REVIEW ARTICLE

PERMEABILITY AND TRANSPORT SYSTEMS IN LIVING CELLS*

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Active and passive Transfer

The interest in permeability was originally more concerned with morphology than with physiology. The question was asked: What is the structure of the cell membrane? Is it a homogeneous layer as the lipid theory postulates or a porous structure with or without electrical charges? Soon, however, new problems arose. Particularly puzzling to earlier workers was the question of how foodstuffs penetrated into living cells. Using methods current at that time the surprising observation was made that the cell seemed to be impermeable to sugars and amino acids, that is to necessary foodstuffs, while appearing highly permeable for a variety of toxic substances including narcotics, alkaloids and other lipid-soluble compounds.

Overton[1] about 50 years ago suggested that sugar is transformed into a lipid-soluble compound, for instance by methylation. Höber[2] in a more general way postulated devices in the cell membrane enabling the cell to regulate the exchange of metabolites at will, a concept that he termed "physiological permeability" as opposed to "physical permeability" and which comes very close to what is called "active transport" today.

Looking back, these two views must now be considered to be prophetic. The past decades have brought not only a large number of observations on secretion across cell layers like intestinal, kidney or gastric epithelia, but have shown that secretory transport of certain substances, particularly ions, appears to be more or less a general property of living cells and that some cells make a highly specialised use of this property. As we know, for example, from the brillant work on the physiology of excitation, carried out mainly in England[3], ion transports in both directions along or against the gradients, and characteristic temporary changes of permeability with a time course of milliseconds are essential parts of the excitation recovery cycle.

In connection with these manifold observations the term "active transport" has been introduced. But the exact meaning that should be assigned to this term has not yet been agreed. When biologists interested in transports come together, they are usually quickly engaged in a discussion of this question.

There is a growing trend to call transports "active" if they occur against the gradient of chemical or of electrochemical activity, or sometimes by a shorter term "uphill". This definition doubtless has

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shortcomings but one definite advantage: it provides an experimental test by which an unequivocal decision can be reached whether a transport system falls within the definition.

The Concept of Carrier Transport

Modified views have been introduced also about the mechanisms of penetration. One of these assumes that molecules or ions may pass the membrane in the form of a complex with a membrane constituent or with a metabolite serving as a carrier; this complex is thought to be formed by special reactions on one side and broken on the other side of the membrane; thereafter the carrier molecule moves back.

This mechanism was for the first time suggested in some detail by Osterhout about 30 years ago[4]. Since that time it has been used more and more in the interpretation of various biological transports. One of the reasons is the fact that it explains a number of characteristics of transports which do not fit into the classical views. Biological transports in contrast to diffusion processes may show a limited transport capacity. In diffusion any increase of the concentration leads to a proportional increase in the rate of translocation. In many biological transports this holds for only low concentrations whereas with higher concentrations a saturation level of the rate is reached which cannot further be increased by raising the concentration. This is true for many absorption and reabsorption processes. It is for instance known to be true for the absorption of sugars and amino acids from the intestine as well as for many reabsorption processes in the kidney where the term of T_m has been introduced[5] to denote maximal transport capacity. It is also applicable to transport mechanisms in single cells like the glucose induced potassium transfer into red cells as shown by Glynn[6]. For more details see reference 7 and 8.

Another observation indicating limited transport capacity is the fact that the presence of one transported substance may inhibit the transport of a second one, in other words phenomena of competition. This also has been early observed for the absorption of sugars from the intestine. In the kidney too, many observations have shown a number of groups of substances to show competition within the same group but not with members of other groups. For details see again references 7 and 8.

In competition experiments some molecular species appear to be more powerful than others. A powerful molecule will be able to reduce markedly the transport rate of a weaker molecule but the reverse is usually untrue. Such observations point to the existence of graded affinities to the transport mechanism.

The carrier mechanism is capable of explaining such observations. If the membrane contains a certain number of carrier molecules the transport will show a limited capacity and molecules differing in their affinity for the carrier will be expected to compete for the mechanism according to their affinity. However, there are certainly other possibilities which might account for a limited capacity and for graded affinity. For instance obligatory enzymatic transformation into a membrane-soluble

molecule without reaction with a carrier would show both the phenomena of competition and of saturation. It appeared useful and necessary therefore to study the corollaries of the carrier concept in more detail in order to appraise its merits and its shortcomings. Today a considerable number of observations have been collected to provide some elements of what may be called carrier transport physiology and the beginning of carrier transport pharmacology.

Most of the experimental observations mentioned so far have been made on active transport passages across the intestinal and the kidney epithelia. It might be assumed therefore that active transports are frequently or always carrier transports and vice versa. This conclusion would certainly not be justified. The carrier mechanism is just one of a number of mechanisms of passage across a membrane. It is certainly a mechanism that may be used for active transportation, but is by no means limited to this type. On the other hand it is a pertinent question to ask whether carrier mechanisms are in some way particularly suited to active transportation and to consider the conditions in which carrier transportation becomes active. These questions will be touched upon later.

Advantages of the Red Blood Cell as a Cell Model

For the study of the elements of a carrier transport physiology a transport system which is not active will have the advantage of simplicity. An object having this advantage and in addition a number of others is the red cell. The erythrocyte is a peculiar kind of cell. Looked at under the microscope it appears as a bag filled with haemoglobin, showing only remote resemblance to a living cell. The red cell, however, is like an old man who has seen better days and who still has a number of memories of those old times. Some of these memories may be vague and if they are reproduced the performance is certainly much less dramatic than that which happens in vital cells. However, if one asks the red cell intelligent questions it gives a number of interesting answers. Parallel and independent studies on sugar transports in red cells have been made particularly by Widdas in England[9–11], LeFèvre in U.S.A.[12–14] and in our laboratory in collaboration with Rosenberg[7,8,15–17,18–28].

Sugar Transport in Red Cells

The erythrocyte (to carry on our analogy) of man and ape is the only adult mammalian red cell with vivid memories of glucose transport[29]. It takes up or releases various sugars according to existing gradients ending with equal concentrations inside and outside the cell. No active transport is observed under normal conditions. Widdas has made the interesting observation that in foetal red cells of species whose adult cells are impermeable to glucose, very effective sugar transport systems are found.

Nevertheless the red cell shows phenomena of limited capacity and graded affinity similar to those in the epithelia of intestine and kidney. The rate of entry is not proportional to the external concentration as would be expected from diffusion processes but falls off with higher concentrations. Plotting the rate against the concentration yields curves

of the saturation type[23]. Such a curve is shown in Figure 1. Limited capacity is shown by the fact that the total amount of sugar entering from a mixture of three sugars in equal concentrations falls much below the calculated sum for the individual penetration rates to be expected from

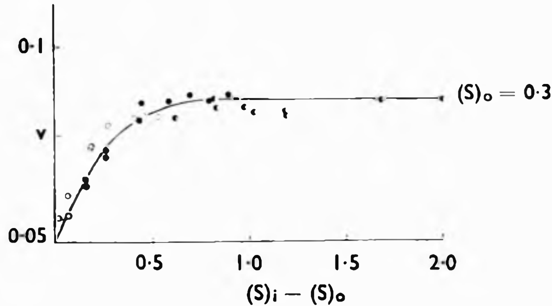


FIG. 1. Dependence of the transport rate v on the internal sugar concentration $(S)_i$ in an experiment on sugar exit from human red cells. Ordinate in units of transport rate v; abscissa in concentration difference.

a consideration of rates of independent penetration[23]. If two sugars are added successively rather than simultaneously they show graded effectiveness: the penetration of glucose in the presence of sorbose is practically unchanged whereas that of sorbose in the presence of glucose is strongly inhibited[13].

Transport Kinetics

A closer study reveals unexpected peculiarities. A striking one is the strong effect of the concentration on the side of the membrane towards which penetration proceeds and which has been termed the trans-side. Variation of the trans-concentration would be expected to affect the rate very little, if it is low compared with the cis-concentration. Actually the rate of exit of glucose from red cells depends largely on the low external concentration into which the sugar enters[8,11,14]. Plotting from such experiments the rate against the internal, that is, the cis-concentration (Fig. 2) one obtains curves of the saturation type in which the maximum rate largely depends on the trans-concentration[23].

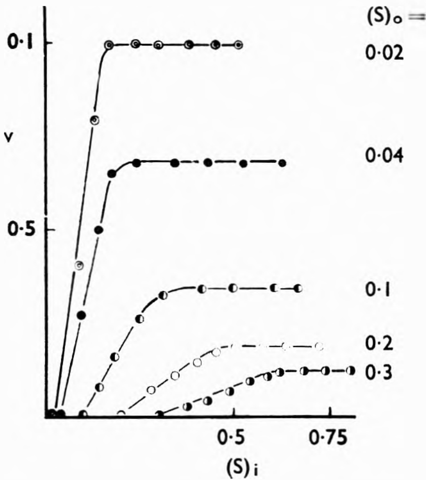


FIG. 2. Dependence of rate of transport on cis-concentration $(S)_i$ in exit experiments in human red cells for different trans-concentrations $(s)_o$. Ordinate: rate of transport, abscissa: cis-concentration $(S)_i$.

It can be shown that this behaviour is a kinetic consequence of the carrier mechanism under special conditions[11,16,21]. If the reaction between

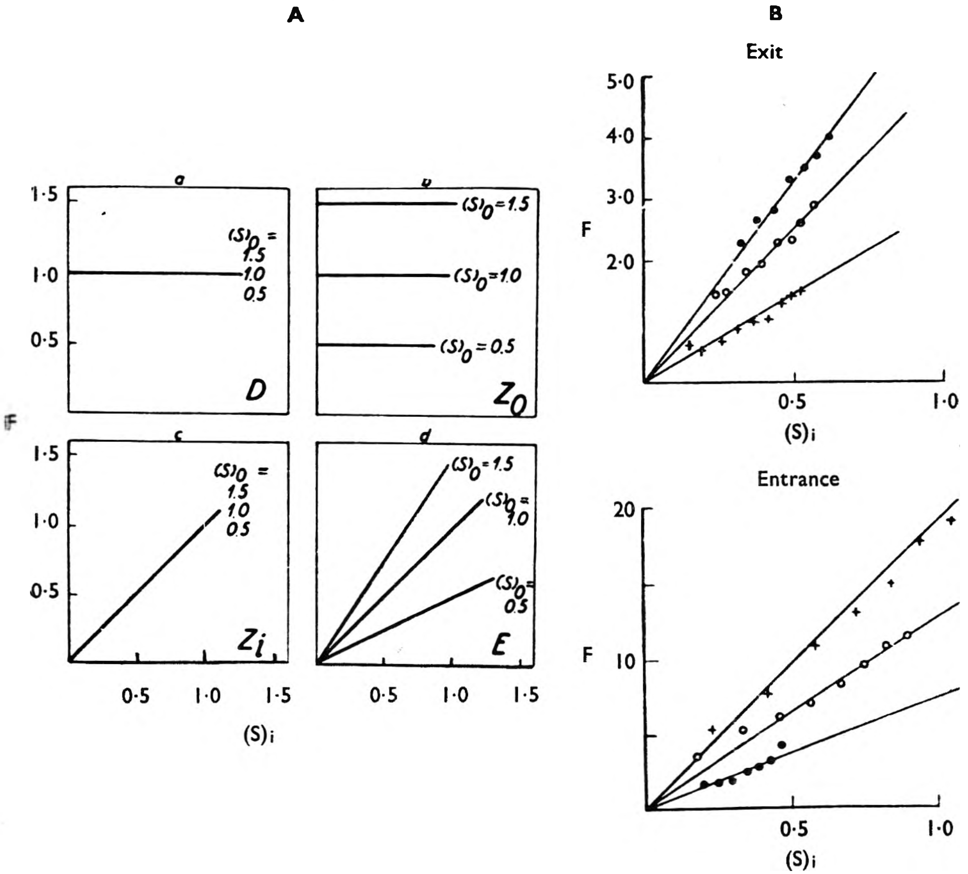


FIG. 3. F-test for E-kinetics.

A. Theoretical plots of the penetration resistance F against the internal glucose concentration $(S)_i$ for the different kinetic types, calculated. The constants K_D, K_{Z_o}, K_{Z_i} and K_E have arbitrarily been chosen as unity. Curves are given for $(S)_o = 0{\cdot}5$, 1·0 and 1·5.

B. Plot of the observed transport resistance F against internal glucose concentration $(S)_i$ for various values of $(S)_o$.
The plots only agree with E-kinetics.

Exit $(S)_o = 0{\cdot}3$: ●; 0·2: ○; 0·1: +.
Entrance $(S)_o = 0{\cdot}5$: ●; 1·0: ○; 1·5: +.

carrier and substrate is rapid so that equilibrium is established on both sides of the membrane the rate will be proportional to the difference between two Michaelis-Menten terms,

$$\frac{dS}{dt} = v = D'(C)_T \left\{ \frac{(S)_I}{(S)_I + K_S} - \frac{(S)_{II}}{(S)_{II} + K_S} \right\} \quad \ldots \quad (1)$$

(with $\frac{dS}{dt}$ = amount of substrate penetrating unit area per unit time
v = transport velocity, D' = diffusion coefficient of the complex in the membrane, divided by the thickness of the membrane, $(S)_I$ and $(S)_{II}$ = substrate concentrations on the two sides of the membrane, K_S = Michaelis constant of the carrier substrate complex).

The kinetics depend on the degree of saturation of the carrier. Far from saturation, the kinetics will be of the diffusion type:

$$v = \frac{D'(C)_T}{K_S}\left\{(S)_I - (S)_{II}\right\} \qquad (2)$$

the rate being proportional to the difference of the two substrate concentrations.

Near saturation a special type of kinetics emerges which has been termed E-kinetics and in which the rate is proportional to the difference of the reciprocals of the concentrations rather than of the concentrations themselves:

$$v = D'(C)_T K_S\left\{\frac{1}{(S)_{II}} - \frac{1}{(S)_I}\right\} \qquad (3)$$

Since under these conditions the trans-concentration will be dominating over a wide range the strong influence of this concentration now becomes understandable.

More stringent tests to see whether E-kinetics hold for glucose penetration can be performed in several ways. One is to plot the "transport resistance" ($F = \frac{(S)_I - (S)_{II}}{v}$) against $(S)_I$ which for the two types of kinetics in question (D and E) should yield the results shown in Figure 3A. The plot from experimental data agrees well with the kinetics type E as shown in Figure 3B.

Another possible test is the comparison of observed penetration curves with theoretical curves obtained by integration of the equations (2) and (3) for the conditions of the penetration experiment. This can best be done by plotting a suitable function of the experimental parameters against time, which should give a straight line if the differential equation chosen is correct. The fit is good with E kinetics for glucose in higher concentrations, whereas for sorbose a similar plot for D-kinetics rather than for E-kinetics yields a good fit[11]. Since sorbose has a low affinity it is expected to be far from saturation in concentrations in which glucose is near saturation. For glucose in low concentrations apparently D-kinetics is approached, as can be seen, from a plot of "permeability constants" calculated on the basis of diffusion against sugar concentration[23]. Whereas in the range of high concentrations the "constants" are far from constant (indicating that the transfer does not follow diffusion kinetics), in the range of low concentrations the "constants" tend to actually become constant indicating an approach towards diffusion kinetics. The prediction of D-kinetics in the range of low saturation thus agrees with several observations.

Enzymic Reaction Between Carrier and Substrate

If the reaction between carrier and substrate is enzymatic the general pattern of the kinetics does not chage markedly. However, under these circumstances two additional sites of possible saturation appear in the two enzymes on the two sides of the membrane. E-kinetics then emerge under conditions under which at least two sites are near saturation, either the carriers on the two sides of the membrane or the carrier on one side of the membrane and the enzyme on the other side; but not if the two enzymes are near saturation[16].

There are a number of reasons for assuming enzymatic reactions to be involved. One is the surprisingly large structural specificity for sugar penetration. For instance under certain conditions (+)-xylose penetrates readily, where (—)-xylose does not and (—)-arabinose penetrates quickly, whereas (+)-arabinose does not[19]. This type and degree of stereospecificity is well-known in enzymatic reactions.

Another indication for enzymatic reactions is the effect of enzyme inhibitors which may block the transport partially or in many cases completely[21,23]. They include phlorizine and related substances, mercury and various mercury compounds, lachrymators, narcotics, tannic acid, formaldehyde and a number of other substances. None of these inhibitors is sufficiently specific to indicate the participation of an individual enzyme.

Since the sugar transport is not uphill no energy yielding metabolism would be expected to be required. This makes it probable that the inhibited enzymes are involved in the transport mechanism itself. Inhibitors interfering with glycolysis like iodoacetate do not inhibit glucose penetration. Shapiro's view that phlorizine inhibition of glucose reabsorption in the kidney is due to inhibition of dehydrogenases[30], would hardly be applicable to the sugar transport in the red cell.

The fact that enzyme inhibitors may block transportation completely has some bearing on the possible mechanisms involved. It seems to indicate that unless the enzymatic part of the mechanism works, the membrane is impermeable to the sugar molecule. Any role for an enzyme which is accelerating only, like a trapping mechanism maintaining steep gradients, would therefore appear to be improbable.

Affinity to the Transport System

With respect to the affinity of various sugars to the transport mechanism, an order has been established from experiments on competitive inhibition by LeFèvre. According to him the order of decreasing affinity is glucose, mannose, xylose, galactose, sorbose. The determination of numerical values for the dissociation constants of the sugar carrier meets with the difficulty that the equation for the rate of transport contains two further unknown quantities besides the dissociation constant: the total concentration of the carrier and its diffusion coefficient in the membrane. Several ways of eliminating these unknowns have been suggested. Widdas determined the constant from experiments in which glucose inhibited the penetration of sorbose[11]. Assuming that the two sugars use the same

mechanism, he arrived at a figure for the constant for glucose of about 10 mM.at 37°.

Transport Rate and Affinity

An interesting question is the relation between affinity and rate of transport. One might expect proportionality. There are, however, observations which would rather point to a reciprocal relationship. Forster and others[31] found in experiments on the transport of dyestuffs across kidney tubules that powerful competitors are slowly transported and vice versa. The same observation was made by Wiseman in experiments on amino acids transport across intestinal cells[32].

A kinetic analysis shows that the relationship to be expected depends on the saturation conditions. Under conditions of low saturation, under which D-kinetics hold, the rate becomes proportional to the affinity, that is, inversely proportional to the dissociation constant as shown by equation (2). Near saturation, however, the rate is proportional to the dissociation constant, that is, inversely proportional to the affinity as shown by equation (3). This result leads to the prediction that the order of rates for different substrates should depend on the conditions of saturation. Near saturation the transport of the substrate with the highest affinity should be the slowest, far from saturation, the fastest one.

Experiments on sugar transports in red cells bear out this prediction[22]. In high concentrations the order of increasing penetration rates is glucose, mannose, galactose, arabinose, and sorbose; in low concentrations, however, it is completely reversed: sorbose, arabinose, galactose, mannose, and glucose.

The experiments by Forster and colleagues have been carried out in a range of dyestuff concentrations in which a further increase of concentration did not affect the rate of penetration perceptibly, that is they were near saturation. Under these conditions Forster's observations agree well with those of carrier kinetics.

Lipid-soluble Sugar Complexes

If, as concluded from the effect of enzyme inhibitors, the membrane is impermeable to the unchanged substrate molecule, what type of reaction then will be suitable to change the molecule into a transport form capable of penetration? One possibility appears to be the transformation into a lipid-soluble molecule, as suggested by Overton as early as 1902.

As a model of this type of glucose complex, a neutral lipid-soluble ester, glucose benzoate, has been synthesised and tested experimentally[15]. If glucose penetrates in the form of a lipid-soluble complex the fact that some red cells are impermeable to glucose probably will not be due to their impermeability to this complex because lipid solubility is a general condition for easy penetration. More likely the difference will lie in the ability to perform the substrate carrier reaction. The lipid-soluble glucose complex then will be expected to penetrate not only human cells but also cells which are impermeable to glucose. If, furthermore, inhibitors affect the reaction between substrate and carrier they will not

be expected to inhibit the penetration of the lipid-soluble complex. Actually glucose benzoate was found to penetrate human cells as well as beef cells and all other cells that have been tested, and not to be affected by phlorizine.

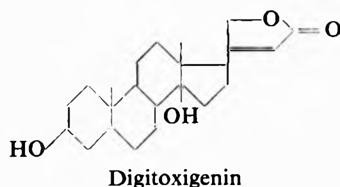
The Possible Chemical Nature of Carrier Molecules

The question of the possible chemical structure of the supposed carrier molecules is unanswered as yet, although suggestions have been made. With respect to sugar transport in red cells at present only a negative statement appears justified: it is highly improbable that the carrier is inorganic phosphate as it is assumed to be in the widely discussed phosphorylation theory of sugar absorption from the intestine and in the kidney tubules.

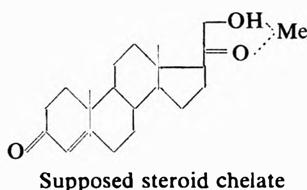
Neither glucose-6-phosphate nor glucose-1-phosphate nor other hexosephosphate esters which were tested are capable of penetrating the red cell membrane[15].

Steroids as Possible Carrier Molecules for Ions

With respect to ion transports a suggestion has been made recently that may be mentioned briefly: the possibility that corticosteroids might act as carriers for cations.



Digitoxigenin



Supposed steroid chelate

FIG. 4. Molecular structures of digitoxigenin and of a supposed chelate of desoxycorticosterone.

