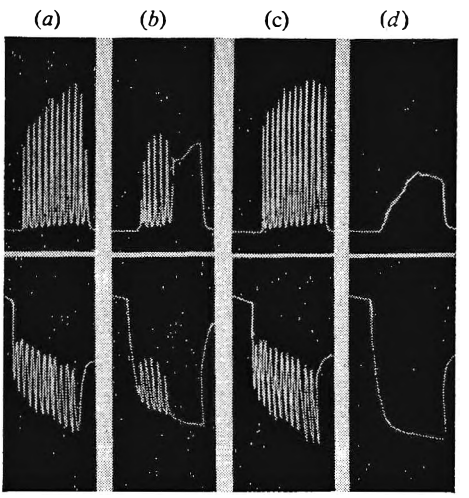


FIG. 4. Effect of Tolpronine on peristaltic reflex. Guinea pig ileum; 70 ml. bath; Trendelenberg preparation. Upper record-longitudinal contractions; lower record-volume changes. (*a*) Normal record, (*b*) one minute after 0·2 mg. Tolpronine, (*c*) normal record, (*d*) one minute after 0·4 mg. Tolpronine.

Defaecation. Tolpronine, 240 mg./kg., had no effect on the number of pellets passed in the first 8 hours. In the next 16 hours the number of faecal pellets passed was reduced to 35 per cent of the controls. There was little or no effect at lower doses.

Cat jejunum in situ. Tolpronine, 0·25 to 2 mg./kg., produced a considerable increase in the tone of the jejunum of an anaesthetised male cat weighing 2·7 kg. The increase occurred within a few seconds of injection and persisted for several minutes before a rapid fall to initial level. Amounts below 0·25 mg./kg. had no effect. Figure 5 illustrates a typical response.

Action on the Cardiovascular and Respiratory Systems

Cat blood pressure. Four cats weighing between 2·7 and 4·8 kg. were used. Amounts from 0·5 to 2·0 mg./kg. caused falls in blood pressure which did not exceed 50 mm. Hg., followed by a gradual return towards the initial level. The fall in blood pressure was not abolished by the intravenous injection of 2 mg. atropine sulphate nor by section of both vagi. Figure 6 shows a typical response following 1 mg./kg. of Tolpronine and codeine phosphate. No effects were observed following 20 mg./kg. of metamizolum.

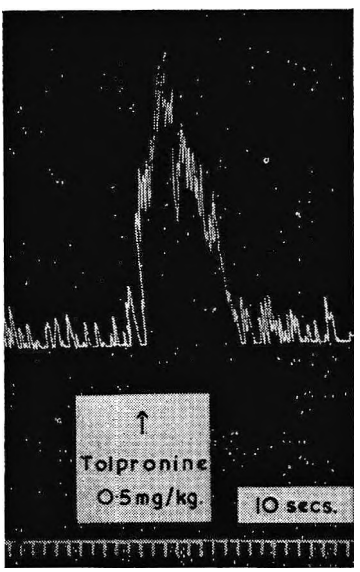


FIG. 5. Effect of intravenous injection of Tolpronine on intact jejunum of 2·7 kg. cat anaesthetised with chloralose, 60 mg./kg. intravenously.

Isolated rabbit heart. 2 mg. of Tolpronine caused a 50 per cent reduction in the amplitude of contraction of the isolated rabbit heart with recovery in approximately 90 seconds. Smaller amounts had little effect.

Respiration of the anaesthetised cat. Respiratory changes in three cats used in the blood pressure experiments were also recorded. Doses of Tolpronine from 0·55 to 1·1 mg./kg. usually produced a transient slight reduction in respiratory minute volume although the rate was increased. Figure 6 illustrates a typical response and also shows the slight respiratory depression produced by a similar amount of codeine phosphate.

