THE CROONIAN LECTURES ON THE PHARMACOLOGICAL ACTION AND THERAPEUTIC USES OF THE NITRITES AND ALLIED COMPOUNDS.

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LECTURE I.

In the Croonian Lectures for 1890 Dr. Lauder Brunton set forth with a master's hand the position of our knowledge with regard to the relationship which exists between chemical composition and physiological action. He pointed out the structure of the leading types of organic compounds and explained the influence which both structure and composition have in determining their physiological action.

In the lectures I have the honour of being invited to give my rôle will be a more limited one. I propose to take up one of the many series of groups with which Dr. Brunton dealt, and, if possible, add something to our knowledge concerning it.

PURPOSES OF LECTURES.

The Croonian Lectures have been instituted for the promotion of knowledge bearing on the prevention, control, and cure of disease, and in those I am about to give I intend to set forth the pharmacology of the nitrites and some allied compounds, and to show how our knowledge of their actions may be utilised in the treatment of disease. But an essential object of these lectures will be to indicate the influence or physiological action which certain small groups of atoms or molecules of oxygen and nitrogen entering into the composition of the nitrites and allied compounds directly and indirectly exert.

ACTION OF ELEMENTS AND GROUPS OF ELEMENTS.

When a medicinal substance enters into the circulation the molecules of which it is constituted, or those resulting from such chemical modification as it undergoes, come into contact with certain tissues to which they are specially related, and, influencing these tissues, produce the alterations in the function of the various organs which we include under the name of pharmacological action.

Into the cause of the relationship or affinity which seems to exist between the molecules of chemical compounds and the tissues they influence we have not yet penetrated, nor do we know how the ultimate constituents of the tissues are so acted upon that modification of function is produced. But, so far as the chemical compounds themselves are concerned, we have evidence that their effects are in some way the result of the influence of the separate element or groups of elements of which they are built up and of the method in which these elements or groups are conjoined.

Some elements make their presence felt pharmacologically in a distinctive manner, the definite effect of others we can hardly determine. It has been shown that many metals, as, for example, potassium, calcium, and barium, exercise a definite influence on muscular tissues of the body. Chlorine, bromine, and iodine have also, as Binz many years ago pointed out, a special action on nerve cells. In O, H, C, and N we have elements the pharmacological offices of which are so widespread and varied that we cannot at present define their sphere of influence; they, however, unite to form groups of which the specific effect can be distinctly shown. The alcoholic radicles or alkyls, such as methyl, CH₃, tend to give the compounds into which they enter a power of depressing the functions of the higher cerebral centres, and hence producing sleep or general anaesthesia.

The group NH₂, like ammonia compounds generally, has a stimulant effect on the medulla and spinal cord. There are even indications that the small group hydroxyl, OH, has itself a peculiar influence, though probably this influence is wide-

spread and varied. It must be confessed that our knowledge of the conditions and limits of this influence is of a rudimentary kind. But, imperfect though it is, we owe to it some of the most valuable of the recent additions to the means at our disposal for the prevention, control, and cure of disease, as well as a better knowledge of the method in which some of the older methods may act.

GROUPS CONTAINING OXYGEN AND NITROGEN.

Among the many groups concerning which more information is wanted are those which contain oxygen and nitrogen. They enter into the composition of many substances used in medicine and of a still greater number which may be found to have therapeutic value. In these nitrogen is combined with one or more atoms of oxygen. The following table sets forth the groups of these two elements to which I propose to call attention:—

GROUPS.	EXAMPLES.
O—N=O Nitrites Na—O—N=O	NaNO ₂ Sodium nitrite
$\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C}-\text{O}-\text{N}=\text{O} \\ \\ \text{H} \end{array}$	CH ₃ NO ₂ Methyl nitrite
$\begin{array}{c} \text{O} \\ // \\ -\text{O}-\text{N} \\ // \\ \text{O} \end{array}$ Nitrates Na—O—N $\begin{array}{c} \text{O} \\ // \\ \text{O} \end{array}$	NaNO ₃ Sodium nitrate
$\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C}-\text{O}-\text{N} \\ // \\ \text{O} \end{array}$	CH ₃ NO ₃ Methyl nitrate
$\begin{array}{c} \text{O} \\ // \\ -\text{N} \\ // \\ \text{O} \end{array}$ Nitro compounds $\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C}-\text{N} \\ // \\ \text{O} \end{array}$	CH ₃ NO ₂ Nitro methane
$\begin{array}{c} \text{O} \\ // \\ \text{O}=\text{N} \\ \\ \text{C} \\ \\ \text{CH} \end{array}$	C ₆ H ₅ NO ₂ Nitro benzene
$\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C} \\ \\ \text{H} \end{array}$ Nitrosamines $\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C}-\text{N}-\text{NO} \\ \\ \text{H} \end{array}$	(C ₂ H ₅) ₂ NNO Dimethyl nitrosamine
—ON=N—O— Hypo-nitrites Na—O—N=N—O—Na	Na ₂ N ₂ O ₂ Sodium hyponitrite
$\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C} \\ \\ \text{H} \end{array}$ Oximes $\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C}=\text{N}-\text{O}-\text{H} \\ \\ \text{H} \end{array}$	CH ₃ CHNOH Aldoxime
$\begin{array}{c} \text{O} \\ // \\ \text{C} \\ \\ \text{CH} \end{array}$	C ₆ H ₄ ONOH Nitroso-phenol