The role of corticosteroids in ion transports is well known particularly from the physiology of renal excretion. The chemical structure of corticosteroids contains in the side chain a configuration from which the ability of chelate formation may be expected. The comparison between a supposed steroid chelate and the genin of a cardiac glycoside (Fig. 4) reveals a certain degree of structural similarity[33]. Thus the possibility of a competitive antagonism between the two molecular species seemed worth a test. This test was first performed by Schatzmann[34] on cold-stored red cells which on rewarming in the presence of glucose perform an active transport of sodium and potassium across the membranes (another example of their "memories from old times"). Schatzmann found an

inhibition of the transport by cardiac glycosides which has since been confirmed and extended in a number of laboratories[35,36,37]. The inhibition does not appear to be related to the energy yielding metabolism since glycolysis[34] and the production of ATP[38] are unimpaired, in contrast to to the inhibition by iodoacetate and the like. Glycoside thus seems to act on the transport mechanism, possibly on the carrier.

Schatzmann in 1954[39] found no enhancing effect of corticosteroids in the absence of glycosides. On the contrary, desoxycorticosterone in very high concentrations showed some inhibition. It appeared, however, that cells of adrenalectomized animals transported slightly less actively than did normal cells.

These experiments have recently been taken up again by Sulser and Kunz. Schatzmann's observation of the lower transport activity in cells of adrenalectomised rats was confirmed and extended[40]. Taking the hematocrit reading as a measure of adrenal insufficiency and plotting transport rate against this figure a clear negative correlation was seen: the higher the degree of insufficiency the slower the transport.

With respect to the supposed antagonism between corticoids and cardiac-glycosides it appeared possible that the action of corticoids could only be observed in the presence of glycoside. Actually Sulser and Wilbrandt[33] found that desoxycorticosterone as well as other corticosteroids under certain conditions do show an effect opposite to that of cardiac glycoside but only in the presence of an inhibitory concentration of glycoside. This result would be expected if the cells were saturated with corticosteroids (a condition that may explain the ineffectiveness of steroids in other cases as well).

The assumption that cardiac glycosides compete with steroid chelates has kinetic consequences that can be tested. The competitive antagonism in this instance should not only depend on the concentration of glycoside and steroid but also on that of the ions to be chelated. The antagonistic action of glycosides should be diminished by high concentrations of these ions. This is what Glynn found experimentally for the inhibition of K influx in red cells by cardiac glycoside. Kinetical equations for this influence of cation concentrations can be derived. The result is that for a given concentration of steroid the ratio between cation concentrations with equal transport rates in the presence and the absence of cardiac glycosides should be constant. Glynn's experiments have born out this quantitative prediction in a very satisfactory way. A qualitatively similar dependence on the potassium concentration was observed by Caviezel[42] for the glycoside action on heart muscle.

One of the main sites of physiological action of corticosteroids is the kidney. In this organ a definite antagonism between cardiac glycosides and steroids was found in the adrenalectomised rat[43]. While desoxy-corticosterone induces the well-known retention of sodium, cardiac glycoside causes a considerable increase in the excretion of sodium which is antagonised by desoxycorticosterone very effectively. In normal (not adrenalectomised) animals the same type of antagonism can be demonstrated clearly after the administration of adrenocorticotrophic hormone,

ACTH. The sodium retention induced by ACTH is antagonised by intravenous injections of K-strophanthoside in a very rapid response. With high doses of strophanthoside the effect is dramatic, but transient. It can be repeated at short intervals. In heart muscle comparable antagonisms have been observed by several authors and under different conditions. Sulser and Kunz[41] have found that the potassium loss induced by cardiac glycoside in the perfused guinea pig heart is completely antagonised by aldosterone. Pöldre and Taeschler[44] described a considerable increase of the toxicity of cardiac glycosides in adrenalectomised animals, that was counteracted by corticosteroids. Similar effects have been observed by Greeff[45].

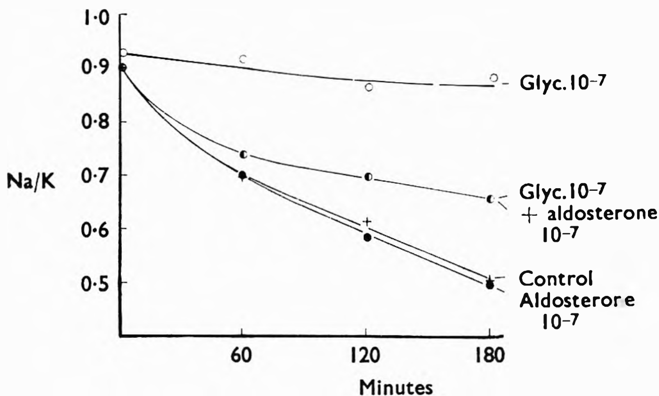


FIG. 5. Antagonism between K-strophanthoside (Glyc.) and aldosterone with respect to their influence on active transport of Na and K across the red cell membrane. Ordinate: intracellular concentration ratio Na/K of cold stored cells, incubated at 37° in the presence of glucose.
The ratio decreases due to active transport of K into and Na out of the cells. The transport is inhibited by glycoside and reactivated by aldosterone.

In summary, some experimental support for the suggestion of steroids acting as carriers in ion transports is available. Up to now, however, the evidence, while suggestive, is indirect and further experimental analysis is to be awaited.

Uphill Transfer by Counter-Transport

Returning to the general features of carrier transports the previously raised question as to which special features distinguish the uphill transport may now be taken up again. A discussion of this question leads back to the sugar transporting systems.

The sugar transport across the red cell membrane is not an uphill transport. In other cells and cell layers, particularly in the intestine and in the kidney it is or may be active. What is the essential difference?

Rosenberg[46] has given a most valuable discussion of the thermodynamics of active transports. He pointed out that an uphill transport is one special case of a movement of a thermodynamic quantity from a lower to a higher level of thermodynamic potential, and that two thermodynamic

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conditions exist for such a movement. One is the simultaneous movement of another thermodynamic quantity from higher to lower level of thermodynamic potential, the second is a coupling link between the two transports. The second thermodynamic quantity may for instance be electricity moving between two levels of electrical potential, or entropy between two temperatures or again molecules moving from higher chemical potential to lower.

In the case of red cells there appear to be several sugars using the same transport mechanism which then could serve as the coupling link. Thus a first sugar moving in one direction across the cell membrane should be able to induce an active transport of a second sugar in the opposite direction. The experimental test was carried out with labelled glucose and with unlabelled mannose[17]. A concentrated cell suspension was equilibrated with labelled glucose and then mannose was added to the external solution in high concentration. A temporary rise of activity in the external solution indicated the movement of labelled glucose from the cells into the external solution against the gradient, an effect that was reversible with a time course corresponding to the penetration of mannose. Unlabelled glucose produced the same effect (Fig. 6). A similar experiment was performed by Park and others[47] using glucose and xylose.

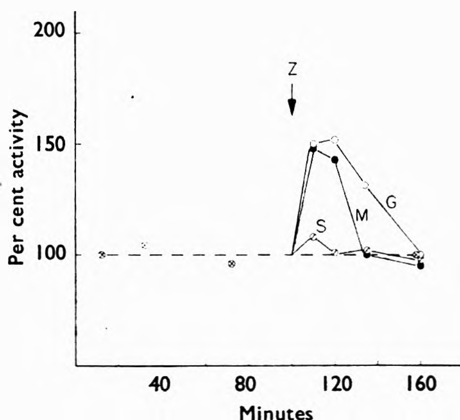

FIG. 6. Countertransport of labelled glucose out of red cells induced by a flow of mannose into the cells.
Ordinate: activity in the external medium of a suspension of red cells, equilibrated with ^{14}C-glucose. Z: addition of mannose (M) or glucose (G) or sucrose (S) in high concentration to the medium to produce a gradient into the cell. The transient rise of external activity induced by M or G indicates a transient uphill contertransport of ^{14}C-glucose from equilibrium out of the cells lasting for the time of mannose (or glucose) penetration into the cells. Correction has been made for activity changes due to dilution.

Thus movements of substrates across a common transport mechanism appears to be one possibility for the mechanism of active carrier transports. If it is a general mechanism the question may be raised what then are the conditions for active transports of high efficiency like the hydrogen ion transport in the stomach which is performed against a gradient of $1:10^6$? If the immediate source of energy for transports are gradients, these must be very steep indeed. This, in combination with the high turn-over numbers of some transports appears to make the participation of enzymes indispensable. The free energy of an enzymatic reaction then produces the "pressure" which is necessary to create steep gradients.

Efficiency of Uphill Carrier Transport

Another remark may be made with respect to the question raised above—whether carrier transports have any particular advantages for active transports. One such advantage would appear to be the fact that they act or may act across membranes which are impermeable to the unchanged substrate molecules. If this were not so, back diffusion of the substrate would be considerable. This would mean poor efficiency and the necessity of keeping the machine running continuously at high speed to diminish losses. Apparently this is not so in the case of active sugar transports. In the intestine[48] as well as in the kidney[49], observations have shown that if the active transport of glucose is blocked by phlorizine there is no back diffusion of glucose into the lumen as would be expected if the transport operated across a system with permeable membranes.

Flux Ratios in Carrier Transports

Finally the question of flux ratios may be touched briefly. Ussing[50] has shown that in free membrane diffusion the ratio of influx to outflux equals the ratio of the chemical activities outside to inside, or in the case of ions the ratio of the electrochemical activities. He also pointed out[51] that in cases where the substrate penetrates in combination with a carrier while the carrier cannot move uncombined (for example, from reasons of electric charge), the flux ratio will be equal to one. He introduced the term "exchange diffusion" for such a situation. Exchange diffusion has since been assumed to explain a number of observations, particularly with low flux ratio.

Such interpretations occasionally evoke the feeling that exchange diffusion is visualised as a complicating additional feature, that is, as a second mechanism operating parallel to and independent of the particular transfer in question, be the latter free diffusion or active transport. It seems worth pointing out that in carrier transports of the type discussed here any value of flux ratios between one and the activity ratio may be expected depending on the degree of saturation of the carrier. With very low degrees of saturation the flux ratio will approach the activity ratio, near saturation it will approach unity. Thus it appears quite unnecessary to invoke exchange diffusion as an additional "revolving door" in cases where unexpected values are found for the flux ratios, as long as they are within the range between unity and the activity ratio.

A consideration of the kinetics and the flux ratios to be expected in simple carrier systems might in some systems be helpful.

Conclusion

In summary the concept of a carrier transport appears to have a number of quantitative consequences which have been tested experimentally with positive results. The question of course can be raised whether or not other interpretations may be found to provide an equally good quantitative fit for some of these features. There are certainly other mechanisms that will show similar transport kinetics. E-kinetics will be observed in all systems with a linear relation between rate of transport and the difference

of two Michaelis-Menten terms. They include for instance under certain conditions, systems with two adsorption layers on the two sides of the membrane. Such an "adsorption membrane", however, would not be able to be used for uphill transports induced by counterflow, because this mechanism requires a coupling link with a mobile element as can be shown thermodynamically as well as kinetically[17]. A mechanism which does contain a mobile element is the rotating molecule discussed by Danielli[52].

With respect to alternative mechanisms, however, another remark may be pertinent. What appears to favour the carrier concept is not so much its exclusiveness as, rather the fact that it works with elements none of which requires essentially new assumptions[7]. It is well known that enzymes may be located on the surface of the membrane both within and without the cell. It is equally well known that substrate complexes move from one enzyme protein to the other and longest of all it has been known that lipid-soluble molecules move across cell membranes. Thus, one might be led to wonder how carrier transports could be avoided rather than whether they are probable. Nevertheless it should clearly be kept in mind that although the carrier concept may be a useful hypothesis, still it is but a hypothesis.

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RESEARCH PAPERS

CHEMOTHERAPEUTIC PROPERTIES OF SOME NEW QUATERNARY AMMONIUM SALTS

PART I. CHEMISTRY

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The preparation of a large number of new heterocyclic quaternary ammonium salts for biological screening is described. Certain of the quaternary salts possess amoebicidal, antibacterial, antifilarial and trypanocidal activity.

SOME of the chemotherapeutic properties of certain heterocyclic bis-quaternary ammonium salts have already been described[1-5]. It appeared desirable to investigate the effect of introducing one or more amino groups into the nuclei, and the present paper records the preparation and physical properties of a large number of new quaternary salts which have been examined in these laboratories during the past few years. The antifilarial activity of selected compounds will be described by Dr. F. Hawking and Dr. R. J. Terry of the National Institute for Medical Research in Part II[6], and some of the antimicrobial properties and toxicities of certain of the compounds will be recorded by Dr. H. O. J. Collier and his colleagues in Part III[7] of this series. For ease of reference, the abbreviated nomenclature first employed by Barlow and Ing[8] will be used; thus, BQn, BIQn, BAIQn and BACn refer respectively to the bis-quinolinium, -*iso*quinolinium, -7-amino-*iso*quinolinium and -4-amino-cinnolinium series, where n is equal to the number of methylene groups in the chain.

The heterocyclic amines and amides used were prepared by standard methods. The nitration product of 2-ethylquinoline-*N*-oxide was assumed to be the 4-nitro derivative following the work of Ochiai[9] on the chemistry of *N*-oxides. According to John[10], acetylation of 4-amino-2-phenyl-quinoline with acetic anhydride yields the 4-acetamido derivative. We have found that acetylation under these conditions gives 4-diacetylamino-2-phenylquinoline, but that the monoacetyl derivative is obtained by acetylation of the amine with a mixture of acetic anhydride and acetic acid. The quaternary salts were generally prepared by refluxing an excess of the appropriate base with a polymethylene dihalide in an organic solvent, for example, benzene, ethanol or ethyl methyl ketone. Where the base contained a free primary amino group in addition to the nuclear nitrogen, the former was generally protected by acylation; the resulting amide was then quaternised, and the product hydrolysed to give the

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TABLE I

POLYMETHYLENE BISQUINOLINIUM AND BIS*iso*QUINOLINIUM DI-IODIDES

(BQ and BIQ series)

$R^+.(CH_2)_n.R^+ \; 2I^-$

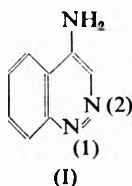
n	Reaction solvent and time (hr.)		m.p.	Cryst. form	Crystn. solvent	Found per cent C	Found per cent H	Found per cent N	Found per cent I	Formula	Required per cent C	Required per cent H	Required per cent N	Required per cent I
R^+ = *iso*Quinolinium														
22	EtOH	120	144°	Yellow granules or plates	EtOH	58·3	7·25	3·45	31·0	$C_{40}H_{58}N_2I_2$	58·5	7·1	3·4	31·0
23	MeCOEt	150	144–146°	Yellow microcrystals	EtOH	58·25	7·05	3·4	30·5	$C_{41}H_{60}N_2I_2$	59·0	7·25	3·4	30·5
24	MeCOEt	110	[1] 152–154°	Yellow plates	EtOH	59·7	7·7	3·0	29·5	$C_{42}H_{62}N_2I_2$	59·4	7·4	3·3	29·95
26	MeCOEt	100	114–117°	Yellow plates	EtOH	60·5	7·5	3·25	28·85	$C_{44}H_{66}N_2I_2$	60·3	7·6	3·2	29·0
30	MeCOEt	150	Sinters 100–104°, m.p. 120–124°	Yellow plates	EtOH	61·4	8·3	3·1	27·3	$C_{48}H_{74}N_2I_2$	61·8	8·0	3·0	27·25
32	MeCOEt	120	Sinters 122°, m.p. 144–146°	Yellow plates	MeOH	62·3	7·9	2·9	26·6	$C_{50}H_{78}N_2I_2$	62·5	8·2	2·9	26·5
36	MeCOEt	90	Melts to a glass at 126–128°, flows at 184°	Yellow plates	MeOH	64·4	8·6	2·6	24·6	$C_{54}H_{86}N_2I_2$	63·8	8·5	2·8	25·0
40	MeCOEt	90	Melts to a glass at 128–130°, flows at 210°	Yellow plates	MeOH	64·5	9·0	2·6	23·8	$C_{58}H_{94}N_2I_2$	64·9	8·8	2·6	23·7
R^+ = Quinolinium														
6	MeCOEt	140	228–229°	Orange-yellow needles	EtOH	48·5	4·5	4·6	42·6	$C_{24}H_{26}N_2I_2$	48·3	4·4	4·7	42·6
22	EtOH	100	166–168°	Yellow plates	MeOH-Et_2O	58·9	7·0	3·5	30·5	$C_{40}H_{58}N_2I_2$	58·5	7·1	3·4	31·0
24	MeCOEt	210	166–168°	Orange-yellow microcrystals	EtOH	59·5	7·5	3·1	29·85	$C_{42}H_{62}N_2I_2$	59·4	7·4	3·3	29·95
32	MeCOEt	210	165–166°	Yellow plates	MeOH	63·0	8·15	2·55	25·8	$C_{50}H_{78}N_2I_2$	62·5	8·2	2·9	26·5

[1] Also forms various solvated crystals of lower m.p.

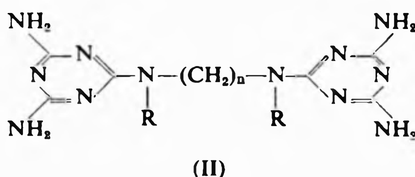
required quaternary salt. In some cases this precaution was found to be unneccessary; thus the products obtained by treating 4-aminoquinoline with either hexamethylene or decamethylene di-iodide were identical with those obtained by hydrolysis of the reaction product of 4-acetamido-quinoline with hexamethylene or decamethylene di-iodide respectively.

Attempts to quaternise the following were unsuccessful, possibly due to steric hindrance: 2-aminoquinoline, 8-acetamidoquinoline, 5:6- and 6:8-diacetamidoquinoline, 4-amino-6-acetamidoquinaldine and 3-acet-amido-*iso*quinoline. Analysis of the reaction product of 5:7-diacet-amidoquinoline with an alcoholic solution of decamethylene di-iodide corresponded reasonably with that of the hydriodide of the amide. This may be a solvent effect, since the amide was readily quaternised by, for example, *p*-xylylene di-iodide, when the reaction was carried out in ethyl methyl ketone solution. We have already reported numerous unsuccessful attempts to prepare decamethylene bisquaternary derivatives of 5-aminoacridine and related compounds[11].

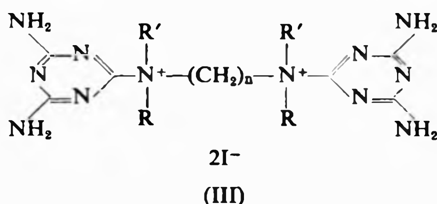
We have not proved the constitution of the bisquaternary ammonium salts derived from 4-aminocinnoline derivatives, but have assumed that quaternisation has occurred at N (1) and not at N (2) (I), since Simpson[12] has already advanced evidence of quaternary salt formation at N (1) for 4-amino-6-chlorocinnoline. Although Atkinson and Taylor[13] have shown that quaternisation of 4-amino-6-nitrocinnoline occurred at each of the ring nitrogens, they found that only the N (1) quaternary salt was formed when 4-acetamido-6-nitrocinnoline was used. All our cinnolinium salts have been prepared from 4-acetamidocinnoline derivatives; moreover, paper chromatographic investigation of a random selection indicated that the final products were single substances.



(I)



(II)



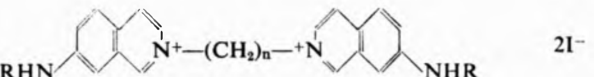
(III)

Melamine derivatives were prepared by reacting 2-chloro-4:6-diamino-*s*-triazine with the requisite *NN'*-dialkylpolymethylene diamine, and treating the product (II) with an alkyl iodide. Here again, we have not established the final identity of the quaternary products, but have assumed the structure shown by III. Attempts to prove this by reacting 2-chloro-4:6-diamino-*s*-triazine with *NNN'N'*-tetra-alkylpolymethylene diamines

TABLE II

POLYMETHYLENE BIS[(7-ACYLAMIDO-) AND (7-AMINO-)*iso*QUINOLINIUM IODIDES]

(BAIQ series)



Reaction solvent = ethyl methyl ketone

R	n	Reaction time (hr.)	m.p.	Cryst. form	Crystn. solvent	Found per cent C	Found per cent H	Found per cent N	Found per cent I	Formula	Required per cent C	Required per cent H	Required per cent N	Required per cent I
Ac	3	24	321–323°	Yellow microcrystals	EtOH/H_2O	45·1	4·2	8·7	38·2	$C_{25}H_{26}O_2N_4I_2$	44·9	3·9	8·4	38·0
H	3	—	290–292° after softening at 165°	Yellow needles	MeOH/Et_2O	43·3	3·8	9·45	43·2	$C_{21}H_{22}N_4I_2$	43·15	3·8	9·6	43·5
Bz	4	40	335–337°	Yellow microcrystals	EtOH/H_2O	54·2	4·3	6·75	30·8	$C_{36}H_{32}O_2N_4I_2$	53·6	4·0	6·95	31·5
Ac	5	24	313–314°	Yellow microcrystals	MeOH/Et_2O	46·6	4·5	7·8	36·3	$C_{27}H_{30}O_2N_4I_2$	46·55	4·3	8·05	36·5
H	5	—	192–194°	Yellow needles	MeOH/EtOH	42·9	4·8	8·85	39·6[1]	$C_{23}H_{26}N_4I_2.1\frac{1}{2}H_2O$	43·2	4·6	8·8	39·75
Ac	6	24	320–321°	Yellow microcrystals	MeOH/Et_2O	46·9	4·6	8·1	36·1	$C_{28}H_{32}O_2N_4I_2$	47·3	4·5	7·9	35·8
H	6	—	Softens at 90°, m.p. 224–226°	Yellow micro-needles	EtOH/Et_2O	43·1	4·85	8·7	38·5[2]	$C_{24}H_{28}N_4I_2.2H_2O$	43·5	4·9	8·5	38·4
Ac	8	24	278–279°	Yellow nodules	MeOH/Et_2O	48·7	5·0	7·7	34·25	$C_{30}H_{36}O_2N_4I_2$	48·8	4·9	7·6	34·4
H	8	—	226–228°	Tan platelets	MeOH/Et_2O	48·0	4·9	8·2	34·4	$C_{26}H_{32}N_4I_2$	47·7	4·9	8·6	38·8
Ac[3]	9	24	—	—	—	—	—	—	—	—	—	—	—	—
H	9	—	226–228°	Yellow granules	MeOH/Et_2O	48·5	5·0	8·5	38·0	$C_{27}H_{34}N_4I_2$	48·5	5·1	8·4	38·0
Ac	10	24	[4]206–208° with efferves-cence	Pale pink microcrystals	MeOH/EtOH/Et_2O	50·1	5·5	7·2	33·0	$C_{32}H_{40}O_2N_4I_2$	50·1	5·3	7·3	33·2
H	10	—	239–240°	Yellow microcrystals	MeOH/Et_2O	48·9	5·5	8·3	37·3	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
Ac	12	24	204–206°	Yellow microcrystals	MeOH/Et_2O	51·5	5·7	7·05	31·85	$C_{34}H_{44}O_2N_4I_2$	51·4	5·6	7·05	32·0
H	12	—	267–268°	Yellow microcrystals	MeOH/Et_2O	50·7	5·65	7·8	35·7	$C_{30}H_{40}N_4I_2$	50·7	5·7	7·9	35·8
Ac	16	24	199–201°	Yellow needles	EtOH	53·95	6·3	6·8	30·7	$C_{38}H_{52}O_2N_4I_2$	53·65	6·2	6·6	29·9
H	16	—	230–232°	Yellow microcrystals	EtOH/Et_2O	54·0	6·25	6·9	32·5	$C_{34}H_{48}N_4I_2$	53·3	6·3	7·3	33·2
Ac[3]	19	43	—	—	—	—	—	—	—	—	—	—	—	—
H	19	—	225–227°	Yellow microcrystals	EtOH/Et_2O	54·4	6·6	7·2	31·6	$C_{37}H_{54}N_4I_2$	54·95	6·7	6·9	31·4
Ac	$(CH_2)_{11}$	18	289–290°	Pink microcrystals	EtOH/H_2O	53·1	4·3	9·8	27·3[5]	[6]$C_{30}H_{30}O_2N_4Br_2$	53·2	4·5	9·6	27·3[5]
H	But-2-ene		249–250°	Yellow microcrystals	MeOH/H_2O/Et_2O	44·4	3·95	9·4	42·4	$C_{22}H_{22}N_4I_2$	44·3	3·7	9·4	42·6

[1] Sample lost $\frac{1}{2}H_2O$ when dried at 100° *in vacuo*, and as analysis then corresponded to crystals containing $1\frac{1}{2}H_2O$, the original material must have been a di-hydrate.
[2] Analysis of material dried *in vacuo* at room temperature.
[3] Hydrolysed directly to the amino quaternary without purification.
[4] Monohydrate.
[5] Bromine.
[6] Bromide.

were unsuccessful, unidentifiable products containing no halogen being obtained. However, the fact that the compounds (assumed to be III) are reprecipitated unaltered by the addition of excess potassium iodide to an alkaline aqueous solution indicates that they are definitely quaternary salts and that methylation at the primary amino groups has not occurred.