DISCUSSION

Tolpronine possesses significant analgesic properties in mice by the subcutaneous, oral and intraperitoneal routes. The compound is most active subcutaneously, having approximately 20 times the activity of metamizolum on a weight for weight basis. The therapeutic indices of Tolpronine and metamizolum are 14·3 and 4·3 respectively, giving Tolpronine a wider margin of safety. Subcutaneously, the duration of action of the compounds is similar, but the new compound appears to have a rather more rapid onset of action. Compared with codeine phosphate, Tolpronine is approximately 0·7 times as active and has a

therapeutic index of 8·7 compared with 7·3 for codeine phosphate. Given orally, tolpronine is approximately 11 times as active as metamizolum; their therapeutic indices are similar, being 2·5 and 2·6 respectively. Tolpronine is also approximately 11 times as active as metamizolum when injected intraperitoneally.

It appears that there is a decrease in toxicity and an increase in activity of Tolpronine by the subcutaneous as compared with the oral and intraperitoneal routes, thus changes in activity and toxicity do not parallel

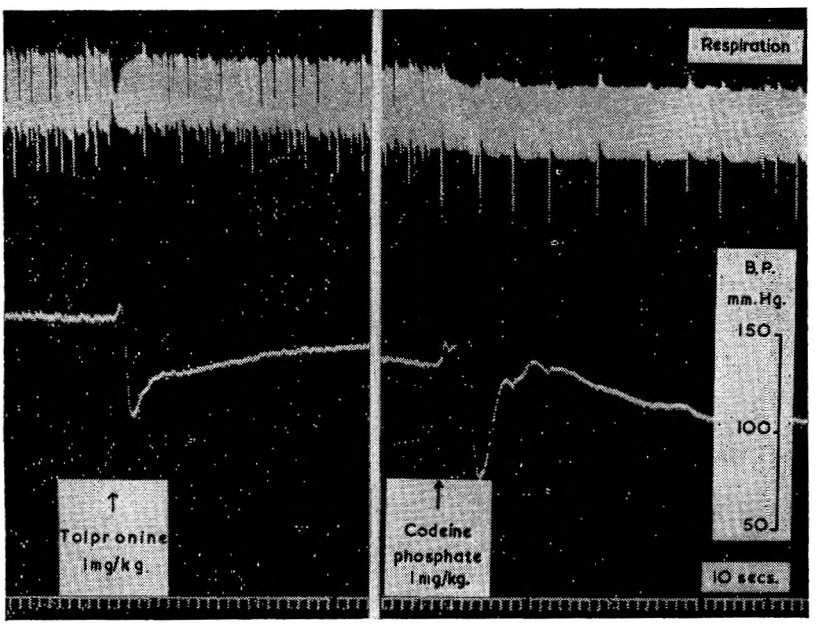
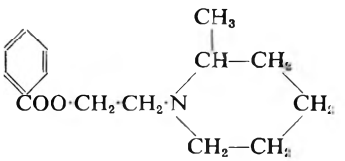


FIG. 6. Effects of intravenous injection of Tolpronine and codeine phosphate on blood pressure and respiration of chloralosed cat.

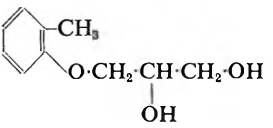
each other. This indicates that the analgesic action of Tolpronine in mice is not merely a manifestation of general toxicity. Bianchi and Franceschini[6] and Jackson[11], using mice and rats respectively, have reported similar findings with other analgesics.

The tail pinch technique, which is rapid and easy to carry out, appears to be particularly useful for screening new compounds with moderate analgesic activity.

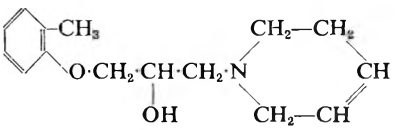
In addition to its analgesic activity, Tolpronine also possesses local anaesthetic properties, being approximately 1·5 times as active as procaine hydrochloride in the guinea pig intracutaneous wheal test. It is, however, at least twice as toxic by the subcutaneous route. The local anaesthetic action is perhaps not surprising as Tolpronine has structural similarities with piperocaine and mephenesin, the latter also having local anaesthetic activity of the same order as procaine.