The formulæ given for these groups and their compounds are founded on their chemical reactions and decompositions, and are those which are generally accepted as representing their structure. The first or nitrite group consists of an atom

of triad nitrogen which has two of its affinities satisfied by an oxygen, whilst the third is attached to a second oxygen. The second oxygen atom has thus one of its links left free.

Nitrogen it is well known at times plays the part of a pentad element, and it is supposed by chemists to do so in the second or nitrate group. Here four of the affinities of the pentad nitrogen atoms are satisfied by two oxygen elements, whilst to the fifth link is attached a third atom of oxygen which serves to couple the molecule with other groups. In the next or nitro group the nitrogen is also probably pentad, and has attached to it two atoms of oxygen. It will be noted that this group is isomeric with the nitrite group, but owing to the difference in structure, the corresponding compounds into which the group enter have not the same pharmacological, chemical, or physical properties.

In the nitrosamines, as in the nitrite group, we have a triad nitrogen, two links of which are satisfied by an oxygen atom, the third having attached to it a nitrogen element.

It is usual to regard hyponitrites as containing a group, NO, united directly to the other element of the compound, hyponitrous acid being looked upon as HNO , and sodium hyponitrite as NaNO . But the recent researches of Zorn and others, seems to have proved that the acid and its salts are diazo compounds, and I have thus represented them in the table.

The last group, the oximes, consists of a group, NOH, the nitrogen of which is united to a carbon element.

It will be seen that the groups of nitrogen and oxygen given on the list enter into metallic, fatty, and aromatic compounds. I propose, however, confining my remarks almost entirely to the metallic and fatty compounds containing them, and even concerning these the observations I have to record must be regarded as the result of a preliminary investigation only of their properties.

METHODS IN WHICH GROUPS OF ELEMENTS IN COMPOUNDS PRODUCE THEIR EFFECT.

Before speaking of the influence exerted by the groups of molecules to which I have drawn attention, it may be well if I briefly discuss the methods in which they may produce their effects on the tissues. It will be noted that while the compounds into which they enter, nitrite of sodium and nitrate of sodium, for example, have an independent existence, the molecules themselves are not known in a separate form, yet there is evidence that, like the radicles of the fatty series (CH_3 , etc.), amidogen (NH_2), and hydroxyl (OH), their special physiological influence can be traced through the various compounds they enter into, modified, of course, by other molecules with which they are associated. The action of the nitrites of the alkaline and earthy metals on muscular tissue will illustrate this.

When the excised muscles of certain animals, such as the frog, are placed in a 0.6 to 0.75 per cent. solution of sodium chloride, the so-called normal salt solution, they continue to live and behave under stimulation in a fairly uniform manner provided they are exposed to exactly similar conditions. If the contractions of corresponding muscles are registered by a myograph, they always yield characteristic tracings, which, though influenced by method of excision, temperature, season of the year, and many other factors, yet correspond in character, provided the circumstances under which the curves are taken are alike. The duration of the life of the muscle too is, if these conditions are fulfilled, much the same.

Fig. 1 represents a muscle curve taken in normal salt solution. As I shall have occasion to refer frequently to the influence on muscle tissue of the substances I am about to speak of in these lectures, I may here take the opportunity of explaining that I have estimated this influence by means of an apparatus devised by Dr. Wild. It is a modification of the instrument originally employed by Dr. Brunton and Professor Cash in their well-known investigations on chemical constitution and physiological action. The excised gastrocnemius of the frog is surrounded by normal salt solution to be tested, and when it contracts from the effect of a break current the character of the contractions produced is represented in the usual way by a lever writing on the revolving drum.