Certain of the compounds described herein possess interesting amoebicidal (e.g., BAIQ 4), antibacterial (BIQ series in general), antifilarial (e.g., BIQ 20, BAC 20) or trypanocidal activity (e.g., melamine derivatives); these properties will be described in detail in Parts II and III.

Experimental

All m.p.s are uncorrected. Unless otherwise stated, the microanalyses of all quaternary salts were carried out on materials dried at 100° *in vacuo.*

The polymethylene dihalides employed were prepared from the diols by standard methods. 1:23-*di-iodotricosane*, colourless plates from acetone, m.p. 71·5–72°. Found: C, 48·2; H, 8·0; I, 43·1. $C_{23}H_{46}I_2$ requires C, 47·9; H, 8·05; I, 44·1 per cent; 1:26-*di-iodohexacosane*, colourless plates from acetone-ethanol, m.p. 77·5–78°. Found: C, 50·5; H, 8·8; I, 40·6. $C_{26}H_{52}I_2$ requires C, 50·5; H, 8·5; I, 41·1 per cent; and 1:36-*di-iodohexatriacontane*, colourless plates from acetone-benzene, m.p. 89–91°. Found: C, 56·5; H, 9·3; I, 34·0. $C_{36}H_{72}I_2$ requires C, 57·0; H, 9·6; I, 33·5 per cent.

1:23-*Dihydroxytricosane*, colourless plates from benzene, m.p. 106–106·5°. Found: C, 77·4; H, 13·85. $C_{23}H_{48}O_2$ requires C, 77·5; H, 13·6 per cent; and *hexatriacontane*-1:36-*bis*(p-*methoxyphenyl ether*), colourless plates from benzene, m.p. 122°. Found: C, 79·6; H, 11·3. $C_{50}H_{86}O_4$ requires C, 80·0; H, 11·6 per cent.

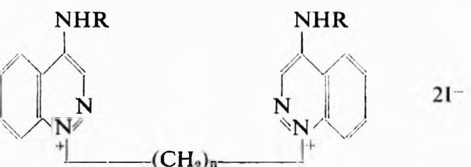
1:21-Dibromoheneicosane was prepared from heneicosa-1:20-dien-11-one[14] by Huang-Minlon reduction to *heneicosa*-1:20-*diene* (colourless oil, b.p. 166°/0·5 mm. Found: C, 86·0; H, 13·45. $C_{21}H_{40}$ requires C, 86·3; H, 13·8 per cent), and subsequent addition of hydrogen bromide in the presence of air.

3-*Acetamido*iso*quinoline*, colourless needles from water, m.p. 122–123°. Found: C, 71·0; H, 5·65; N, 14·75. $C_{11}H_{10}ON_2$ requires C, 71·0; H. 5·4; N, 15·05 per cent; 7-*benzamido*iso*quinoline*, colourless needles from benzene, m.p. 177–179°. Found: C, 77·5; H, 4·9; N, 11·1. $C_{16}H_{12}ON_2$ requires C, 77·4; H, 4·9; N, 11·3 per cent; 5:8-*diacetamido*-iso*quinoline*, colourless needles from water, m.p. 295–296°. Found: C, 64·0; H, 5·2; N, 17·2. $C_{13}H_{13}O_2N_3$ requires C, 64·2; H, 5·4; N, 17·3 per cent.

4-*Iodoquinaldine*, cream coloured needles from aqueous ethanol, m.p. 109–110·5°. Found: C, 44·2; H, 3·0; N, 5·2; I, 47·5. $C_{10}H_8NI$ requires C, 44·6; H, 3·0; N, 5·2; I, 47·2 per cent; 4-*acetamidoquinaldine hydrochloride*, colourless needles from ethanol, m.p. 280–282° (decomp.). Found: C, 60·0; H, 5·7; N, 11·9; Cl, 15·0. $C_{12}H_{13}ON_2Cl$ requires C, 60·9; H, 5·5; N, 11·8; Cl, 15·0 per cent; 4-*acetamido*-6-*methylquinaldine*, colourless needles from aqueous ethanol, m.p. 191–192°. Found: C, 72·75; H, 6·75; N, 12·9. $C_{13}H_{14}ON_2$ requires C, 72·9; H, 6·6; N, 13·1 per cent; 4-*acetamido*-5-*methylquinaldine*, colourless needles from benzene,

TABLE III

Polymethylene bis[(4-acylamido-) and (4-amino)cinnolinium iodides] (BAC series)



Reaction solvent = ethyl methyl ketone

R	n	Reaction time (hr.)	m.p.	Cryst. form	Crystn. solvent	Found per cent C	Found per cent H	Found per cent N	Found per cent I	Formula	Required per cent C	Required per cent H	Required per cent N	Required per cent I
Ac	4	75	262–263°	Orange microcrystals	MeOH/EtOH	42·7	4·2	11·9	36·2	$C_{24}H_{20}O_2N_6I_2$	42·1	3·8	12·3	37·1
H	4	—	277–279°	Yellow microcrystals	MeOH/EtOH	40·2	3·9	14·1	41·7	$C_{20}H_{22}N_6I_2$	40·0	3·7	14·0	42·3
H	4	—	253–254°	Colourless microcrystals	MeOH/EtOH	44·6	4·3	15·2	12·7[1]	[2]$C_{20}H_{22}O_8N_6Cl_2$	44·0	4·1	15·4	13·0[1]
Ac	6	205	223°	Orange-red microcrystals	MeOH/EtOH	43·7	4·7	11·7	35·2	$C_{26}H_{30}O_2N_6I_2$	43·8	4·25	11·8	35·7
H	6	—	281–282°	Yellow prisms	EtOH/H_2O	42·2	3·9	13·2	40·2	$C_{22}H_{26}N_6I_2$	42·0	4·2	13·4	40·45
Ac	7	204	236–238°	Orange-red prisms	MeOH/EtOH	44·1	4·65	11·7	34·8	$C_{27}H_{32}O_2N_6I_2$	44·6	4·4	11·6	35·0
H	7	—	222–224°	Yellow granules	EtOH	43·2	4·6	13·0	39·35	$C_{23}H_{28}N_6I_2$	43·0	4·4	13·1	39·6
Ac	8	232	223–224°	Orange granules	MeOH/EtOH	45·4	4·95	11·4	34·0	$C_{28}H_{34}O_2N_6I_2$	45·4	4·6	11·35	34·3
H	8	—	252°	Dark yellow rosettes	MeOH/EtOH	43·95	4·8	12·7	38·5	$C_{24}H_{30}N_6I_2$	43·9	4·6	12·8	38·7
Bz	10	53	160–162°	Orange plates	EtOH	53·7	4·8	9·3	28·45	$C_{40}H_{42}O_2N_6I_2$	53·8	4·75	9·4	28·5
H	10	—	209–210°	Yellow plates	EtOH/Et_2O	45·65	5·4	11·9	37·05	$C_{26}H_{34}N_6I_2$	45·6	5·0	12·3	37·1
H	10	—	195–197°	Cream needles	EtOH	56·3	6·1	19·9	—	[3]$C_{26}H_{34}O_6N_8$	56·3	6·2	20·2	—
Ac	16	210	156–158°	Buff plates	EtOH/Et_2O	50·8	6·3	9·65	29·8	$C_{36}H_{50}O_2N_6I_2$	50·7	5·9	9·9	29·8
H	16	—	[4]181–183°	Yellow-brown nodules	EtOH	50·3	5·7	10·8	32·7	$C_{32}H_{46}N_6I_2$	50·0	6·0	10·9	33·1
Ac[5]	18	280[6]	—			—	—	—	—	—	—	—	—	—
H	18	—	164–166°	Yellow needle clusters	EtOH	52·0	6·3	10·45	31·2	$C_{34}H_{50}N_6I_2$	51·3	6·3	10·55	31·9
Ac[5]	20	286[6]	—	—	—	—	—	—	—	—	—	—	—	—
H	20	—	186–188°	Yellow granules	EtOH/Et_2O	52·45	6·7	10·2	30·6	$C_{36}H_{54}N_6I_2$	52·4	6·6	10·2	30·8
H	20	—	198–199°	Cream microcrystals	EtOH/Et_2O	67·3	8·4	12·9	11·2[1]	[7]$C_{36}H_{54}N_6Cl_2$	67·4	8·5	13·1	11·1[1]
Ac	22	158	171°	Red plates	EtOH	53·6	6·7	9·1	27·4	$C_{42}H_{62}O_2N_6I_2$	53·85	6·7	9·0	27·1
H	22	—	162–163°	Yellow needles	EtOH	53·8	6·9	10·0	29·7	$C_{38}H_{58}N_6I_2$	53·5	6·9	9·9	29·8

[1] Chlorine. [2] Perchlorate. [3] Nitrate. [4] Monohydrate. [5] Reaction solvent = ethanol. [6] Hydrolysed directly to the amino quaternary without purification. [7] Chloride.

m.p. 173–174°. Found: C, 72·5; H, 6·5; N, 12·9. $C_{13}H_{14}ON_2$ requires C, 72·9; H, 6·6; N, 13·1 per cent.

2-*Ethylquinoline*-N-*oxide*, yellow oil, b.p. 192–194°/14 mm. Found: C, 75·6; H, 6·2; N, 8·35. $C_{11}H_{11}ON$ requires C, 76·3; H, 6·4; N, 8·1 per cent; 4-*nitro*-2-*ethylquinoline*-N-*oxide*, yellow needles from ethanol, m.p. 123–124°. Found: C, 60·4; H, 4·6; N, 12·95. $C_{11}H_{10}O_3N_2$ requires C, 60·55; H, 4·6; N, 12·8 per cent; 4-*amino*-2-*ethylquinoline*, colourless needles from benzene-light petroleum (b.p. 40–60°), m.p. 153–155°. Found: C, 76·65; H, 7·1; N, 15·9. $C_{11}H_{12}N_2$ requires C, 76·7; H, 7·0; N, 16·3 per cent; 4-*hydroxy*-2-*ethylquinoline*, colourless needles from water, m.p. 180–181°. Found: C, 76·05; H, 6·4; N, 7·9. $C_{11}H_{11}ON$ requires C, 76·3; H, 6·4; N, 8·1 per cent.

2-n-*Propylcinchoninamide*, colourless needles from benzene, m.p. 193–194°. Found: C, 72·75; H, 6·7; N, 12·85. $C_{13}H_{14}ON_2$ requires C, 72·9; H, 6·6; N, 13·1 per cent; 4-*amino*-2-n-*propylquinoline*, cream coloured needles from benzene, m.p. 141–142°. Found: C, 77·1; H, 7·6; N, 14·8. $C_{12}H_{14}N_2$ requires C, 77·4; H, 7·6; N, 15·05 per cent; 4-*acetamido*-2-n-*propylquinoline*, colourless needles from benzene-light petroleum (b.p. 40–60°), m.p. 135–137°. Found: C, 73·6; H, 6·9; N, 11·9. $C_{14}H_{16}ON_2$ requires C, 73·7; H, 7·1; N, 12·3 per cent; 4-*chloro*-2-n-*propylquinoline*, colourless liquid, b.p. 88–90°/0·1 mm. Found: C, 70·1; H, 5·8; N, 6·8; Cl, 16·9. $C_{12}H_{12}NCl$ requires C, 70·1; H, 5·9; N, 6·8; Cl, 17·3 per cent.

4-*Amino*-2-*phenylquinoline hydriodide*, colourless needles from ethanol, m.p. 249–250° (decomp.). Found: C, 51·9; H, 3·65; N, 7·85; I, 36·65. $C_{15}H_{13}N_2I$ requires C, 51·7; H, 3·8; N, 8·05; I, 36·5 per cent; 4-diacetylamino-2-phenylquinoline, colourless needles from ethanol, m.p. 108–110° (recorded[10] 117°). Found: C, 75·6; H, 5·25; N, 8·7. Calc. for $C_{19}H_{16}O_2N_2$, C, 75·0; H, 5·3; N, 9·2 per cent; 4-acetamido-2-phenylquinoline, colourless needles from ethanol, m.p. 193–194° (recorded[10] 108°). Found, C, 77·6; H, 5·5; N, 10·7. Calc. for $C_{17}H_{14}ON_2$, C, 77·9; H, 5·4; N, 10·7 per cent; *hydriodide*, colourless needles from ethanol, m.p. 279–281° (decomp.). Found: C, 51·75; H, 4·1; N, 6·8; I, 31·7. $C_{17}H_{15}ON_2I$ requires C, 52·3; H, 3·9; N, 7·2; I, 32·6 per cent.

5-*Acetamido*-6-*methylquinaldine*, colourless needles from benzene, m.p. 206–207°. Found: C, 72·4; H, 6·6; N, 13·0. $C_{13}H_{14}ON_2$ requires C, 72·9; H, 6·6; N, 13·1 per cent; 5:6-*diacetamidoquinoline*, cream coloured needles from aqueous ethanol, m.p. 257–258°. Found: C, 64·4; H, 5·15; N, 17·1. $C_{13}H_{13}O_2N_3$ requires C, 64·2; H, 5·4; N, 17·3 per cent; 5:7-*diacetamidoquinoline*, colourless needles from aqueous ethanol, m.p. 283°. Found: C, 64·1; H, 5·25; N, 17·1. $C_{13}H_{13}O_2N_3$ requires C, 64·2; H, 5·4; N, 17·3 per cent; 6:8-*diacetamidoquinoline*, colourless needles from aqueous ethanol, m.p. 243–244°. Found: C, 64·3; H, 5·4; N, 17·5. $C_{13}H_{13}O_2N_3$ requires C, 64·2; H, 5·4; N, 17·3 per cent.

Quinoline-7-*carboxamide*, cream coloured needles from chloroform-light petroleum (b.p. 40–60°), m.p. 178–179°. Found: C, 69·65; H, 4·7; N, 16·0. $C_{10}H_8O_2N$ requires C, 69·8; H, 4·7; N, 16·3 per cent; 7-*acetamido*-4-*aminoquinoline*, colourless needles from water, m.p. 287–288°.

TABLE IV

MISCELLANEOUS DECAMETHYLENE BIS(QUATERNARY AMMONIUM IODIDES)

$[R^{+}.(CH_2)_{10}.R^{+}]2I^{-}$

Reaction solvent = ethyl methyl ketone

R	Reaction time (hr.)	m.p.	Cryst. form	Crystn. solvent	Found per cent				Formula	Required per cent			
					C	H	N	I		C	H	N	I
3 AcNH Q	580	206–207°	Yellow nodules	MeOH/EtOH	50·0	5·3	7·2	33·2	$C_{32}H_{40}O_2N_4I_2$	50·1	5·3	7·3	33·2
3 NH_2 Q	—	159–161°	Yellow microcrystals	EtOH	48·5	5·6	8·2	36·9	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
4 AcNH Q[1]	260	—	—	—	—	—	—	—	—	—	—	—	—
4 NH_2 Q[2]	40	238–239°	Cream microcrystals	EtOH	48·65	5·5	8·2	37·1	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
4 AcNH.6MeO Q	600	215–216°	Yellow needles	EtOH	49·0	5·7	6·7	30·75	$C_{34}H_{44}O_4N_4I_2$	49·4	5·4	6·8	30·75
4 NH_2.6MeO Q	—	253–254°	Yellow needles	$EtOH/Et_2O$	48·5	5·8	7·5	34·1	$C_{30}H_{40}O_2N_4I_2$	48·5	5·4	7·55	34·2
5 AcNH Q[1]	400	—	—	—	—	—	—	—	—	—	—	—	—
5 NH_2 Q	—	281°	Red microcrystals	MeOH/EtOH	49·2	5·5	8·4	36·9	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
5 Cl Q	400	218–220°	Orange-red needles	$MeOH/Et_2O$	46·2	4·4	3·8	45·1[3]	$C_{28}H_{32}N_2Cl_2I_2$	46·6	4·5	3·9	45·1[3]
6 AcNH Q[4]	980	238–240°	Yellow needles or nodules	MeOH	49·7	5·0	7·3	33·5	$C_{32}H_{40}O_2N_4I_2$	50·1	5·3	7·3	33·2
6 NH_2 Q	—	242–243°	Orange prisms	MeOH/EtOH	48·7	5·3	8·3	37·1	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
7 AcNH Q	135	[5]Sinters at 120° m.p. 216–218°	Orange nodules	MeOH/EtOH	49·7	5·1	7·3	33·2	$C_{32}H_{40}O_2N_4I_2$	50·1	5·3	7·3	33·2
7 NH_2 Q	—	249–251°	Orange needles	MeOH/EtOH	49·8	5·4	8·2	36·8	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
4 AcNH iQ	125	214–215°	Yellow needles	$EtOH/Et_2O$	50·0	5·4	7·4	32·9	$C_{32}H_{40}O_2N_4I_2$	50·1	5·3	7·3	33·2
4 NH_2 iQ	—	194–195°	Yellow needles	EtOH/light petroleum b.p. 40–60°	49·4	5·4	8·2	37·05	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
5 AcNH iQ[6]	440	186–188°	Yellow granules	EtOH	50·5	5·0	7·1	32·85	$C_{32}H_{40}O_2N_4I_2$	50·1	5·3	7·3	33·2
5 NH_2 iQ	—	208°	Yellow microcrystals	MeOH/EtOH	49·4	5·3	7·9	36·9	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
5:8 diAcNH iQ[1]	300	—	—	—	—	—	—	—	—	—	—	—	—
5:8 diNH_2 iQ	—	155–156°	Red needles	$EtOH/Et_2O$	46·8	5·7	11·85	35·35	$C_{28}H_{38}N_6I_2$	47·2	5·4	11·8	35·7
4:6 diAcNH C	130	223–225°	Orange granules	MeOH/EtOH	46·4	5·25	12·6	28·45	$C_{34}H_{44}O_4N_8I_2$	46·3	5·0	12·7	28·8
4:6 diNH_2 C	—	209–211°	Yellow microcrystals	MeOH/EtOH	44·0	5·2	15·65	35·3	$C_{26}H_{36}N_8I_2$	43·7	5·1	15·7	35·6
3 AcNH P[4]	96	Sinters 95–97°[5] then melts over a range	Cream coloured granules	EtOH	43·5	5·35	8·5	37·85	$C_{24}H_{36}O_2N_4I_2$	43·2	5·45	8·4	38·1
3 NH_2 P	—	177–178°	Cream coloured micro-crystals	EtOH	41·35	5·7	9·8	43·2	$C_{20}H_{32}N_4I_2$	41·2	5·5	9·6	43·6
3 $CONH_2$ P	70	206–208°	Colourless microcrystals	EtOH	48·4	5·85	9·9	29·2[7]	[8]$C_{22}H_{32}O_2N_4Br_2$	48·5	5·9	10·3	29·4[7]
4 AcNH P	115	173–174°	Cream coloured clusters	EtOH	43·4	5·5	8·7	37·8	$C_{24}H_{36}O_2N_4I_2$	43·2	5·45	8·4	38·1
4 NH_2 P	—	243–244°	Colourless needles	EtOH	41·6	5·3	9·5	43·5	$C_{20}H_{32}N_4I_2$	41·2	5·5	9·6	43·6
4 AcNH.NH.CO P	340	179–181°	Yellow granules	EtOH	42·0	5·4	11·4	33·8	$C_{26}H_{38}O_4N_6I_2$	41·5	5·1	11·2	33·8

Q = Quinolinium. iQ = *iso*Quinolinium. C = Cinnolinium. P = Pyridinium.