Piperocaine



Mephenesin



Tolpronine

Its effect on the gastrointestinal tract is somewhat variable but a weak spasmolytic effect is usually observed. It has an anti-acetylcholine action on the isolated guinea pig ileum, although it has only about 0·1 per cent of the activity of methantheline bromide. In large amounts it causes inhibition of the pendular movements of the isolated rabbit duodenum and of the peristaltic reflex in the isolated guinea pig ileum. It has a constipating effect in normal mice only at very high dose levels. In contrast, the intravenous injection of Tolpronine has a spasmogenic effect on the jejunum of the anaesthetised cat.

Rapid intravenous injection of moderate amounts causes a fall in blood pressure and transient respiratory depression in the anaesthetised cat. The effects produced, however, are no greater than those observed after similar amounts of codeine phosphate.

The continued oral administration of 100 mg./kg. daily to immature rats for six weeks and of 50 mg./kg. daily to rabbits for eight weeks did not produce any untoward effects, apart from some reduction in the growth rate of rats. In particular, there was no deleterious action on the haemopoietic system of rabbits.

The authors wish to thank Professor T. Crawford for carrying out the histological investigations.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

PHARMACY

Transmission of Forces Through a Powder Mass During the Process of Pelleting. D. Train. (*Trans. Instn. Chem. Engrs.*, 1957, **35**, 258.) A manganin wire resistance gauge, protected by glyco-gelatin, has been developed to facilitate the measurement of pressure response within a mass of particulate matter under compaction. A number of such gauges have been used under specified conditions to obtain data so that the movement of isobars or pressure levels could be traced through a cylindrical mass of heavy magnesium carbonate subjected to an increasing pressure up to 2000 kg./sq. cm. from one end. Evidence for the development of a complex pressure pattern was confirmed by determining the relative density distributions in a number of compacts pressed to various levels within the pressure range, and a close relation was found to exist between the pressure developed and the apparent density produced at a point within a compact. A sequence of diagrams is presented for the development of the pressure pattern which is in accord with the work reported previously (*J. Pharm. Pharmacol.*, 1956, **8**, 745) and a tentative explanation is advanced for its development. When pressure is applied through the top punch, an uneven reaction develops in the powder just beneath because of opposing frictional forces at the die wall. This produces a high intensity wedge in the outer edges of the top of the powder mass because more material is impacted into this zone as the punch descends and this can be accommodated only by an increase of the local stresses. Resolution of such stresses indicates that the resultants within the particulate mass will be along an oblique line towards the lower centre of the pressing and coinciding with the ridge of relatively high intensity previously reported. By reason of symmetry the combination of this cone of resultant stresses produces a higher intensity region near the lower centre of the compact.

Further comment is made on the influence of shearing stresses on interparticulate bonding. D. T.

PHARMACOLOGY AND THERAPEUTICS

Analgesics, Strong, Some Ethyl-1-aralkyl-4-phenylpiperidine-4-carboxylates. B. Elpern, L. N. Gardner and L. Grumbach. (*J. Amer. chem. Soc.*, 1957, **79**, 1951.) A series of ethyl-1-aralkyl-4-phenylpiperidine-4-carboxylates have been prepared by treating various aralkyl halides with ethyl 4-phenylpiperidine-4-carboxylate, and by minor modifications of this procedure. Substituted *N*-phenethyl derivatives were of equal or greater potency than the *N*-methyl derivative, the most potent substance carrying the 4-aminophenethyl substituent. Replacement of phenethyl by pyridylethyl enhanced the potency, 4-pyridylethyl being more effective than 2-pyridylethyl. Maximum activity was seen with three methylene groups separating the aryl group from the nitrogen. A double bond in this position still further enhances activity, but a triple bond abolishes activity. J. B. S.