[1] Hydrolysed directly to the amino quaternary without purification. [2] Also prepared by acid hydrolysis of the 4-acetamidoquaternary salt. [3] Total halogen. [4] Reaction solvent benzene. [5] Dihydrate. [6] Reaction solvent ethanol. [7] Bromine. [8] Bromide.

Found: C, 65·1; H, 5·7; N, 20·85. $C_{11}H_{11}ON_3$ requires C, 65·7; H, 5·5; N, 20·9 per cent.

Baker[15] has prepared 4-aminocinnoline in 39 per cent yield from 4-hydroxycinnoline by conversion to the 4-chloro compound and treatment of this with alcoholic ammonia in the presence of copper acetate. We have obtained the amine in 70·5 per cent yield (calculated on the hydroxy compound) by the action of gaseous ammonia on a phenolic solution of 4-chlorocinnoline. The acetyl derivative, cream coloured needles from water, had m.p. 272–273° (as recorded[16]). Found: C, 64·1; H, 4·9; N. 22·35. Calc. for $C_{10}H_9ON_3$, C, 64·2; H, 4·85; N, 22·5 per cent. 4-*Benzamidocinnoline*, cream coloured needles from ethanol, m.p. 222–224°. Found: C, 72·15; H, 4·4; N, 16·75. $C_{15}H_{11}ON_3$ requires C, 72·3; H, 4·45; N, 16·9 per cent.

Preparation of Quaternary Salts

Method (*a*). By refluxing excess of the tertiary base with a dihalide in an appropriate solvent, for example: *Heneicosylenebis*(iso*quinolinium bromide*). 1:21-Dibromoheneicosane (1·0 g.) and *iso*quinoline (1·0 g.) in ethyl methyl ketone (25 ml.) were refluxed for 90 hr. The oil which separated solidified on scratching, and was filtered off, washed with ethyl methyl ketone, then water, and dried. After recrystallisation from methanol-acetone, *heneicosylenebis*(iso*quinolinium bromide*) was obtained as a cream coloured microcrystalline powder, m.p. 147–148°. Found: C, 65·8; H, 7·8; N, 3·9; Br, 22·4. $C_{39}H_{56}N_2Br_2$ requires C, 65·7; H, 7·9; N, 3·9; Br, 22·5 per cent. The properties of related polymethylenebis (-quinolinium and -*iso*quinolinium iodides) are listed in Table I. Other salts listed below were prepared by conventional double decomposition methods: *Dotriacontylenebis*(iso*quinolinium perchlorate*) colourless microcrystalline solid from ethanol, m.p. 124°. Found: C, 65·9; H, 8·6; N, 2·9; Cl, 8·05. $C_{50}H_{78}O_8N_2Cl_2$ requires C, 66·3; H, 8·7; N, 3·1; Cl, 7·85 per cent. *Dotriacontylenebis*(iso*quinolinium methosulphate*), colourless needle clusters from ethanol-acetone, melting over the range 96–120°. Found: N, 3·0; S, 6·7. $C_{52}H_{84}O_8N_2S_2$ requires N, 3·0; S, 6·9 per cent. *Dotriacontylenebis*(iso*quinolinium nitrate*), pale pink microcrystalline solid from ethanol, sinters at 104°, m.p. 138–140°. Found: N, 6·4. $C_{50}H_{78}O_6N_4$ requires N, 6·75 per cent. With suramin, very sparingly soluble products separated, which could not be recrystallised, e.g., *Eicosylenebis*(iso*quinolinium suramin salt*), colourless microcrystalline powder, sintering at 158° and becoming homogeneous at 168–170°. Found: N, 5·8; S, 6·75. $C_{165}H_{196}O_{23}N_{12}S_6$ requires N, 5·8; S, 6·6 per cent and *Docosylenebis*(iso*quinolinium suramin salt*) colourless microcrystalline powder, sintering at 153–155° and becoming homogeneous at 173–175°. Found: N, 6·0; S, 6·55. $C_{171}H_{208}O_{23}N_{12}S_6$ requires N, 5·6; S, 6·4 per cent.

Method (*b*). By refluxing excess of the amide with a dihalide in an appropriate solvent, followed by acid hydrolysis of the resultant quaternary amide, for example: *Tetramethylenebis*(*7-amino*iso*quinolinium iodide*). 7-Acetamido*iso*quinoline (0·4 g.), and tetramethylene di-iodide (0·22 g.) in ethyl methyl ketone (10 ml.) were refluxed for 40 hr. After cooling,

TABLE V

MISCELLANEOUS POLYMETHYLENE BIS(QUATERNARY AMMONIUM IODIDES)

$[R^+.(CH_2)_n.R^+]2I^-$

Reaction solvent = ethyl methyl ketone

R	Reaction time (hr.)	m.p.	Cryst. form	Crystn. solvent	Found per cent				Formula	Required per cent			
					C	H	N	I		C	H	N	I
n = 4													
5 AcNH Q	250	290–291°	Yellow microcrystals	H₂O/EtOH/Et₂O	45·4	4·3	8·15	37·3	$C_{26}H_{28}O_2N_4I_2$	45·75	4·1	8·2	37·2
5 NH₂ Q	—	287–288°	Red needles	MeOH/EtOH	44·2	4·35	9·2	41·9	$C_{22}H_{24}N_4I_2$	44·15	4·05	9·4	42·5
7 AcNH Q	100	303–304°	Orange-yellow needles	MeOH/EtOH	45·9	4·2	8·2	36·85	$C_{26}H_{28}O_2N_4I_2$	45·75	4·1	8·2	37·2
7 NH₂ Q	—	321–322°	Yellow microcrystals	MeOH/EtOH	44·3	4·15	9·6	42·05	$C_{22}H_{24}N_4I_2$	44·15	4·05	9·4	42·5
5 AcNH iQ	40	285°	Yellow microcrystals	MeOH/EtOH	45·5	4·0	8·2	37·1	$C_{26}H_{28}O_2N_4I_2$	45·75	4·1	8·2	37·2
5 NH₂ iQ	—	290–291°	Yellow microcrystals	MeOH/EtOH	43·9	4·2	9·05	42·35	$C_{22}H_{24}N_4I_2$	44·15	4·05	9·4	42·5
7 Cl iQ	24	242–243°	Yellow needles	MeOH/Et₂O	41·1	3·2	4·4	50·8[1]	$C_{22}H_{20}N_2Cl_2I_2$	41·4	3·2	4·4	51·0[1]
3 Br P[2]	24	[3]247°	Yellow needles	H₂O/MeOH	27·0	2·6	4·4	66·1[1]	$C_{14}H_{16}N_2Br_2I_2$	26·8	2·6	4·5	66·1[1]
n = 5													
3 Br P[2]	24	243°	Yellow needles	H₂O/MeOH	28·2	3·0	4·4	64·45[1]	$C_{15}H_{18}N_2Br_2I_2$	28·1	2·8	4·4	64·7[1]
n = 6													
3 Br P[2]	24	218°	Yellow rosettes	MeOH	29·4	3·3	4·3	63·5[1]	$C_{16}H_{20}N_2Br_2I_2$	29·4	3·1	4·3	63·3[1]
4 AcNH Q	400	249–250°	Yellow microcrystals	MeOH/EtOH	47·0	4·7	7·6	35·2	$C_{28}H_{32}O_2N_4I_2$	47·3	4·5	7·9	35·8
4 NH₂ Q[4]	50	274–275°	Cream microcrystals	EtOH	46·0	4·4	8·7	40·6	$C_{24}H_{28}N_4I_2$	46·0	4·5	8·95	40·6
4 PrNH 2 Me Q	380	281–282°	Tan prisms	MeOH/EtOH	51·7	6·1	7·6	34·2	$C_{32}H_{44}N_4I_2$	52·0	6·0	7·6	34·4
4-n-Heptyl-amino 2 Me Q	336	208–209°	Pink prisms	MeOH/Et₂O	56·4	7·0	6·4	29·7	$C_{40}H_{60}N_4I_2$	56·5	7·1	6·6	29·9
4 AcNH 2:6 di-Me Q	1030	275°	Cream microcrystals	EtOH/MeOH	49·6	5·5	7·2	33·3	$C_{32}H_{40}O_2N_4I_2$	50·1	5·3	7·3	33·2
4 NH₂ 2:6 di-Me Q-4	260	332°	Cream granules	MeOH/EtOH	49·3	5·6	8·0	36·9	$C_{28}H_{36}N_4I_2$	49·3	5·3	8·2	37·2
5 AcNHQ	350	334–336°	Orange needles	MeOH/Et₂O	47·2	4·7	7·5	35·0	$C_{28}H_{32}O_2N_4I_2$	47·3	4·5	7·9	35·8
5 NH₂ Q	--	298–299°	Red needles	H₂O/MeOH	46·2	4·8	8·55	40·3	$C_{24}H_{28}N_4I_2$	46·0	4·5	8·95	40·6
5 AcNH 6 Me Q	260	256°	Orange-red microcrystals	EtOH	48·8	4·95	7·2	34·4	$C_{30}H_{36}O_2N_4I_2$	48·8	4·9	7·6	34·4
5 NH₂ 6 Me Q	--	288°	Red microcrystals	MeOH/EtOH	47·9	5·05	8·55	38·55	$C_{26}H_{32}N_4I_2$	47·7	4·9	8·6	38·8
7 AcNH Q	96	283–284°	Yellow microcrystals	MeOH/EtOH	47·4	4·7	7·7	35·5	$C_{28}H_{32}O_2N_4I_2$	47·3	4·5	7·9	35·8
7 NH₂ Q	--	257–259°	Yellow needles	MeOH/EtOH	45·75	4·55	8·8	40·4	$C_{24}H_{28}N_4I_2$	46·0	4·5	8·95	40·6
4:6 diAcNH C[5]	260	—	—	—	—	—	—	—	—	—	—	—	—
4:6 diNH₂ C	--	291°	Yellow granules	MeOH/EtOH	40·2	4·4	17·3	38·6	$C_{22}H_{28}N_8I_2$	40·1	4·3	17·0	38·6
n = 8													
4:6 diAcNH C	280	[3]Slow 267–269° rapid 276°	Yellow granules	MeOH/EtOH	45·0	5·2	12·9	29·8	$C_{32}H_{40}O_4N_8I_2$	45·0	4·7	13·1	29·7
4:6 diNH₂ C	--	[6]Sintered 155–157° m.p. 205–207°	Yellow granules	MeOH/EtOH	41·7	5·05	16·6	36·7	$C_{24}H_{32}N_8I_2$	42·0	4·7	16·3	37·0
5 AcNH Q	200	231–233°	Orange microcrystals	MeOH/Et₂O	48·6	5·0	7·8	34·3	$C_{30}H_{36}O_2N_4I_2$	48·8	4·9	7·6	34·4
5 NH₂ Q	--	290–292°	Red microneedles	MeOH/EtOH	48·3	4·8	8·3	38·4	$C_{26}H_{32}N_4I_2$	47·7	4·9	8·6	38·8
7 AcNH Q	100	277°	Orange needles	MeOH/EtOH	49·4	5·2	7·7	34·5	$C_{30}H_{36}O_2N_4I_2$	48·8	4·9	7·6	34·4
7 NH₂ Q	—	215–216°	Orange needles	EtOH/Et₂O	47·5	4·95	8·7	38·6	$C_{26}H_{32}N_4I_2$	47·7	4·9	8·6	38·8

[1] Total halogen. [2] Reaction solvent benzene. [3] Monohydrate. [4] Also prepared by acid hydrolysis of the 4-acetamido quaternary salt. [5] Hydrolysed directly to the aminoquaternary without purification. [6] Trihydrate.

the solid was filtered off, washed with ethyl methyl ketone, dried and recrystallised from water, giving *tetramethylenebis(7-acetamido-*iso*quinolinium iodide)* as a pale yellow microcrystalline powder, m.p. 309–310°. Found: C, 45·6; H, 4·1; N, 8·25; I, 37·2. $C_{26}H_{28}O_2N_4I_2$ requires C, 45·75; H, 4·1; N, 8·2; I, 37·2 per cent. 0·45 g. of this was heated on the steam bath with hydrochloric acid (3 ml.) for 10 minutes, the mixture diluted with water, filtered hot, made alkaline with ammonium hydroxide, boiled, and solid potassium iodide (8 g.) added. After cooling, the precipitate was filtered off, washed with water, dried, and recrystallised from aqueous acetone, giving *tetramethylenebis(7-amino*iso*quinolinium iodide)* as a bright yellow microcrystalline powder, m.p. 300–301°. Found: C, 43·9; H, 4·1; N, 9·4; I, 42·15. $C_{22}H_{24}N_4I_2$ requires C, 44·15; H, 4·05; N, 9·4; I, 42·5 per cent. The following salts were prepared by double decomposition: *chloride*, yellow microcrystalline powder, from methanol-ethanol, m.p. 306–307° (decomp.). Found: C, 63·3; H, 6·2; N, 13·7; Cl, 17·3. $C_{22}H_{24}N_4Cl_2$ requires C, 63·6; H, 5·8; N, 13·5; Cl, 17·1 per cent; *nitrate*, yellow needles from aqueous acetone, m.p. 289–290°. Found: C, 56·65; H, 4·9; N, 18·0. $C_{22}H_{24}O_6N_6$ requires C, 56·4; H, 5·2; N, 17·95 per cent; *perchlorate*, yellow needles from aqueous acetone, m.p. 271–272°. Found: C, 48·4; H, 4·4; N, 10·3; Cl, 13·25. $C_{22}H_{24}O_8N_4Cl_2$ requires C, 48·6; H, 4·5; N, 10·3; Cl, 13·1 per cent.

The majority of the quaternary salts were prepared by method (*b*) and their properties are listed in Tables II–VI.

Preparation of Melamine Derivatives (BM series)

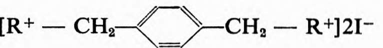
The *NN'*-dialkyl- and *NNN'N'*-tetra-alkyl polymethylene diamines employed have all been reported in the literature[17–21]. We found that the most convenient preparation (but not necessarily the best) was to treat an ethereal solution of a polymethylene dihalide with an excess of the appropriate amine at room temperature for 1–2 weeks. With primary amines, the yields were poor, large quantities of high boiling fractions being formed, but with secondary amines, e.g., diethylamine, excellent yields of the order of 80–85 per cent resulted.

NN'-*Dimethyl*-NN'-*bis*(4:6-*diamino*-s-*triazino*)-*decamethylene diamine* (II, R = Me, n = 10)

A mixture of 2-chloro-4:6-diamino-s-triazine[22] (1·455 g., 2 mol.), *NN'*-dimethyldecamethylene diamine (1·0 g., 1 mol.), anhydrous potassium carbonate (0·69 g., 1 mol.) and dimethylformamide (30 ml.) was heated under reflux on a steam bath for 36 hr. (the observed slight ammoniacal smell may have been due to some decomposition of the amide by the potassium carbonate). After cooling, the colourless crystals which separated were filtered, washed with dimethylformamide, then with cold water and dried. The product (2·05 g.) was then recrystallised from dimethylformamide, giving the required *base* as colourless microcrystals, m.p. 232–234°. This lost 25·6 per cent by weight at 100° *in vacuo*. $C_{18}H_{34}N_{12}$·2HCON$(CH_3)_2$ requires 25·9 per cent. Found for the dried material: C, 51·6; H, 8·2; N, 40·3. $C_{18}H_{34}N_{12}$ requires C, 51·7; H, 8·2; N, 40·2

TABLE VI

p-XYLYLENE BIS(QUATERNARY AMMONIUM IODIDES)



$[R^+ — CH_2$—C₆H₄—$CH_2 — R^+]2I^-$

Reaction solvent = ethyl methyl ketone

R	Reaction time (hr.)	m.p.	Cryst. form	Crystn. solvent	Found per cent				Formula	Required per cent			
					C	H	N	I		C	H	N	I
5:7 diAcNH Q[1]	250	—	—	—	—	—	—	—	—	—	—	—	—
5:7 diNH$_2$ Q	—	>320°	Orange-red microcrystals	EtOH/H_2O	46·0	4·2	12·0	36·9	$C_{26}H_{26}N_6I_2$	46·15	3·9	12·4	37·6
7 AcNH iQ	20	327–328°	Yellow microcrystals	MeOH/H_2O/Et_2O	49·1	4·2	7·6	34·7	$C_{30}H_{28}O_2N_4I_2$	49·3	3·9	7·7	34·8
7 NH$_2$ iQ	—	278–279°	Yellow microcrystals	MeOH/H_2O/Et_2O	48·5	3·8	8·7	39·0	$C_{26}H_{24}N_4I_2$	48·3	3·7	8·7	39·3
4 AcNH C[1]	24	—	—	—	—	—	—	—	—	—	—	—	—
4 NH$_2$ C	—	278–279°	Yellow nodules	MeOH/EtOH	44·5	3·4	12·7	39·15	$C_{24}H_{22}N_6I_2$	44·4	3·4	13·0	39·2

Q = Quinolinium. iQ = *iso*Quinolinium. C = Cinnolinium.

[1] Hydrolysed directly to the aminoquaternary without purification.

per cent. Treatment of this with methyl iodide (2·1 mol.) in dimethylformamide at room temperature for 4 weeks gave the *methiodide* (III, R = R′ = Me, n = 10) as cream coloured granules from ethanol-ether, m.p. 252–253°. Found: C, 34·4; H, 5·7; N, 23·5; I, 36·3. $C_{20}H_{40}N_{12}I_2$ requires C, 34·2; H, 5·7; N, 23·9; I, 36·2 per cent; *perchlorate*, colourless granules from ethanol-ether, m.p. 226–228°. Found: C, 37·7; H, 6·4; N, 26·1; Cl, 10·9. $C_{20}H_{40}O_8N_{12}Cl_2$ requires C, 37·1; H, 6·2; N, 26·0; Cl, 11·0 per cent.

*NN′-dimethyl-*NN′*-bis*(4:6*-diamino-*s*-triazino)octamethylene diamine*, colourless granules from dimethylformamide, m.p. 216–218°. This lost 27·0 per cent by weight at 100° *in vacuo*. $C_{16}H_{30}N_{12}2HCON(CH_3)_2$ requires 27·2 per cent. Despite repeated recrystallisations and drying at 100° *in vacuo*, it was impossible to obtain a satisfactory nitrogen analysis. Found: C, 49·2; H, 8·1; N, 41·3. $C_{16}H_{30}N_{12}$ requires C, 49·2; H, 7·75; N, 43·1 per cent; the corresponding *methiodide*, cream coloured granules from ethanol-ether, m.p. 252–254° was also difficult to analyse. Found: C, 33·35; H, 5·7; N, 25·2; I, 35·5. $C_{18}H_{36}N_{12}I_2$ requires C, 32·05; H, 5·4; N, 24·9; I, 37·7 per cent, although the *perchlorate* obtained from it by double decomposition as colourless microcrystals from ethanol-ether, m.p. 202–204°, analysed satisfactorily. Found: C, 35·0; H, 6·0; N, 27·35; Cl, 11·3. $C_{18}H_{36}O_8N_{12}Cl_2$ requires C, 34·9; H, 5·9; N, 27·1; Cl, 11·5 per cent; NN′*-dimethyl-*NN′*-bis*(4:6*-diamino-*s*-triazino)hexamethylene diamine*, cream coloured microcrystalline powder from dimethylformamide-ether, m.p. 124–126°. It was not possible to drive off all the solvent of crystallisation, and this material was therefore analysed after drying at room temperature. Found: C, 46·2; H, 7·5; N, 38·8. $C_{14}H_{26}N_{12}.2HCON(CH_3)_2$ requires C, 47·2; H, 7·9; N, 38·6 per cent; *methiodide*, cream coloured microcrystals from ethanol-ether, m.p. 272°. Found: C, 29·85; H, 5·3; N, 25·7; I, 38·8. $C_{16}H_{32}N_{12}I_2$ requires C, 29·7; H, 5·0; N, 26·0; I, 39·3 per cent.

Attempt to Prepare the Di-ethochloride of NN′*-Diethyl-*NN′*-bis*(4:6*-diamino-*s*-triazino)decamethylene diamine* (III, R = R′ = Et, n = 10) *by an Unambiguous Route*

A mixture of 2-chloro-4:6-diamino-*s*-triazine (1·5 g., 2 mol.) and *NNN′N′*-tetraethyl decamethylene diamine (1·46 g., 1 mol.) in dimethylformamide (80 ml.) was refluxed for 260 hr. The solvent was then distilled off *in vacuo*, the residue treated with ether and left overnight, when the brown oily product had become almost solid. The solvent was then decanted off, the residue dissolved as far as possible in ethanol, filtered, concentrated to small bulk and cooled. After filtering, the resultant brownish product was recrystallised from ethanol (after charcoal), giving 0·4 g. of unidentified colourless needles or octahedra, m.p. 305–307°, which contained no halogen. Found: C, 39·2; H, 6·4; N, 54·5; Cl, 0 per cent. When *NNN′N′*-tetramethyl hexamethylene diamine was used, the unidentified crude product again contained no halogen.

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CHEMOTHERAPEUTIC PROPERTIES OF SOME NEW QUATERNARY AMMONIUM SALTS

PART II. ANTIFILARIAL ACTION AGAINST *Litomosoides carinii*

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Several new quaternary ammonium salts have been tested for antifilarial activity against *Litomosoides carinii*, filarial parasite of the cotton rat. Many of these compounds are active in killing *L. carinii*; the most promising (BIQ 20) eicosane 1 : 20-bis(*iso*quinolinium iodide) and (BAC 20) eicosane 1 : 20-bis(4-aminocinnolinium iodide) possess an index (LD50 mice/minimum effective dose cotton rats) of 19 and 39 respectively. The compounds have a direct filaricidal action on the adult worms while the microfilariae are comparatively unaffected.

THIS paper describes the results of testing a series of quaternary ammonium salts against *Litomosoides carinii*, filarial parasite of the cotton rat. The chemistry and some of the pharmacological, and antimicrobial properties of these compounds are described in other papers of this series[1,2]. The abbreviated nomenclature of Barlow and Ing[3] is used in this paper; thus BQn, BIQn, BAIQn, BAQDn and BACn refer respectively to the bis-quinolinium, -*iso*quinolinium, -7-amino*iso*quinolinium, -4-aminoquinaldinium and -4-aminocinnolinium series, where n is equal to the number of methylene groups in the chain.

The cotton rats used in these tests were bred at this Institute and were infected in the laboratory by the method of Hawking and Sewell[4]. The tests were usually made with cotton rats which were in the early stages of infection (80–100 days after exposure to infected mites); this practice lessened the risk of the results being confused by the natural death of some of the worms. The compounds were synthesized by Dr. E. P. Taylor and his colleagues of Messrs. Allen and Hanburys Ltd.; the toxicity tests were carried out by Dr. H. O. J. Collier and his colleagues.

The *in vivo* antifilarial tests were based on the method of Sewell and Hawking[5]. Briefly, the rats were given five daily doses of the drug intraperitoneally at a level known to be non-toxic to mice. Soluble compounds were dissolved in sterile physiological saline; insoluble compounds were finely ground and suspended in 5 per cent gum acacia. Measured samples of tail blood were taken immediately before treatment and 7 and 14 days after the first dose to investigate the effect of the drugs on the numbers of circulating microfilariae. On the 14th day the rats were killed with coal gas and the worms were removed from the pleural cavities and placed in sterile Ringer's solution. If the worms were not actively moving, they were placed in an incubator at 37° for 30 minutes and re-examined. If again no movement was seen, the worms were deemed to have been killed by the drug. Where a compound was found

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to be active, additional rats were treated at a fraction (usually 0·5) of the previous dose. The minimum effective dose of a compound was taken to be the smallest dose which killed all the worms in two or more cotton rats.

Selected compounds were tested against the adults of *L. carinii in vitro*. The worms were removed from the pleural cavities under aseptic conditions and placed in Carrel flasks containing a simple medium consisting of one-third horse serum and two-thirds Tyrode's solution; penicillin and aureomycin were added to the flasks, each at a final concentration of 0·1 mg./ml. At least two male and two female worms were placed in each flask. In this simple medium *L. carinii* will remain alive and active for periods up to a fortnight, although the production of microfilariae ceases after about 24 hours.[6] The selected compounds were dissolved in sterile saline and added to the flasks in serial dilution. The minimum lethal concentration was arbitrarily taken as the lowest concentration of the drug which killed all the worms in the flask within 24 hours.

EXPERIMENTAL

Action on Adult L. carinii in vivo

Table I summarises the results of testing the several series of compounds against *L. carinii in vivo*. Table II illustrates the protocols of the tests made on two of the more active compounds. The most thoroughly explored series has been the BIQ compounds where there is a definite link between filaricidal activity and the length of the polymethylene chain.

TABLE I

THE ACTION OF QUATERNARY AMMONIUM SALTS ON ADULT *L. carinii in vivo*

Compound		LD50 mice mg./kg. i.p.	MED cotton rat mg./kg. daily × 5	Index LD50 mice/MED cotton rat
BIQ	12		>10·0	
	13	16·1	1·5	10·7
	14	9·8	0·8	12·2
	15	6·5	0·8	8·1
	16	5·8	0·8	7·2
	17	3·5	0·4	8·7
	18	2·3	0·2	11·5
	19	2·7	0·2	13·5
	20	2·8	0·15	18·7
	22	2·4	0·3	8·0
	24	2·6	0·3	8·7
	32	>64	>10·0	—
	40	>64	>10·0	—
BAC	20	19·5	0·5	39·0
	22	>40·0	4·0	>10·0
BQ	12	5·0	2·0	2·5
	14	4·0	1·6	2·5
	16	3·3	0·6	5·5
	18	2·8	0·8	3·5
	20	1·8	0·8	2·3
BAIQ	19	9·4	1·0	9·4
BAQD	10	20·9	10·0	2·1
	16	4·9	1·0	4·9
	20	—	1·0	—
BIQ	16 Suramin complex	400+	25·0 (single dose)	16+
	20	400+	12·5 (single dose)	32+
	22	400+	15·0 (single dose)	27+

No activity could be detected in compounds having 12 or less methylene groups, but as the chain length increased from the 13 to 20, the total minimum effective dose decreased from 7·50 to 0·75 mg./kg. BIQ 20 seems to be the peak of antifilarial activity; BIQ 22 and BIQ 24 were slightly less active and the higher chain lengths BIQ 32 and BIQ 40 were inactive. The peak of acute toxicity in mice occurred at BIQ 18.

The related BQ series also showed antifilarial activity but to a lesser extent; the peak of activity occurred at BQ 16.

Individual BQ compounds were more toxic to mice than were the corresponding members of the BIQ series. Several compounds belonging to the BAIQ, BAQD and BAC series were tested; all series showed activity. but except for BAC 20 no compound was as promising as BIQ 20.

It is known that complexes of the trypanocidal compounds ethidium and pentamidine with suramin show decreased toxicity when compared with the parent compounds whilst at the same time the prophylactic activity is increased, presumably due to the formation of depots of insoluble drug which are slowly absorbed by the body. Williamson[7] has recently reviewed the work in this field. It was thought that the high toxicity of the quaternary ammonium compounds might be reduced in this way and accordingly, suramin compounds of BIQ 16, 20 and 22 were prepared. These compounds were much less toxic and yet were effective in killing adult *L. carinii* when given in single doses at the level shown in Table I.

TABLE II

ILLUSTRATIVE PROTOCOLS OF THE TESTS ON BIQ 20 AND BAC 20

Compound	Rat	Dose (mg./kg. × 5)	No. of worms killed/No. of worms present
BIQ 20	6431	0·3	14/14
	6916	0·2	all/50+
	6602	0·15	all/50+
	6605	0·15	16/16
	6520	0·15	5/5
	6526	0·15	all/50+
	6914	0·1	half/50+
	6467	0·1	8/8
	6665	0·1	7/16
BAC 20	6620	1·0	all 50+
	6621	0·5	all/50+
	6658	0·5	7/7
	6669	0·5	18/18
	6804	0·5	all/50+
	6801	0·2	about 20/50+
	6805	0·2	8/12
	6800	0·1	0/50+

Action in vitro *on Adult* L. carinii

A few compounds were tested *in vitro* against *L. carinii* to determine whether the drugs were killing the worms directly or whether their action was mediated through the host, as in the case of diethylcarbamazine. The results of these tests are shown in Table III. All compounds were effective in killing the worms at low concentrations and it would seem that the lethal action is a direct one. The minimum lethal concentrations of the drugs *in vitro* are of about the same order as the minimum effective

doses *in vivo*. The uterine contents of female worms, exposed to concentrations slightly less than the minimum lethal concentration, were examined with the phase contrast microscope. According to Dr. A. E. R. Taylor many malformed and aborted embryos were found; the primary oocytes appeared to be particularly affected by the BIQ compounds. The other tissues were apparently normal and the muscles were still actively contractile.

TABLE III

THE ACTION OF SOME QUATERNARY AMMONIUM SALTS ON *L. carinii in vitro*

Compound	Minimum lethal concentration (24 hr.) µg./ml.	MED *in vivo* mg./kg.
BIQ 16	8	0·8
17	6	0·4
18	1	0·2
BAIQ 19	10	1·0
BAQD 16	100	1·0

Action on the Microfilariae

Estimates of the numbers of circulating microfilariae before and after treatment showed only small differences. Seemingly the drugs do not directly affect the microfilariae which have reached the circulation. The small decreases in numbers which sometimes occurred (maximum reduction 20 per cent) are presumably due to the drugs killing the adult worms and so preventing the replenishment of those microfilariae which are eliminated by the host.

DISCUSSION

The results given show that some of the bis*iso*quinolinium and related compounds possess considerable antifilarial activity, the compounds with the most favourable relation between toxicity and activity being BIQ 20.

$$\frac{\text{LD50 mice}}{\text{MED cotton rats}} \quad \left.\frac{2\cdot8}{0\cdot15}\text{ i.e. } 18\cdot7\right\}\text{and BAC 20}\left\{\frac{19\cdot5}{0\cdot5} = 39\cdot0\right\}\text{(See Table I).}$$

This index of activity is not completely satisfactory as therapeutic tests and toxicity tests have not been made on the same species; cotton rats are too expensive to use for toxicity work. Nevertheless the index does provide a reasonable estimate of the therapeutic value of any compound which is sufficiently reliable for biological screening work of this type.

As regards the relation between chemical structure and antifilarial activity, it may be concluded that in the BIQ series, activity increases as the length of the carbon chain increases; the peak of activity probably occurs at a length of 20 carbon atoms. In the BQ series, the peak of activity may occur at a carbon length of 16 atoms. These peaks are not sharply defined and the data could also be interpreted as indicating a plateau of activity at 18–22 carbon atoms for the BIQ series and at 16–20 carbon atoms for the BQ series. The *iso*quinolinium group at the end of the chain produces somewhat greater activity than the quinolinium

group. With the other groupings, it is not known what the optimum length of carbon chain is, so that it cannot be definitely decided how the activity of the terminal groupings compares with those of the quinolinium and *iso*quinolinium.

The compounds have a direct filaricidal action upon the adult worms, while the microfilariae are comparatively unaffected. The most susceptible parts of the adult female worms seem to be the developing embryos and oocytes; this also occurs with many other filaricidal drugs (but not with diethylcarbamazine) and is probably due to these cell-groups having the highest metabolic turnover in the worm. Similarly in schistosomes, the part most susceptible to the lethal actions of lucanthone and of antimony salts is the gonad.

Besides being active, these compounds are also toxic, but in the most promising compounds (BIQ 20 and BAC 20) there is a fair margin between the minimum curative dose and the maximum tolerated dose. A dog tolerated four intravenous injections of BAC 20 at a dose of 10 mg./kg.; a rhesus monkey tolerated 10 mg./kg. but was killed by 12·5 mg./kg., death being apparently due to poisoning of the respiratory centre. The antifilarial activity deserves investigation in larger animals and ultimately (if possible) in man, but it is too soon at present to prophesy whether or not it will possess practical utility.

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THE EFFECT OF SUBSTANCE P ON THE PERISTALTIC REFLEX WHEN ACTING ON THE OUTSIDE OF THE ISOLATED GUINEA PIG ILEUM

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Substance P (30–100 units) when acting on the outside of the isolated guinea pig ileum abolished the peristaltic reflex. Small doses (4 units) occasionally potentiated peristalsis. The block produced by high doses could not be overcome by intraluminal injections of 5-HT or substance P itself. The effect of nicotine was reduced or abolished in the presence of high doses of substance P. The high doses can also block the response of the gut to the small doses of the same substance. It is concluded that the blocking effect of substance P takes place between the synapse in the peristaltic reflex arc and the effector cell in the plain muscle.

PREVIOUSLY[1] we reported that the introduction of substance P into the lumen of the isolated guinea pig ileum caused an increase in the number and amplitude of the peristaltic waves. It also restored peristalsis in preparations in which the peristaltic reflex was abolished by fatigue, by 5-HT, intraluminally or in the bath, or by lowering the temperature of the bath.

Small amounts of substance P applied externally to the small intestine *in situ* also increases the tone and the rhythmic spontaneous activity[2].

This work reports the effect of large doses of substance P in the bath on the peristaltic reflex of the guinea pig ileum.

MATERIALS AND METHODS

The peristaltic activity was recorded as described previously[1]. The reflex was tested by raising the intraluminal pressure (varying from 30 to 60 mm. in different experiments) for 90 seconds at 10 minute intervals. The ileum was kept in Tyrode's solution at 37° in a bath volume of 30 ml. and oxygenated with a mixture containing 97 per cent O_2 and 3 per cent CO_2. Intraluminal injections were made in a volume of 0·1 to 0·2 ml.

The drugs used were 5-hydroxytryptamine creatinine sulphate, nicotine hydrogen tartrate and a sample of substance P containing 1 unit/mg.

RESULTS

The Effect of Increasing Doses of Substance P

The addition of small doses of substance P into the bath caused an increase in amplitude of the peristaltic waves in three out of seven experiments. In several other experiments the doses of substance P were gradually increased, and a typical response is shown in Figure 1. The addition of 20 units of substance P (at P in A) to the bath caused an immediate reduction in the number of peristaltic waves which was

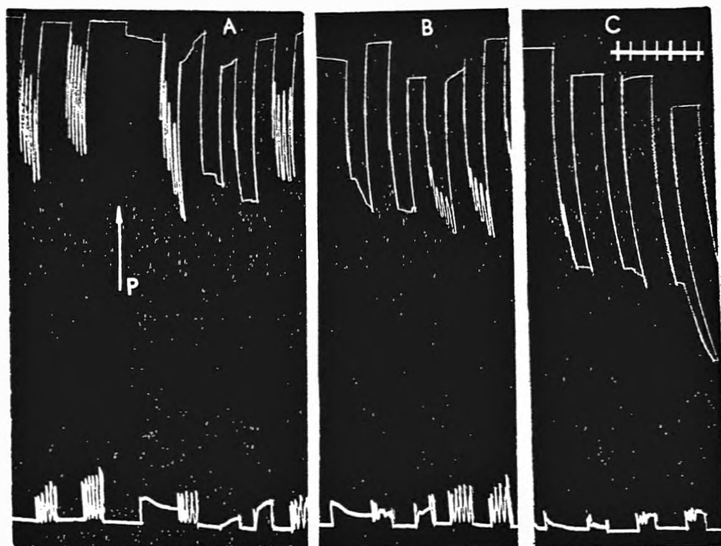

FIG. 1. The effect of increasing doses of substance P on peristalsis. Upper record, peristalsis. Lower record, contractions of the longitudinal muscle. At P in A, 20 units of substance P added to the bath. Between A and B, 30 units and between B and C, 40 units of substance P added to the bath. Time in 1 minute intervals.

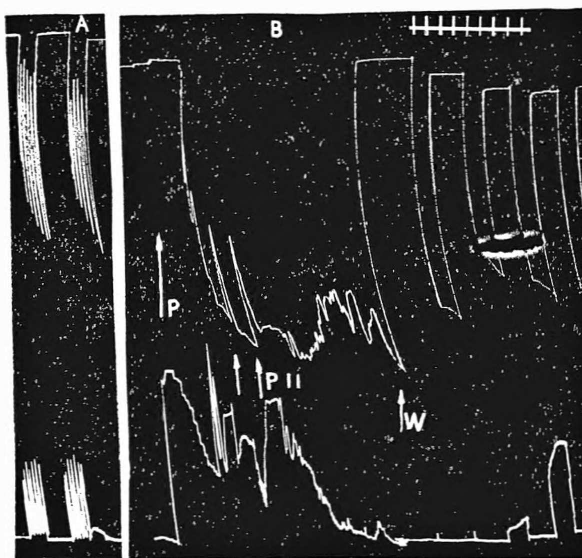

FIG. 2. The effect of intraluminal injection of substance P. Records as in Figure 1. A, the control response. At P in B, 30 units of substance P added to the bath. At the arrow, 0·2 ml. of Tyrode solution introduced intraluminally. At P II, 30 units of substance P introduced intraluminally. W, washing out the bath and gut. Time in 1 minute intervals.

followed 15 to 25 minutes later by complete abolition of the reflex, which was reversible, as shown in A. Between A and B, 30 units of substance P were added to the bath, causing a complete block of the reflex with an incomplete recovery, as shown in B. Between B and C, 40 units of substance P was added into the bath, and this dose produced a complete abolition of the reflex lasting more than 60 minutes, as shown in C.

The Effect of Intraluminal Injection of Substance P

To study the site of blocking action of substance P, it was injected intraluminally into gut in which the reflex was blocked by the previous addition of substance P to the bath. Peristalsis was not restored. In some experiments the intraluminal injection produced only irregular and unco-ordinated peristaltic waves. This type of response is shown in Figure 2. At P in B, 30 units of substance P was added into the bath, producing an abolition of the reflex. At P II. in B the same dose of substance P was injected intraluminally. After 2 minutes only the irregular and unco-ordinated peristaltic waves were recorded. The complete block of the reflex was still present 1 hour after washing out substance P.

When larger doses of substance P were used to block peristalsis the intraluminal injection produced no effect.

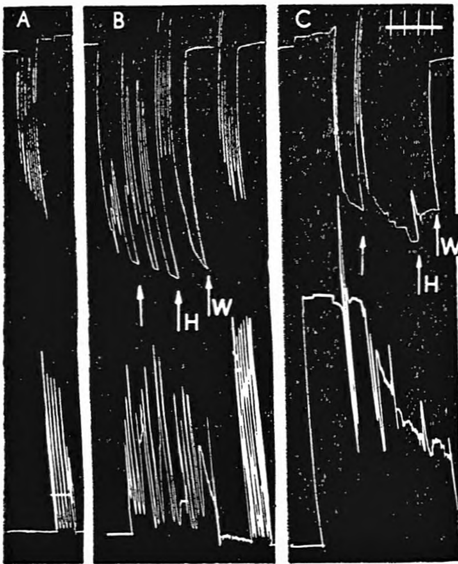

FIG. 3. The effect of intraluminal injection of 5-HT. Records as in Figure 1. A, the control response. Between A and B, 30 units and between B and C, 50 units of substance P added to the bath. At the arrow, 0·2 ml. of Tyrode solution introduced intraluminally. At H, 2 μg. of 5-HT introduced intraluminally. W, washing out the bath and gut. Time in 1 minute intervals.

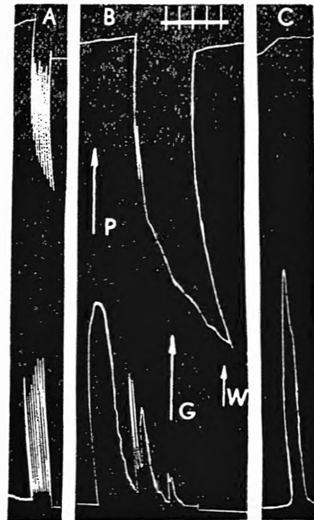

FIG. 4. The effect of nicotine. A, control response. Records as in Figure 1. At P, 100 units of substance P added to the bath. At G, 0·2 mg. of nicotine added to the bath. W, washing out the bath. C, 0·2 mg. of nicotine added to the bath without raising the intraluminal pressure. Time in 1 minute intervals.

The Effect of Intraluminal Injection of 5-HT

Intraluminal injection of substance P had been found to restore peristalsis previously blocked by high doses of 5-HT in the bath[1], but we have now found that intraluminal injection of 5-HT restored peristalsis only if small doses of substance P were used. With large doses of substance P in the bath, the intraluminal 5-HT failed to produce peristalsis. A typical experiment is shown in Figure 3. Between A and B, 30 units of substance P was added to the bath. This dose caused only a partial block of peristalsis, and the intraluminal injection of 2 μg. 5-HT (at H in B) caused peristalsis. Between B and C, 50 units of substance P was added to the bath and caused a complete block on which 2 μg. of 5-HT had no effect, as shown in C. Similar findings were observed with doses of 5-HT up to 0·2 mg.

The Effect of Nicotine

After blocking the peristaltic reflex with a high dose of substance P in the bath, nicotine added to the bath caused no effect. This is shown in Figure 4. The response to 0·2 mg. of nicotine in the presence of 100 units of substance P is shown in B. The same dose of nicotine was added to the bath 25 minutes later, after substance P had been washed out, and it caused a contraction.

Where doses of substance P less than 100 units were used to abolish peristalsis, the response to nicotine was reduced but not abolished.

Abolition of the Response to Substance P by Substance P Itself

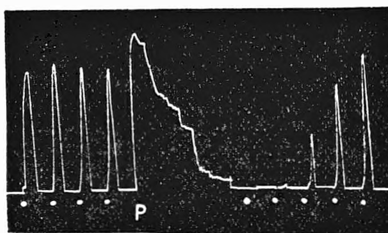

FIG. 5. Abolition of the response to substance P by a high dose of substance P itself. At dots, 4 units of substance P added to the bath (10 ml.). At P, 50 units of substance P added to the bath.

The isolated guinea pig ileum was arranged to record contractions of the longitudinal muscle only. It was found that a high dose of substance P abolished the response of the gut to the small doses of the same substance. A typical experiment is shown in Figure 5. The small dose of substance P (4 units) was added to the bath at 6-minute intervals. At P, 50 units substance P was added to the bath and washed out after 7 minutes. The response to the small dose of substance P was abolished; it became normal 25 minutes after washing out the high dose.

DISCUSSION

Small doses of substance P when acting on the outside of the intestine *in situ* can increase the tone and spontaneous rhythmic activity[2]. The present work on the isolated guinea pig ileum has in part confirmed this finding, and it has also been found that high doses of substance P may abolish the peristaltic reflex when acting on the outside of the isolated

guinea pig ileum. This was not preceded by a stimulating action, which is in contrast to the response to intraluminal substance P, where it always caused an increase of peristaltic activity before abolition of the reflex[1].

It was also found on the Magnus preparation that a high dose of substance P can block the response of the gut to a small dose of the same substance. This effect is probably due to the saturation of the substance P receptors in the plain muscle cells. So far it seems that the blocking effect of substance P takes place somewhere between the synapse in the peristaltic reflex arc and the effector cell.