Glycyrrhetinic Acid, A Non-steroidal Anti-Inflammatory Agent in Dermatology. E. Colin-Jones and G. F. Somers. (*Med. Press*, 1957, **238**, 206.) This paper reviews the pharmacology of glycyrrhetinic acid and reports on its anti-inflammatory activity as evidenced by tests on experimental animals and an extensive clinical investigation in a variety of skin diseases. Glycyrrhetinic acid is a complex triterpene derived from glycyrrhizic acid, the sweet constituent

of liquorice. The authors have demonstrated the anti-inflammatory action of the biologically active isomers of glycyrrhetinic acid in established experimental methods in laboratory animals:—the cotton pellet method of Meier, Schuler and Desaulles, inhibition of tuberculin reaction in B.C.G. infected guinea pigs, the rat foot test and the granuloma pouch test. It is also reported that it heals experimentally induced inflammatory lesions on the skin of the rabbit and reduces induced inflammation in the rabbit eye. It was also demonstrated that a "commercially pure" glycyrrhetinic acid did not suppress the tuberculin reaction in B.C.G. infected guinea pigs, and the authors postulate that this may be due to method of extraction and purification. Clinically, the topical application of ointments containing the active isomers of glycyrrhetinic acid (Biosone G.A.) in 254 cases has shown its value in a variety of dermatological conditions. A trial against the inert base gave a probability figure $P < 0{\cdot}001$. In a comparison with hydrocortisone ointments it compared favourably in subacute and chronic conditions. The ointment was found to be most effective in flexural eczema, traumatic and contact dermatitis, napex neurodermatitis, disseminated neurodermatitis, pruritus associated with psoriasis and in pustular psoriasis. Some value was found in infantile eczema, nummular eczema and in pruritus vulvae and ani. Glycyrrhetinic acid has been shown to act synergistically with the antibiotic neomycin, giving excellent results in impetigo and impetiginised eczema. In psoriasis the depressive action of glycyrrhetinic acid on the inflammatory process, combined with the anti-eczematous action of tar and the keratolytic action of salicylic acid gave more effective and speedier results than any of these used alone. The active form of glycyrrhetinic acid brought relief in cases which had hitherto been intractable to other forms of treatment. It was concluded that certain fractions of the acid have a marked anti-inflammatory action and were effective in a variety of skin diseases, and compared favourably in subacute and chronic conditions, with hydrocortisone. G. F. S.

Iron, Effect of Massive Overload in the Rat. L. Golberg, J. P. Smith and L. E. Martin. (*Nature, Lond.*, 1957, **179**, 734.) Iron-dextran complex ("Imferon") was administered intramuscularly to rats over a period of 18 months, the total dose being 1,650 mg. of iron per kg. Control animals were given equivalent amounts of dextran, in the same way. The iron-loaded rats remained in good health, although growth rate, particularly in males, was less than with the controls. At intervals during the period of treatment groups of rats were killed. The dextran-treated control animals showed no abnormality; in those receiving the iron-dextran there was organ siderosis, but no haemochromatosis. The only changes in the iron-treated animals resembled the characteristic signs of vitamin E deficiency in the rat: rapid post-mortem renal autolysis (from 9 weeks onwards), brown uterus (from 50 weeks onwards) and testicular atrophy (in two instances, only, at 47 and 52 weeks). The most striking feature in all the animals was massive accumulation of ceroid-like pigments, particularly in the kidney. Comparison of iron-treated and vitamin E deficient rats revealed some differences. The increase in tissue non-protein nitrogen accompanying renal autolysis seen in the vitamin deficiency was absent in the iron-treated group. Nor did the kidneys of the iron-loaded rats have a higher Q_{O_2}. Some of the most important signs of vitamin E deficiency were not evident in the animals receiving the iron-dextran complex; muscles, nervous and adipose tissue and incisor teeth were normal. The absence of these characteristics suggests that if a vitamin E deficiency exists in iron-loaded rats this must be localised in susceptible tissues. G. P.