Acknowledgements. 5-Hydroxytryptamine creatinine sulphate was kindly supplied by Farmitalia, Milan, and substance P by Dr. B. Pernow, Stockholm.

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A COMPARISON BETWEEN THE VASCULAR RESPONSES TO ADRENALINE AND NORADRENALINE IN INDIVIDUAL SKELETAL MUSCLES OF THE CAT

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The effects of intra-arterially and intravenously administered adrenaline and noradrenaline on the venous outflow from individual muscles in the hind limbs of cats under chloralose anaesthesia were studied. The various responses to these amines were shown to depend on the dose administered, the route of injection, the general arterial blood pressure and the vasomotor tone in the muscle. They did not vary with the type of muscle studied. When the vasomotor tone was high, intra-arterially administered adrenaline caused vasodilatation, vasoconstriction or a compound response according to the dose administered. Intra-arterially administered noradrenaline caused only vasoconstriction. However, both intravenously administered adrenaline and noradrenaline in small doses caused vasodilatation in skeletal muscles, the former being the more potent. That this effect depended on intact nervous connections was confirmed in cross-circulation experiments in which the muscle of one cat was perfused entirely by blood from a donor cat. When the vasomotor tone in the muscle was low, either naturally or as a result of acute denervation, it was difficult to produce any dilatation with adrenaline, and noradrenaline always caused vasoconstriction. Under these conditions, the increase in blood flow produced by the intravenous administration of the amines was shown, by means of a blood pressure stabiliser, to be a passive effect caused by the rise in blood pressure forcing more blood through the muscles.

It is generally believed that the intravenous or intra-arterial administration of small doses of adrenaline produces vasodilatation in skeletal muscles while the administration of larger doses causes vasoconstriction[1–8]. Nevertheless, many workers have been unable to obtain such results. Some recorded only vasoconstriction after the intra-arterial administration of minimal effective doses of adrenaline[9,10]. Others[11,12] recorded a two-fold response of dilatation followed by constriction or dilatation preceded by constriction. Several workers[10,13,14] have reported that the only effect of intravenously administered adrenaline on skeletal muscle blood vessels is a vasoconstriction which may be preceded by a short-lasting passive increase in flow caused by the rise in blood pressure.

There is unanimous agreement that intra-arterially administered noradrenaline causes vasoconstriction in skeletal muscle vessels[6,8,10,15–18], but results differ about its effects when administered intravenously. Some workers have reported an immediate reduction in the blood flow following intravenous administration[19–22]. Others[10,17], described an initial increase in flow which they attributed to the rise in blood pressure and which was immediately followed by a decrease in flow. Still others[7,8,23,24] observed that intravenously administered noradrenaline, like adrenaline,

produced an increase in blood flow which was followed by vasoconstriction only when large doses were administered.

In the past, most workers have recorded blood flow changes in the muscles of the whole limb or in other large groups of muscles. The present experiments were designed to find out whether different types of skeletal muscles respond differently to these amines, thereby possibly accounting for some of the discrepancies. It was considered possible that during emergency states, adrenal medullary hormones might cause an increase in the blood supply to the quick acting white muscles, such as tibialis anterior but might have a different effect on the slow acting red muscles, such as soleus, which are known to contain their own oxygen storing pigments.

The results show that the various responses to adrenaline and noradrenaline depend on the dose administered, the route of injection, the general arterial blood pressure and the vasomotor tone in the muscle vessels but that they do not vary with the type of muscle studied.

Methods

Cats were anaesthetised with chloralose only (80 mg./kg.) injected into the subcutaneous vein of the forelimb or into the internal saphenous vein. The blood flow in the femoral vein of one hind limb was then restricted to include only that from one of three muscles, the tibialis anterior, the gastrocnemius-plantaris or the soleus. The method used for recording the venous outflow from the tibialis anterior or the soleus muscle has been previously described[25]. When the blood flow from the gastrocnemius-plantaris muscle was studied, a similar procedure was carried out except that the circulation of this muscle was left intact while both arteries and veins supplying all other muscles were ligated. The blood flow in the femoral vein was recorded by means of the drop-chamber described by Hilton[26,27] and the Gaddum drop recorder[28]. To denervate the muscle during the experiment, the sciatic nerve was exposed high in the thigh.

In cross-circulation experiments, the gastrocnemius-plantaris muscle of one cat was prepared as described. A mass ligature was then made round the thigh just above the knee but excluding the femoral artery and vein and the sciatic nerve. Blood from the femoral artery of an anaesthetised, heparinised, donor cat was then led through polythene tubing into the cut peripheral end of the femoral artery of the first cat. The venous outflow from the perfused gastrocnemius-plantaris muscle was allowed to pass through the drop-chamber and then returned to the central end of the cut femoral vein of the donor cat. Thus the cross perfused muscle entirely lacked vascular connections with the recipient animal, only the nervous connections through the sciatic nerve being intact.

Blood pressure was recorded by means of a mercury manometer attached to a cannula in the right common carotid artery. In experiments in which the effects of intravenously administered adrenaline and noradrenaline were studied, the same doses were administered both

before and after connecting a blood pressure stabiliser[29] to the carotid artery.

Drugs were administered intravenously via a cannula in the jugular vein or intra-arterially by means of a needle cannula in the cut central end of a branch of the femoral artery—usually the small artery supplying the gracilis muscle. Before being tied and clamped in position, the needle cannula was inserted into the artery until its tip was level with the junction of the cannulated artery and the femoral artery. A micro-syringe was used for all intra-arterial injections. The drugs used were (—)-adrenaline bitartrate and (—)-noradrenaline bitartrate. Solutions were made in 0·9 per cent w/v NaCl saline. The doses quoted in the text refer to the quantity of amine calculated as base.

At the end of each experiment, Indian ink was injected through the arterial cannula to ascertain that the blood flow was restricted to the required muscle.

RESULTS

Preliminary experiments showed that the intra-arterial administration of volumes of 0·9 per cent saline, greater than 0·02 ml., themselves usually caused an increase in venous outflow. For this reason adrenaline and noradrenaline were administered in volumes which did not exceed 0·01 ml. and control injections of saline were made throughout each experiment.

Throughout the experiments, similar responses were obtained whatever the muscle under study. The results to be described, therefore, apply to all three muscles, the tibialis anterior, the gastrocnemius-plantaris and the soleus.

Intra-arterially Administered Adrenaline and Noradrenaline

The effect of intra-arterially administered adrenaline depended on the magnitude of the dose and the vasomotor tone in the muscle. When the vasomotor tone was shown to be high by the fact that later in the experiment acute denervation caused a marked and prolonged increase in venous outflow, the sequence of responses to ascending doses was as follows. Minimal effective doses (0·001–0·01 μg.) produced a short-lasting increase in flow only and this response at first increased as the dose was increased. With slightly larger doses (0·04–0·4 μg.), however, the increase in flow became progressively less and was followed by a small decrease in flow. As the dose was further increased, the initial increase in flow became still smaller and the following decrease larger, until, over a small range of doses (0·4–1·5 μg.), only a decrease in flow was recorded. Larger doses (greater than 1–2 μg.) produced an initial decrease in flow which was usually followed by an increase in flow of variable duration (from 90 seconds to 12 minutes). With the range of doses employed in this type of experiment the general arterial blood pressure usually remained unaltered. Figure 1 shows a characteristic sequence of responses to increasing doses of adrenaline.

In 2 out of 15 experiments, minimal effective doses caused an initial increase in flow which was followed by a decrease, that is, the pure dilator phase was absent. In 3 of the experiments minimal effective doses caused only a decrease in flow. These responses occurred in cats in which the vasomotor tone in the muscle was shown to be low by the fact that acute denervation later in the experiment caused only an initial short-lasting increase in flow. When the vasomotor tone was high, acute denervation of the muscle caused an initial short-lasting but marked increase in venous outflow accompanied by a rise in blood pressure of similar duration. The rate of flow subsequently subsided to a level 1·5–3 times greater than the flow from the innervated muscle, remaining there for several hours (Fig. 2).

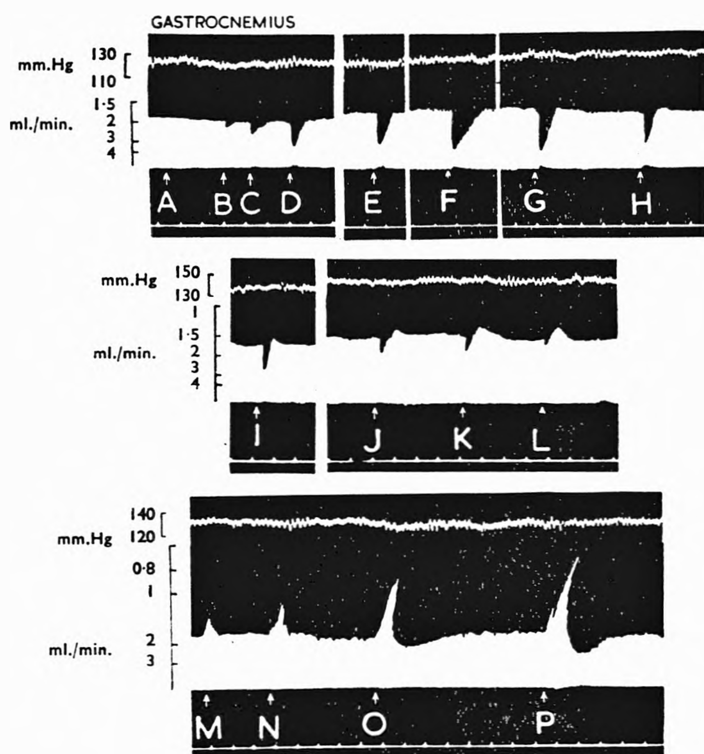

FIG. 1. Cat, 3·4 kg. The effect of intra-arterially administered adrenaline on the venous outflow from innervated muscle. Upper record: blood pressure; lower record: venous outflow. Time scale in min. At A, 0·0005; B, 0·001; C, 0·002; D, 0·004; E, 0·008; F, 0·01; G, 0·02; H, 0·04; I, 0·05; J, 0·1; K, 0·2; L, 0·4; M, 0·5; N, 1; O, 2; P, 3 μg.

After acute denervation, it was occasionally still possible to produce some dilatation with small intra-arterial doses of adrenaline. If, before denervation, the peak of the increase in flow caused by a certain dose of adrenaline was higher than the increased level of flow produced by

denervation, the adrenaline still had a slight dilator effect after denervation. Doses of adrenaline which produced in the innervated muscle a two-fold response of dilatation followed by constriction, caused only vasoconstriction during the hyperaemia produced by acute denervation (Fig. 2). Denervation did not always prevent the secondary increased flow which usually followed the vasoconstriction produced by large doses of adrenaline. When the vasomotor tone was low, the responses to intra-arterially administered adrenaline were the same before and after denervation.

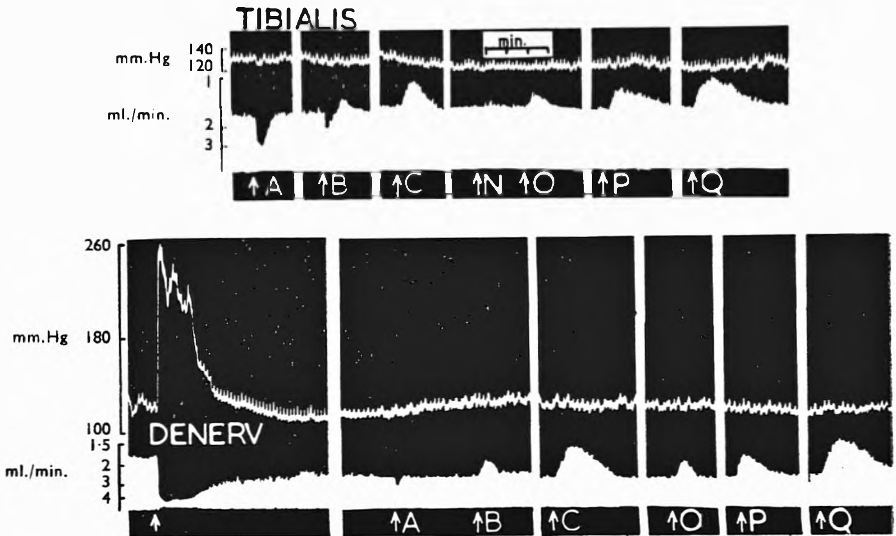

FIG. 2. Cat, 3·7 kg. The effect of acute denervation on the responses to intra-arterially administered adrenaline and noradrenaline. At A; 0·05, B, 0·2 and C, 0·5 μg. adrenaline. At N, 0·05; O, 0·1; P 0·2 and Q 0·5 μg: ncradrenaline. At DENERV, scientic nerve severed. Time scale in min.

Minimal effective intra-arterial doses of noradrenaline (0·01–0·1 μg.) caused vasoconstriction only in the skeletal muscles and this response increased with increase in the dose. Doses of noradrenaline equivalent in size to purely dilator doses of adrenaline, were without effect (Fig. 2). In general, noradrenaline produced a greater and slightly longer lasting reduction in venous outflow than that produced by adrenaline (Fig. 2). However, in one animal in which the vasomotor tone in the muscle was later shown to be low, the vasoconstriction produced by adrenaline was equal to that produced by noradrenaline and was not altered by denervation. When denervation caused an increased flow through the skeletal muscle, noradrenaline subsequently injected produced a proportionately greater reduction in flow than when injected before denervation (Fig. 2).

Intravenously Administered Adrenaline and Noradrenaline

In general, the effects of intravenously administered adrenaline and noradrenaline were similar except that the vasopressor responses and the

reductions in venous outflow produced by noradrenaline were slightly greater than those produced by adrenaline, while the dilator effects were considerably weaker. The effects of the amines, when administered intravenously, depended on the general arterial blood pressure and the vasomotor tone in the muscle under study.

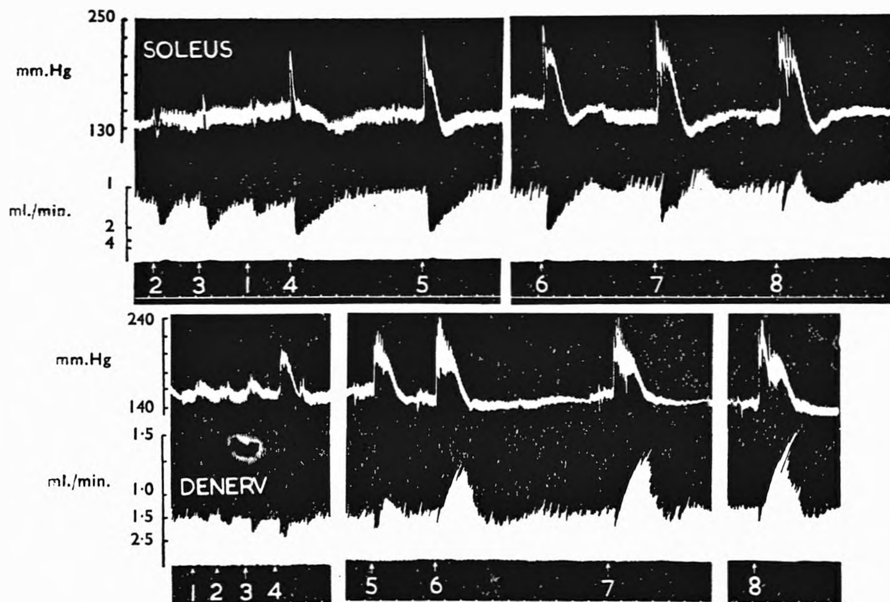

FIG. 3. Cat, 4·2 kg. The effect of acute denervation on the responses to intravenously administered adrenaline. Upper tracings: innervated muscle; lower tracings: acutely denervated muscle. Time scale in min. At **1**, 0·25; **2**, 0·5; **3**, 1; **4**, 5; **5**, 10; **6**, 20; **7**, 30; **8**, 50 μg.

In 21 out of 30 experiments in which the effects of intravenously administered adrenaline were studied, the blood pressure was greater than 100 mm. Hg and the vasomotor tone in the muscle was high, as shown by the fact that later in the experiment acute denervation caused a prolonged increase in venous outflow from the muscle. In these experiments both depressor and pressor doses of adrenaline, from 0·03 to 5–10 μg./kg., usually caused only an increase in venous outflow. This response increased with increase in dose. With larger doses (greater than 5–10 μg./kg.) the increase in flow became progressively less and was followed by a rapid return either to, or slightly below the normal level. Following this reduction in flow, even in preparations in which the flow was not reduced below normal, there was often a further increase in flow of longer duration (Figs. 3 and 5). After acute denervation of the skeletal muscle, it was usually still possible with small doses (up to 2–3 μg./kg.) to produce a small increase in venous outflow (Fig. 3). With larger doses, however, there occurred an initial short-lasting increase in venous outflow as the blood pressure rose, and this was followed immediately by vasoconstriction (Fig. 3). The vasoconstriction usually

occurred while the blood pressure was still rising, or at its maximum. Increase in dose caused an increase in vasoconstriction. Thus, a dose which was purely dilator in the innervated muscle, could cause a marked constriction in the acutely denervated muscle (Fig. 3). As in the innervated muscle an increase in flow often followed the vasoconstriction.

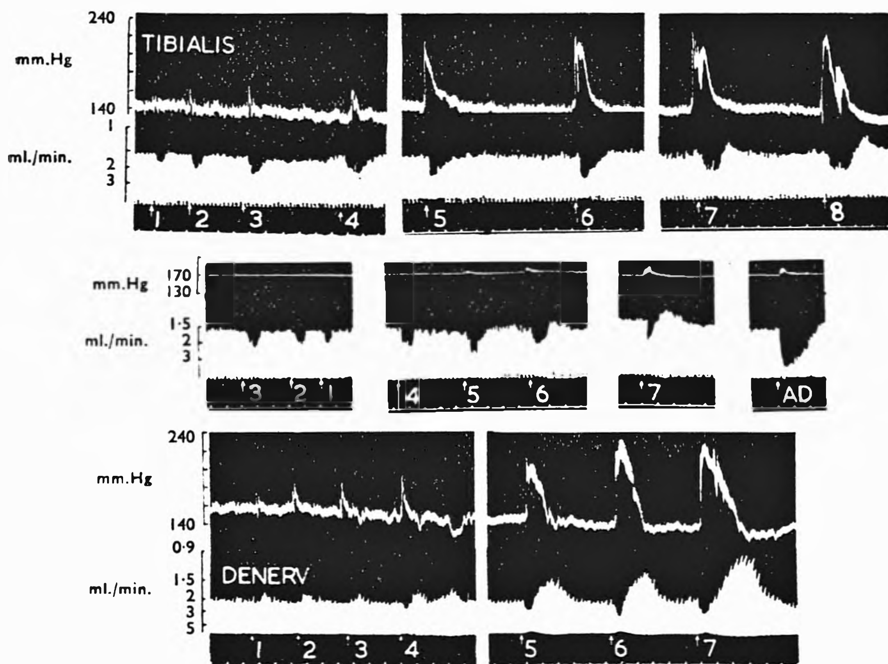

FIG. 4. Cat, 3·8 kg. The effect of stabilising the blood pressure and of acute denervation on the responses to intravenously administered noradrenaline. Upper tracings: innervated muscle; middle tracings; innervated muscle, blood pressure stabilised; lower tracings: acutely denervated muscle. Time scale in min. At **1**, 0·1; **2**, 0·25; **3**, 0·5; **4**, 1; **5**, 5; **6**, 10: **7**, 20; **8**, 40 μg. noradrenaline. At **AD**, 10 μg. adrenaline for comparison.

In 22 out of 28 experiments in which the effects of intravenously administered noradrenaline were studied, the blood pressure and vasomotor tone were high. In 12 of these 22 experiments intravenously administered noradrenaline produced effects similar to those described for adrenaline except that the increases in flow were smaller, while the decreases in flow were larger and usually occurred with smaller doses. Figure 4 illustrates one of these experiments. Unlike the effect of adrenaline, vasoconstriction produced by large doses of noradrenaline was not followed by a secondary increase in flow. In none of the experiments did small doses of noradrenaline cause a fall in blood pressure like that reported by West[30], but in two experiments in which the general arterial blood pressure was exceptionally high, the smallest doses of noradrenaline which increased the venous outflow from the muscle, were without effect on the blood pressure (Fig. 4). In the remaining 10 of these 22

experiments, intravenously administered noradrenaline in all effective doses produced only vasoconstriction. Intravenously administered adrenaline on the other hand, produced vasodilatation even in these experiments. Acute denervation later in these experiments caused a prolonged increase in the venous outflow from the muscle but it was much smaller in extent than that which occurred in the other 12 experiments. After acute denervation, in all experiments, effective doses of noradrenaline produced a vasoconstriction which, particularly with large doses, was preceded by a short-lasting increase in flow as the blood pressure rose (Fig. 4).

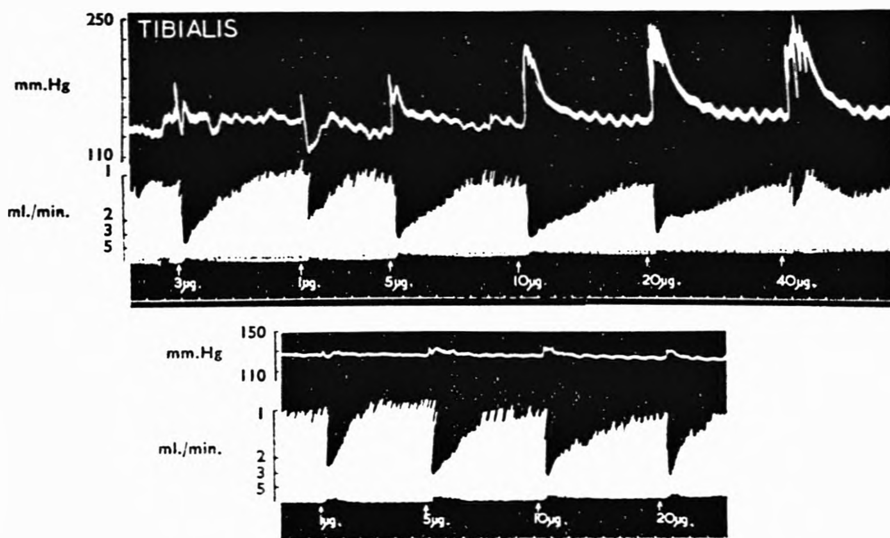

FIG. 5. Cat, 3·8 kg. The effect of stabilising the blood pressure on the responses to intravenously administered adrenaline in the innervated muscle. Time scale in min.

When the blood pressure stabiliser was connected to the carotid artery, the dilatation produced by intravenously administered adrenaline was only slightly reduced in extent and duration (Fig. 5). The dilator response to noradrenaline was reduced but not abolished when the blood pressure was prevented from rising (Fig. 4). With the stabiliser connected, even large doses of adrenaline and noradrenaline, of about 20 µg./kg., produced a rise in blood pressure of only 5–10 mm. Hg. After denervation, the reduction in blood flow produced by adrenaline and noradrenaline was slightly greater than that produced when the blood pressure was allowed to rise, and with large doses of adrenaline and all doses of noradrenaline there was no initial short increase in flow preceding the vasoconstriction.

The results so far described indicate that both adrenaline and noradrenaline can produce a vasodilatation in skeletal muscles which is not due to a direct action on the vessels but which is activated through

the nerves. Further support for this conclusion was provided by experiments in which the effects of intra-arterially and intravenously administered adrenaline were compared. In such experiments, it was always possible to produce a greater increase in venous outflow from the skeletal muscle with intravenously administered adrenaline than with intra-arterially administered adrenaline. Even in the range of intravenous vasodepressor doses, it was always possible to find a dose which produced a greater increase in venous outflow than any dose administered intra-arterially. Figure 6 illustrates such an experiment.

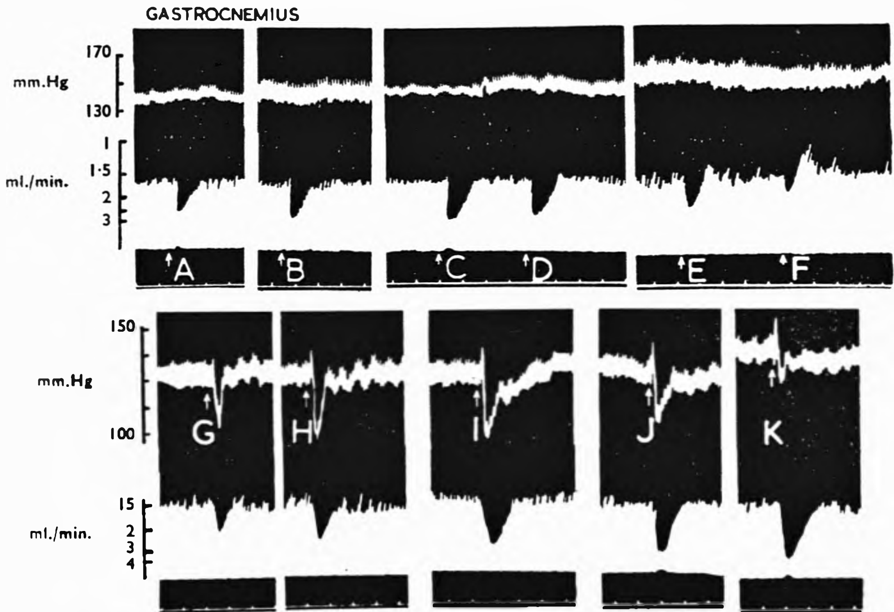

FIG. 6. Cat, 4·1 kg. Comparison between responses to intra-arterially and intravenously administered adrenaline in the innervated muscle. Upper tracings: intra-arterial administration; lower tracings: intravenous administration. Time scale in min. At A, 0·025; B, 0·04; C, 0·06; D, 0·08; E, 0·15; F, 0·3; G, 0·1; H, 0·25; I, 0·5; J, 1; K, 2 μg.

Cross-circulation experiments confirmed that the vasodilatation produced by intravenously administered noradrenaline and part of that produced by adrenaline, is dependent on intact nervous connections. In 3 out of 5 such experiments, the intravenous administration of adrenaline to the recipient cat caused vasodilatation in the skeletel muscles and in 2 of them, noradrenaline caused a similar but smaller response. Figure 7 illustrates an experiment in which both adrenaline and noradrenaline caused such a vasodilatation. These effects occurred despite the fact that there was no circulation connection between the recipient cat and the muscle under study. The absence of a pressor response in the donor cat confirmed the lack of circulatory connection. In the other experiments the amines were without effect when administered to the

recipient cat. In these experiments the vasomotor tone in the perfused muscle was later shown to be low. After acute denervation of the muscle the administration of the amines to the recipient animal was without effect in every experiment. With the doses used, the administration of the amines to the donor cat caused vasoconstriction in the perfused muscle both before and after denervation. The vasoconstriction was preceded by a brief passive increase in flow as the blood pressure of the donor animal rose (Fig. 7).

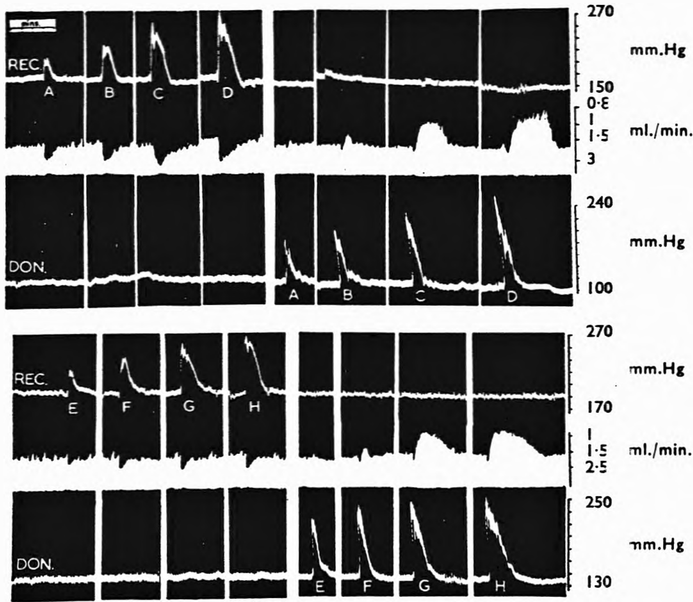

FIG. 7. Recipient cat, 3 kg; donor cat, 3·7 kg. Cross-circulation experiment. REC:—blood pressure of recipient cat. DON:—blood pressure of donor cat. Middle record:—venous outflow from perfused muscle. At A, B, C, D, 5, 10, 20 and 30 μg. adrenaline. At E, F, G, H, 5, 10, 20 and 30 μg. noradrenaline. The doses are marked under the blood pressure record of the cat to which they were intravenously administered.

In 9 out of 30 experiments with adrenaline and in 6 out of 28 with noradrenaline, the blood pressure was lower than 100 mm. Hg and the vasomotor tone in the muscle under study was later shown to be low. In these experiments minimal effective doses of adrenaline and noradrenaline (0·02–0·1 μg./kg.) produced a rise in blood pressure and an increase in venous outflow from the muscle. These effects increased in magnitude with increase in dose, up to a maximum with doses of 5–20 μg./kg. Larger doses caused an increase in flow which was followed by vasoconstriction (Fig. 8). The vasoconstriction occurred as the blood pressure was returning to normal. A further dilatation of longer duration followed the vasoconstriction produced by adrenaline on only 2 occasions (Fig. 8). However, on several occasions the vasoconstriction produced by both adrenaline and noradrenaline showed two distinct peaks. That

is, a short-lasting dilatation of variable extent was superimposed on the vasoconstriction 20 to 60 seconds after its onset. This dilatation, which occurred less often and was always smaller with noradrenaline, was then followed by a further vasoconstriction. Acute denervation did not alter the responses to adrenaline and noradrenaline in these experiments. By connecting the blood pressure stabiliser to the carotid artery the initial increase in venous outflow was prevented and all effective doses of adrenaline and noradrenaline caused an immediate vasoconstriction which was slightly greater than that which occurred when the blood pressure was allowed to rise. However, the vasoconstriction still often showed two distinct peaks. Figure 8 illustrates an experiment in which a single large

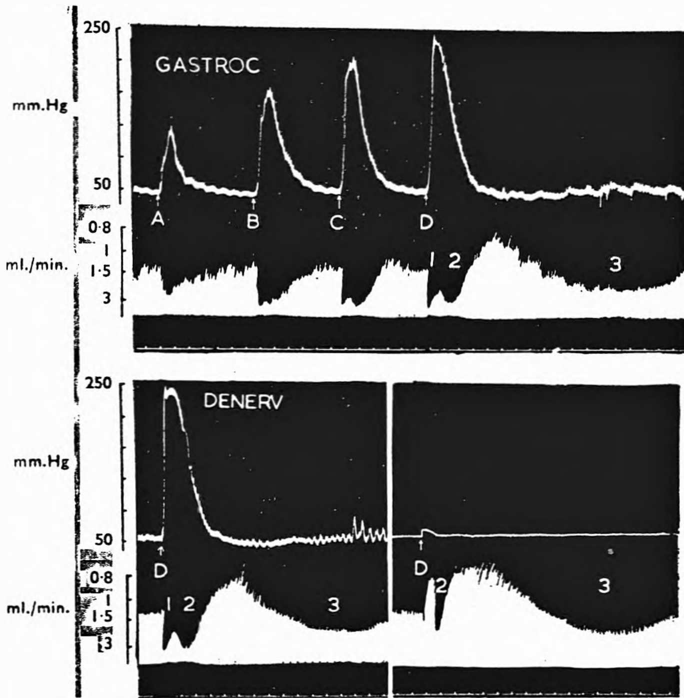

Fig. 8. Cat, 3·9 kg. The effect of intravenously administered adrenaline when blood pressure and vasomotor tone low. Upper tracing: innervated muscle; left lower tracing: acutely denervated muscle; right lower tracing: acutely denervated muscle, blood pressure stabilised. Time scale in min. At A, B. C and D, 2, 10, 20 and 40 μg. adrenaline.

vasoconstrictor dose of adrenaline produced three distinct and separate increases in flow; an initial increase as the blood pressure rose, a second increase which occurred immediately after the onset of vasoconstriction and a third increase which followed the vasoconstriction. The same response was produced in the acutely denervated muscle. When the blood pressure stabiliser was connected, the initial increase was absent and vasoconstriction occurred immediately but the second increase was still superimposed upon it and the third increase still occurred after the

vasoconstriction. These increases in flow are marked 1, 2 and 3 in Figure 8.

Discussion

When vasomotor tone is high, the intra-arterial administration of small amounts of adrenaline causes vasodilatation in skeletal muscles. With increase in dose, the response changes through a dual effect of dilatation followed by constriction, to a pure reduction in venous outflow. Girling[14] recorded a similar sequence of responses to ascending doses of adrenaline in rabbits. In most experiments the vasoconstriction produced by large doses of adrenaline was followed by a secondary increase in blood flow, an effect previously described in the dog by Green[31] and by Dörner[8]. Dörner[8] thinks no great importance should be attributed to the vasodilatation produced by small intra-arterial doses of adrenaline. He showed that even physiological saline injected intra-arterially may cause vasodilatation in the femoral artery. In the present work, the volume of injections administered intra-arterially was restricted to 0·01 ml. and control saline injections of the same volume as the adrenaline solutions were without effect. Furthermore, the intra-arterial administration of noradrenaline causes only vasoconstriction, the minimal effective dose being roughly equal to the smallest dose of adrenaline which shows some vasoconstrictor action. Doses of noradrenaline equivalent in size to purely dilator doses of adrenaline are without effect on the blood flow. The dilatation must, therefore, be attributable to a specific effect of adrenaline.

Lundholm[32] and Celander[18] have suggested that the dilatation of the blood vessels of skeletal muscles produced by adrenaline might be due to the increased lactic acid concentration resulting from changes in the carbohydrate metabolism. However, from the present experiments it appears unlikely that lactic acid can account for the initial dilatation obtained with small amounts of adrenaline. The onset of this dilatation is rapid, in many cases starting before the injection is completed. A direct action on the walls of the vessels is, therefore, more probably the cause since a secondary effect due to metabolic changes would involve first the penetration of adrenaline from the lumen of the vessel to the skeletal muscle, secondly, the break-down of glycogen and finally the return of lactic acid to the blood stream. Increased lactic acid formation may, however, account for the secondary dilatation which usually follows vasoconstriction produced by adrenaline. The time lag between injection and the appearance of secondary dilatation is more in accordance with what might be expected if the effect were due to metabolic changes. It is unlikely that the secondary dilatation is caused by the concentration of adrenaline, as it passes from the circulation, again entering the dilator range. Vasoconstriction is abruptly cut short by the secondary dilatation and if the above were the explanation, a more gradual change from constriction to dilatation might be expected. Furthermore, secondary dilatation often occurred in acutely denervated muscles in which no initial dilatation with small doses of adrenaline could be demonstrated. A

comparison between the secondary dilatation and the reactive hyperaemia which follows a short period of ischemia resulting from mechanical occlusion of the femoral artery, showed, in accordance with the results of Lanier and others[33] that, while the adrenaline effect in different experiments lasted from 90 seconds to 12 minutes, the duration of the reactive hyperaemia rarely exceeded 45 seconds. Moreover, the vasoconstriction produced by noradrenaline was not succeeded by a secondary increase in flow. Finally, the secondary adrenaline dilatation often occurred after the intravenous administration of large doses of adrenaline which, however, did not reduce the rate of flow below the normal level.

Acute denervation of the muscle usually caused an increase in venous outflow which on some occasions was as great as three times that of the flow from the innervated muscle. The extent of the increase in flow after acute denervation provided some indication of the degree of vasomotor tone, dependent on autonomic nerves. It was occasionally still possible to cause some dilatation in the acutely denervated muscle with small doses of adrenaline administered intra-arterially but constriction was the more usual response. Thus when the vasomotor tone is low and the vessels are consequently widely dilated, it is difficult to produce any further dilatation with intra-arterially administered adrenaline.

Noradrenaline, when administered intra-arterially, produced an immediate vasoconstriction. These results confirm the findings of others[6,8,10,15-17]. In most of my experiments, the vasoconstriction produced by noradrenaline was slightly greater and longer lasting than that produced by adrenaline, but it is difficult to determine the relative potency of the two amines in producing vasoconstriction because the effect of adrenaline is the overall result of two opposing actions. In the experiments of Wakim and Essex[10] adrenaline and noradrenaline were equally potent in producing vasoconstriction and this was found to be so in one of the present experiments in which the vasomotor tone in the muscle was low. It is therefore probable that adrenaline is at least as potent a vasoconstrictor substance as noradrenaline but when the vasomotor tone is high this action is masked to some extent by its vasodilator action.

When the general arterial blood pressure and the vasomotor tone in the muscles are high, intravenously administered adrenaline, and, to a smaller extent, noradrenaline cause, except in large doses, an increase in the blood flow through skeletal muscles. The use of the blood pressure stabiliser showed that only a small part of this effect is brought about through the rise in blood pressure and the buffer reflexes. With the intravenous administration of small amounts of adrenaline, the direct vasodilator action, as seen after intra-arterial injection must take part in the effect. However, a direct action on the blood vessels cannot account for the vasodilatation produced by the intravenous administration of noradrenaline or of large amounts of adrenaline. That the dilatation is partly activated through the nerves is shown by the following results.

1. After acute denervation of the muscle, intravenous doses of adrenaline or noradrenaline, which previously caused vasodilatation, frequently

produced a marked vasoconstriction, the flow often being reduced to a level below that of the normal flow from the innervated muscle. Several workers have explained this effect with adrenaline on the grounds that the widely dilated vessels of the acutely denervated limb cannot be further dilated. This explanation, however, does not account for the marked vasoconstriction which occurs after denervation. Certainly it cannot be said that acute denervation sensitises the vessels to the constrictor action of adrenaline because no such marked sensitisation occurs when it is administered intra-arterially.

2. The dilatation produced by intravenously administered adrenaline, even when it caused a fall in blood pressure, was very much greater than that produced by any dose administered intra-arterially. Similar results were obtained in the dog by Dörner[8].

3. When the vasomotor tone was high, the intravenous administration of adrenaline or noradrenaline to the recipient cat in a cross-circulation experiment caused vasodilatation in the muscle despite the fact that the only connection between the muscle and the recipient cat was through the nerves. After acute denervation of the muscle, a similar administration to the recipient cat was without effect on the muscle blood vessels. On the other hand, the intravenous administration of adrenaline or noradrenaline to the donor animal caused, after an initial passive increase in flow, only vasoconstriction in the perfused muscle. These results confirm those obtained in the dog by several other workers[2,34–38] with adrenaline and those of Gruhzit and others[38] with noradrenaline.

Even large doses of adrenaline administered intravenously often caused only an increased flow through the muscle, although, as shown by cross-circulation experiments in which adrenaline was administered to the donor animal, the local action of such doses is vasoconstriction. Thus in the innervated muscle, the dilatation due to nervous activity is often sufficiently great to mask completely the local constrictor action. Gruhzit and others[38] showed that, in the dog, mechanoreceptors along the course of the thoracic aorta, activated by the inotropic cardiac action of adrenaline and noradrenaline may be the afferent source of a reflex vasodilatation produced in skeletal muscles.

When the general arterial blood pressure and the vasomotor tone in the muscle were low, intravenously administered adrenaline and noradrenaline did not cause reflex dilatation in the skeletal muscles. A similar result was obtained in dogs by Gruhzit and others[38] The marked increases in venous outflow produced by adrenaline and noradrenaline under these conditions was unaltered after acute denervation and was shown, by the use of the blood pressure stabiliser, to be simply a passive effect caused by the large rise in blood pressure.

Several workers have been unable to record any vasodilatation with intra-arterially or intravenously administered adrenaline or with intravenously administered noradrenaline. Such results were occasionally obtained in the present experiments but only in animals in which the vasomotor tone in the muscles was shown to be low. Species difference

may account for the observations of some of these workers. For example, the experiments of Girling[14] were made on rabbits, animals which, according to Hartman, Kilborn and Lang[39] lack vasodilator mechanisms sensitive to intravenously administered adrenaline. Others[19,20,22], worked with human subjects and there is no evidence that adrenaline or noradrenaline causes reflex dilatation in the skeletal muscles of man. However, some workers[9–12,17] used the cat or the dog as their experimental animals and it seems probable, therefore, that the vasomotor tone in the muscles must, for some reason, have been low. Several factors are known to influence vasomotor tone. For example, Folkow[40] has shown that the use of many types of arterial flow meter causes a loss of vasomotor tone in the muscle vessels and the importance of anaesthetics in its maintenance has often been emphasised[8,24]. In the present experiments the blood flow was continuously recorded on the venous side; chloralose was the anaesthetic used and it is known that under chloralose anaesthesia vasomotor tone is well maintained.

From a physiological point of view the results are of interest since they indicate that, in emergency states, the relatively small amounts of both adrenaline and noradrenaline liberated from the adrenal medullae will cause vasodilatation in skeletal muscles.

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Couch Grass Ergot, Study on. L. C. Schramm and J. L. Beal. (*J. Amer. pharm. Ass., Sci. Ed.*, 1958, **47**, 326.) Ergots found growing on couch grass in central Ohio were assayed for total and water-soluble alkaloids by a modification of the method of Silber and Schulze (*Pharmazie*, 1953, **8**, 675) in which the defatted material was first extracted with ammoniated ether in a Soxhlet apparatus. The ergotoxine content was above, and the ergometrine content below the U.S.N.F.X. minimum requirements. Parts of the sample infested with a small beetle-like insect had a lower content of alkaloid and fat. By means of partition chromatography it was shown that the ergots contained ergometrine, and probably ergotamine, ergotaminine, ergosine, ergosinine, ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine and ergocryptinine, together with lysergic acid. G. B.

***Sedum acre*, Alkaloid Mixtures Isolated under Different Conditions from.** L. K. Bergane and A. Nordal. (*Medd. Norsk. Farm. Sels.*, 1958, **20**, 70.) Extracts of *Sedum acre* were submitted to paper chromatography, using butanol saturated with water, or butanol 4, acetic acid 1, water 5, as solvent, and examining the spots after treatment with a modified Dragendorff's reagent. This procedure was satisfactory for the separation of sedamine and sedridine. Canadian, Dutch, German and Norwegian specimens, collected at different times of year, were all found to yield sedamine and sedridine. Sedamine was the main alkaloid of the plant, from which it could be extracted by a simple process. A special technique was required for the extraction of sedridine.

G. B.

ANALYTICAL

Adrenaline and Noradrenaline, Fluorimetric Determination of, in Aqueous Solution. S. Roston. (*Analyt. Chem.*, 1958, **30**, 1363.) This method depends upon the differential transformation of adrenochrome and noradrenochrome into the corresponding fluorescent products adrenolutine and noradrenoutine. Two identical samples of mixture were taken and oxidised under the same conditions with ferricyanide to form mixtures of adrenochrome and noradrenochrome. One sample was then treated so as to form preferentially noradrenolutine and the other to form preferentially adrenolutine and the fluorescence measured in each case. From two simultaneous equations based on these readings and standard curves of the pure substances, the concentration of each in the mixture was calculated. The conditions for these preferential reactions depended upon interaction with varying amounts of alkali and ascorbic acid and have been carefully worked out. For 0·03–1 μg. of adrenaline and 0·05–1 μg. of noradrenaline the recovery of the amines was within 10 per cent of the amount added. As little as 0·01 μg. of adrenaline and 0·02 μg. of noradrenaline could be determined to within an error of $\pm$20 per cent.

D. B. C.

Chloramphenicol and Chloramphenicol Palmitate, Assay of, by Non-aqueous Titration. B. Salvesen. (*Medd. Norsk. Farm. Sels.*, 1958, **20**, 65.) Chloramphenicol and its palmitate may be assayed by hydrolysis at the amide linkage, followed by non-aqueous titration of the resulting aminopropanediol against perchloric acid. Chloramphenicol is readily hydrolysed with 25 per cent hydrochloric acid, but the ester must be dissolved in alcohol and boiled with hydrochloric acid. In either case the dichloroacetic acid and excess hydrochloric acid are removed by evaporating to dryness, after which the aminopropanediol hydrochloride is titrated with perchloric acid in the presence of mercuric acetate, using crystal violet as indicator. Direct titration of chloramphenicol or its palmitate with perchloric-acetic acid provides a basis of a rapid purity test to determine small quantities of aminopropanediol which may be produced during storage. A suitable technique is described, and 0·05 ml. of 0·01N perchloric acid is suggested as a suitable limit when using 0·1 g. of chloramphenicol or 0·2 g. of palmitate. G. B.

Prednisone in Tablets, Polarographic Determination of. H. P. Deys and J. A. C. van Pinxteren. (*Pharm. Weekbl.*, 1958, **93**, 760.) Preliminary experiments with prednisone showed that 10 mg., dissolved in 4 ml. of ethanol and 4 ml. of an acetate buffer, pH 5, and the mixture deoxygenated, gave a distinct wave between -1 V. and $-1{\cdot}5$ V. When applied to tablets, it was found that the tablet excipients had a small but constant influence. Provided the temperature was controlled, a 5 mg. tablet could be assayed with an accuracy to within ± 1 per cent of the mean, and the method required only half an hour since no extraction process was necessary. Prednisolone could be similarly assayed and could be distinguished from prednisone in that its half-wave potential was 0·05 V. more negative than the latter. In the case of prednisolone, the method was not applied to tablets, although the authors feel confident that it could be. D. B. C.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

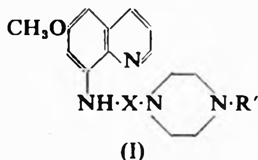
5-Hydroxytryptamine and Hyperglycaemia. G. B. West. (*Nature, Lond.*, 1958, **182**, 182.) It has been suggested that the hyperglycaemic substance present in the blood of the pancreatico-duodenal vein of animals treated with growth hormone is 5-hydroxytryptamine (5-HT). However, injections of 5-HT into normal dogs fail to produce a significant rise in blood sugar, although similar injections into depancreatised animals result in hyperglycaemia. Studying the distribution of 5-hydroxytryptophan decarboxylase, it was found that the pancreas possesses a considerable degree of enzyme activity. This was true for the rat, guinea pig, rabbit, cat and dog. In some instances the values compared favourably with those of the kidney, the richest known source of the enzyme. The method used was that of Gaddum and Giarman, substituting iproniazid for choline *p*-tolyl ether, as the inhibitor of monoamine oxidase. When 5-HT was used as the substrate and iproniazid omitted it was possible to estimate the monoamine oxidase activity of the homogenates. Again the pancreas was found to have a high value. This observation probably explains why the 5-HT content of the pancreas is low. It should now be possible to identify the hyperglycaemic substance in the pancreatico-duodenal blood after injections of growth hormone, and to test its action on the blood sugar of animals treated with monoamine oxidase inhibitors. M. B.

5-Hydroxytryptamine in Blood Platelets, Increase of, by Iproniazid. A. Pletscher and A. Bernstein. (*Nature, Lond.*, 1958, **181**, 1133.) It has been found that iproniazid causes an increase in the 5-hydroxytryptamine (serotonin, 5-HT) and noradrenaline content of the brain in various species. A significant rise in the catechol amine content of the myocardium of guinea pigs has been observed after the administration of iproniazid. These effects are probably due to the inhibition of monoamine oxidase, an enzyme present in brain and heart and which is markedly inhibited by iproniazid. Isoniazid, a compound which inhibits monoamine oxidase much less than iproniazid, causes little or no rise in the noradrenaline or 5-HT content of brain or heart. This paper deals with the effect of iproniazid on the 5-HT content of blood platelets. The amount of 5-HT was measured by a spectrophotofluorimetric method. In rabbits the daily administration of 30 mg./kg. of iproniazid for 4 days caused a marked rise in the blood 5-HT. In man the daily oral administration of 5–8 mg./kg. of iproniazid caused the platelet 5-HT to rise gradually. Isoniazid caused a much smaller rise. Thus it seems probable that this effect is connected with monoamine oxidase inhibition. It is possible that certain pharmacological actions of iproniazid are mediated by the increase of 5-HT in platelets. This might be true for certain effects of iproniazid on the heart, since the cardiovascular system is relatively sensitive to 5-HT. M. B.

Noradrenaline, Demonstration of 3-Methoxy Analogue of, in Man. A. Sjoerdsma, W. M. King, L. C. Leeper and S. Udenfriend. (*Science*, 1958, **127**, 876.) Recent studies have shown that a major metabolite of noradrenaline and adrenaline found in human urine is 3-methoxy-4-hydroxymandelic acid. Subsequently it was shown that the 3-hydroxy position of both noradrenaline and adrenaline can be methylated by animal tissues to yield the corresponding 3-methyoxy analogues. Since the conversion of catechol amines to their methoxy analogues may play some part in the physiology of these agents it seemed important to determine whether these substances exist in man. It was not possible to demonstrate the methoxy amines in human urine but on subjecting a human phaeochromocytoma to various extraction procedures followed by paper chromatography and spectrophotofluorimetry, 25 μg./g. of the 3-methoxy analogue of noradrenaline was detected. Preliminary studies suggest that these tumours contain the enzyme which transfers the methyl group from *S*-adenosylmethionine to the 3-hydroxy group of the catechol amines. M. B.

CHEMOTHERAPY

Leishmanicides, New Series of. E. Beveridge, L. G. Goodwin and L. P. Walls. (*Nature, Lond.*, 1958, **182**, 316.) A series of compounds of structure (I), in which X is a straight or branched alkylene chain and R is H, alkyl or hydroxyalkyl, have been shown to possess activity against *Leishmania donovani* in hamsters. The active members were more effective than pentavalent antimonials and aromatic diamidines when given subcutaneously, and moreover are also effective orally. The introduction of a third basic group in the alkylene chain as in I [X = $\cdot(CH_2)_2NH(CH_2)_2\cdot$] leads to inactivity, as does replacement of the terminal piperazine group by morpholino, piperidino or pyrrolidino rings. The 6-hydroxy-, 6-ethoxy- and 5:6-dimethoxy-compounds are also active, but the 6-chloro- and 6-unsubstituted compounds



(I)

are inactive. High activity is shown by compounds where X is —$(CH_2)_2$—. Some compounds of the series possess high activity against *Plasmodium gallinaceum* in chicks. J. B. S.

PHARMACOGNOSY

Cinchona Alkaloids, Biosynthesis of. J. Relijk. (*Pharm. Weekbl.*, 1958, **93**, 625.) Evidence is presented in support of the view that the crystalline alkaloids of cinchona bark are derived from amorphous alkaloidal precursors with an indole structure which are first formed in the leaves and later transported to the bark. This is in accord with a biosynthesis propounded by Woodward which starts from tryptamine and *m*-hydroxyphenylacetaldehyde. Evidence of an indole structure in a mixture of amorphous leaf alkaloids (which could not be further purified) is based on the similarity of the ultra-violet absorption spectrum to compounds such as cinchonamine, tryptophane and yohimbine which contain an indole structure. Many colour reactions of the above substances and the R_F value of cinchonamine are also similar to those of the leaf alkaloids. D. B. C.

PHARMACOLOGY AND THERAPEUTICS

Amiphenazole and Morphine in the Production of Analgesia. S. Gershon, D. W. Bruce, N. Orchard and F. H. Shaw. (*Brit. med. J.*, 1958, **2**, 366.) The combination of amiphenazole and morphine has been used for the treatment of intractable pain in terminal carcinoma in more than 500 patients over a period of 4 years. In the presence of amiphenazole (*a*) morphine may be given in large doses (200 mg. four times daily at 6-hourly intervals) without any risk to the patient, provided small doses are given initially; (*b*) drowsiness does not develop unless there is gross liver damage; (*c*) addiction to morphine does not develop (there is no euphoria, no craving, and no withdrawal symptoms on abrupt cessation of morphine administration). Complete analgesia for 24 hours in the day can be obtained. The dose of amiphenazole must be reduced at night to avoid insomnia. In some cases, where the patient becomes aware of his hopeless condition, and there is rapid deterioration, it is necessary to omit the amiphenazole. The mechanism of the combined action is still unknown, but it is suggested that the drugs combine, probably in the liver, to form a non-narcotic analgesic substance. The doses of amiphenazole used at present are much greater than those previously recommended; as much as 200 mg. three or four times daily may be given either orally or intramuscularly. Ten case histories are given. S. L. W.

Digitalis Glycosides, Effect of, on the Oxygen Consumption of Heart Muscle *In Vitro*. E. Peschel and C. Schlayer. (*J. Lab. clin. Med.*, 1958, **52**, 417.) The problem here was to see whether an increase of oxygen consumption is associated with the action of digitalis glycosides on heart muscle *in vitro*, and whether a difference could be demonstrated for the action of these substances on heart muscle as compared with other muscle. All experiments were done using the direct method of Warburg, employing rat heart slices. In any one experiment all slices were from the same animal. Homogenised or minced heart tissue was unsatisfactory. It was found that the digitalis glycosides stimulate the oxygen consumption of the rat heart slices by 12–19 per cent.

The effective concentration is about 10^{-5} M. The oxygen consumption of other tissues, such as kidney and liver, is slightly depressed by corresponding concentrations of the glycoside. The oxygen consumption of the diaphragm is depressed by even very small concentrations of the glycoside. This depression, increasing with dosage, is probably an indication of temporary cell damage. This might explain the liberation, by therapeutic doses of digitalis in man, of intracellular potassium without any effect on the sodium and potassium balance of the heart. M. B.

Fibrinolysin in the Treatment of Thromboembolic Disease. K. M. Moser. (*J. Amer. med. Ass.*, 1958, **167**, 1695.) A study of the effects of intravenously administered fibrinolysin (plasmin) was carried out in 52 patients with various forms of thromboembolic disease, the object being to achieve a level of plasma fibrinolytic activity which would be capable of dissolving an intravascular thrombus without undue toxic effects. The conditions treated included deep venous thrombophlebitis, pulmonary embolism, peripheral arterial occlusion, and superficial thrombophlebitis. The profibrinolysin used was a highly purified derivative of human plasma protein fraction 3. It was activated to fibrinolysin (Actase) with small amounts of purified streptokinase, after which the residual streptokinase was removed and the activated material lyophilised. One fibrinolytic unit of fibrinolysin has been defined as that amount of a standard preparation which will change the turbidity of a standard fibrinogen-thrombin clot by 50 per cent in 10 minutes. The fibrinolysin was dissolved immediately before use in 500–1000 ml. of 5 per cent dextrose in water, the infusion being carried out over 2–4 hours. Thirteen patients received infusions of 30,000 fibrinolytic units (FU), 19 patients 40,000–50,000 FU, and 20 patients 69,000–90,000 FU. In the doses used toxicity appeared to be limited to pyrogenicity, which occurred in 49·2 per cent of the patients. There were 2 cases of delayed allergic skin reaction. No embolic events followed the infusions, and no haemorrhagic phenomena occurred in any patient, including 29 who were simultaneously receiving anticoagulant drugs. Fibrinolytic activity of the plasma was consistently enhanced by the infusions, the intensity and duration of this activity running parallel with the total fibrinolysin dosage. Anticoagulant drugs should be used in all patients to prevent rethrombosis, unless it is clear that the factors which led to thrombosis have subsided. The therapeutic results were encouraging and justify the view that fibrinolysin may prove a safe and effective agent for achieving acute lysis of intravascular clot, but firm conclusions must await completion of large-scale controlled studies.

S. L. W.

Framycetin Sulphate in the Treatment of Skin Infections. D. Burrows. (*Brit. med. J.*, 1958, 2, 428.) Fifty patients with skin infection were treated with framycetin sulphate 1·5 per cent in a water-soluble ointment base. The cases were made up of impetigo 32, infective dermatitis 9, secondarily infected eczema 4, infected papular urticaria 2, folliculitis and sycosis barbae 1. Twenty-two of the cases of impetigo were cured within one week, and only 2 failed to respond —one of impetigo and one of infective dermatitis. The ointment was applied three or four times daily, removing crusts with a 1 per cent solution of cetrimide. In 38 patients all of whom responded, *Staphylococcus aureus haemolyticus* was isolated; in 19 cases *Streptococcus haemolyticus* was isolated and only 5 were sensitive to the antibiotic; this may reduce its effectiveness in general use. No case of contact sensitivity or irritation was observed. S. L. W.

Methylpentynol Carbamate, Susceptibility to. E. Marley. (*Brit. med. J.*, 1958, 2, 493.) Methylpentynol carbamate was administered in a dose of 0·2 g. four times daily to 10 patients who had previously developed toxic symptoms while receiving a 5-day therapeutic trial of methylpentynol, 0·5 g. four times daily. Except in one patient who showed a maximal toxic response, it was shown during a similar 5-day period that the carbamate was much superior in its freedom from incidental toxicity. However, in a population known to be especially sensitive to methylpentynol, the use of the carbamate as an alternative in doses of 1 g. a day or greater is likely to be followed by the early manifestation of toxic symptoms. S. L. W.

Morphine, Inhibitory Action on Guinea Pig Ileum. H. W. Kosterlitz and J. A. Robinson. (*Brit. J. Pharmacol.*, 1958, **13**, 296.) Small concentrations of morphine inhibit the peristaltic reflex of the guinea pig isolated intestine. A study of its effects on the longitudinal muscle has shown that it markedly inhibits the contractions produced by nicotine, barium and 5-HT; while the actions of acetylcholine, carbachol and histamine were hardly affected. After morphine the addition of atropine reduced the residual contractions of nicotine and 5-HT, but had scarcely any effect on barium. The additional effect of atropine on nicotine was always greater than on 5-HT. While hexamethonium depressed the action of barium alone, after morphine the residual contraction was unaffected. With nicotine the residual contraction after morphine was further depressed by hexamethonium. The morphine antagonists nalorphine and levallorphan had both a morphine-like action and at the same time a morphine-inhibiting action, which varied with the antagonist used. The morphine-like actions were most pronounced on the effect of 5-HT, which was antagonised; while the morphine protecting action was most strongly present on the effect of nicotine. G. F. S.

Pentacynium Methylsulphate; Effect on Renal Circulation in Hypertension. J. R. Cox and J. J. Daly. (*Brit. med. J.*, 1958, **2**, 78.) The effect of pentacynium methylsulphate on the renal circulation was studied in 9 patients with hypertension. The drug was given subcutaneously in a dose of 25 mg. in seven patients and 12·5 mg. in two. A fall of blood pressure to normal levels occurred in all subjects and persisted for at least 16 hours. Reduction in effective renal blood flow (E.R.B.F.) and glomerular filtration rate (G.F.R.) occurred initially in all patients in 30 minutes. In 4 patients the mean E.R.B.F. and G.F.R. had risen to 50 per cent of the mean control value by 60 to 110 minutes; in 2 patients with severe renal damage these values had risen to greater than their control values after 110 minutes. Similar changes in renal haemodynamics occur with other ganglion-blocking drugs, but pentacynium has the advantage that it is effective with a single daily administration and therefore causes less frequent changes in these values than other drugs requiring more frequent administration.

S. L. W.

Sodium Diethyldithiocarbamate (Dithiocarb) in Nickel Carbonyl Poisoning. F. W. Sunderman and F. W. Sunderman, Jr. (*Amer. J. med. Sci.*, 1958, **236**, 26.) Eleven workers suffering from accidental exposure to the vapours of nickel carbonyl were successfully treated with sodium diethyldithiocarbamate (dithiocarb), the drug being given by mouth in doses of 0·5 g. three or four times

daily, together with 0·5 g. of sodium bicarbonate and a glass of water. Nickel carbonyl is one of the most toxic chemicals encountered industrially and owing to its high volatility it is difficult to avoid exposure to inhalations during handling. Measurement of nickel concentration in the initial 8-hour collection of urine is a valuable aid in determining the severity of poisoning. The upper normal limit for the concentration of nickel in urine is 3·0 μg./100 ml. If the initial 8-hour specimen has a concentration of less than 10 μg./100 ml. the exposure is classified as mild, and serious delayed symptoms will probably not develop; if the concentration is above 10 μg. but less than 50 μg./100 ml. the exposure is moderately severe, and delayed symptoms are likely to develop; and if the concentration is above 50 μg./100 ml. the exposure is severe and serious illness is likely. In the moderate or severe cases dithiocarb therapy is instituted and continued until the patients are free from symptoms and the concentration of nickel in the urine is less than 10 μg./100 ml. In critical cases it is suggested that dithiocarb may be administered parenterally, in an initial dose of 25 mg./kg. bodyweight, the total amount during 24 hours being limited to 100 mg./kg. bodyweight: so far, no patients have been treated parenterally. In the 11 patients in this series (in one of whom the initial nickel concentration was 200 μg./100 ml.) the symptoms of poisoning were relieved within a few hours after administration of dithiocarb. Delayed reactions were minimal and convalescence uneventful, normal levels of nickel concentration in the urine being reached within 16 days of exposure. This was in marked contrast to the treatment previously of 31 cases of severe nickel poisoning with dimercaprol, in which 2 patients died and the majority required several months convalescence. Patients treated with dithiocarb should abstain from alcohol, or symptoms similar to those following the use of disulfiram may occur. S. L. W.

Substance P—Effect on Peristaltic Reflex. D. Beleslin and V. Varagić. (*Brit. J. Pharmacol.*, 1958, **13**, 321.) Substance P potentiated the peristaltic reflex of the guinea pig isolated ileum when introduced into the lumen. In fatigued preparations substance P produced vigorous peristalsis, it also abolished the depressant action of 5-HT on the reflex. The effect of substance P on peristalsis was abolished by hexamethonium. Injection of substance P into the lumen of the intestine, from which the mucous membrane had been removed, did not restore the peristaltic reflex. The experiments show that substance P may produce peristalsis when the tryptamine receptors have been saturated, and therefore the receptors for this substance may differ from those for tryptamine. G. F. S.

Trifluoperazine in the Treatment of Chronic Psychotics. M. E. Forrester. (*Brit. med. J.*, 1958, **2**, 90.) A short trial of trifluoperazine (Stelazine) was undertaken with 25 chronic psychotic patients. The dosage was built up over a period of two weeks to 30 mg. daily (in 2 patients to 40 and 45 mg. daily respectively). Treatment lasted for 4 weeks and the dosage was then gradually reduced until discontinuance. Benzhexol, 6–8 mg. daily, was used to counteract side-effects. The changes in behaviour were: very slightly improved, 6; no change, 10; worse, 6; much worse, 3. With the dosage used, the side-effects, chiefly of the Parkinson type, were so marked and unpleasant that it is doubtful whether the drug would be acceptable for routine use, and in view of the lag of time between stopping the drug and the subsidence of side-effects it would appear that the drug is cumulative in the body. The amount of improvement in a few cases was not sufficient to encourage further use. S. L. W.

LETTER TO THE EDITOR

Staphylococcal Infection in Histamine and 5-Hydroxytryptamine Depleted Rats

SIR,—It is well known that in the rat much histamine and 5-hydroxytrypt-amine(5-HT) is contained in the skin[1]. The preponderance of these substances in regions which meet the outside world, suggests that they may be concerned in resistance of the animal to infection[2].

We have studied the effect of preferential depletion[3] of histamine or 5-HT on the resistance of the rat to infection by coagulase positive *Staphylococcus aureus*, isolated from a human source. When normal control rats were given intra-peritoneal injection of doses of *Staphylococcus aureus* (18 hours broth culture) and killed 72 hours after, viable organisms could not be recovered from the blood. In these animals no abcesses, macroscopic or microscopic could be detected. The mast cells from subcutaneous tissues, however, showed considerable degranulation and rupture in areas distant from the site of injection. When such injections of *Staphylococcus aureus* were given in rats previously depleted of histamine by repeated injections of polymixin B or depleted of 5-HT by injection of reserpine[3], the results obtained were different. In both these groups of animals, viable organisms were recovered from the blood. There were multiple pyaemic abcesses in the lung, liver and kidney. Organisms could be recovered from these abcesses as well. The mast cell damage produced by injections of polymixin B or reserpine were similar to those described by Parratt and West[3]. Injection of *Staphylococcus aureus* was without any effect on these damaged cells.

The total and differential count of leucocytes were done in control animals and in animals depleted of histamine or 5-HT, before and 24 hours after the injection of *Staphylococcus aureus* and also at autopsy. There was no appreci-able alteration produced in either count by injections of polymixin or reserpine and the counts remained essentially the same in all the three groups after injection of the organisms.

The resistance of rats to infection by *Staphylococcus aureus*, and the presence of histamine and 5-HT in the tissues may be related. The injection of these organisms causes rupture of mast cells with release of histamine and other metabolites which may possibly aid in the defence of the body. Histamine or 5-HT, however, did not inhibit the growth of *Staphylococcus aureus in vitro*, when included in the culture media. The breakdown of the resistance, of the histamine or 5-HT-depleted rats to this organism cannot be accounted for by alteration of the leucocyte response. The mechanism by which the presence of histamine or 5-HT in tissues in rats confers immunity to infection by this organism is obscure. It was of interest therefore to note that 5-HT increased the phagocytic power of monkey leucocytes *in vitro* (Buttle and Northover, personal communication) and injections of histamine caused increased phagocytosis of BCG in rats[4]. Further work is in progress to elucidate this mechanism.

LETTER TO THE EDITOR

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