It is sometimes convenient to estimate the vitality and contractile power of muscles without taking curves. For this purpose contractions are recorded on a stationary drum every

few minutes, the drum being moved a short distance between each new contraction. When a frog's gastrocnemius is surrounded by normal salt for ten to twenty minutes the curves change but little, then they fall gradually until the death of the muscle in about twenty-four hours. This diagram, like all the others, is taken by the camera from an actual tracing; for the sake of clearness only a few of the curves have been reproduced.

Below are represented the contractions of muscle in normal saline taken at regular intervals and recorded on a stationary drum for six hours. The muscle was then left in the solution all night. The small contractions at the right hand, taken twenty-four hours after, were produced by the full strength of the induction current, and simply show that the muscle was not then dead.

Fig. 1.—a Normal muscle curve immediately after preparation; β after standing one hour in normal salt solution; γ four hours after; δ twenty-four hours after.

Fig. 2.—Muscle contractions recorded on stationary drum; α to β at intervals for six hours; γ after twenty-four hours.

The influence which the addition of small quantities of alkaline and earthy metals to normal salt solution has on muscular contraction and life has been investigated by Dr. Brunton and Professors Cash and Ringer. Figs. 3 and 12 show the effect of 1 part of barium chloride and calcium chloride added to 1,000 of normal saline on muscles.

Barium chloride increases the contractile power of the muscle, and alters the shape of the curve; it causes, too, contraction, and kills the muscle in three or four hours.

Calcium increases the contractile power of the muscles, and causes contraction, but tends to maintain the vitality of muscle which lives more than twenty-four hours.

That the effect produced on the contractions is not due to increased density of the fluid surrounding the muscle is proved by the fact that 1 in 1,000 of chloride of sodium added to a normal salt solution does not in any way influence contraction or vitality.

Now if we compare these muscle tracings with those produced by the addition of 1 in 1,000 of the corresponding nitrites, we see at once that the nitrite element has a very distinct effect. In this strength of sodium nitrite solution the contractions become less strong, and in about thirty to forty minutes the muscle fails to respond to stimulation, and is dead. If we place a muscle in a 0.75 per cent. of sodium nitrite, the muscle dies in fifteen to 20 minutes.

Fig. 3.—Muscle curve from gastrocnemius of frog showing the effect of 1 in 1,000 NaNO_2 in normal salt solution; α , normal curve; β , 14 minutes after addition of nitrite; γ , 24 minutes after.

Fig. 4.—Tracing showing amplitude of contraction of muscle in 1 in 1,000 NaNO_2 solution; 1, normal contractions; 2, sodium nitrite introduced; 3, contractions 15 minutes afterwards.

When 1 in 1,000 of barium nitrite is added to the normal salt solution the contractions at first produced by stimulation have the characteristic peculiarity of barium, but not so well

marked as in the case of the chloride. But rapidly, much more rapidly than with the same quantity of chloride of barium, the contracture is annulled. The contractile power decreases, and the muscle dies in thirty minutes. If a smaller quantity of barium nitrite be used, such as 1 in 2,000 to 1 in 5,000, the barium contracture may not occur.

Fig. 5.—Tracing showing amplitude of muscle contraction in 0.75 per cent. NaNO_2 solution; 1, normal contraction; 2, 0.75 of sodium chloride solution replaced by 0.75 of sodium nitrite; 3, 15 minutes afterwards.

Fig. 6.—Tracing showing that nerve stimulation is as effective as direct stimulation of muscle when immersed in 1 in 1,000 NaNO_2 solution; 1, normal muscle contraction; 2 and 3, normal nerve contractions; 4, muscle; 5, nerve; 6, muscle; 7, nerve; 8, muscle; 9, nerve.

Fig. 7.—Barium chloride, 1 in 1,000; 1, curve with normal salt solution; 2, 15 minutes after barium chloride was added; 3, 20 minutes after; 4, 30 minutes after; 5, 45 minutes after; 6, 1 hour after; 7, 2 hours after.

Fig. 8.—Barium nitrite, 1 in 1,000; 1, normal; 2, 6 minutes after barium chloride; 3, 12 minutes after; 4, 24 minutes; 5, 27 minutes; 6, 30 minutes; 7, 33 minutes.

Fig. 9.—Barium nitrite, 1 in 5,000; 1, normal; 2, 10 minutes after barium chloride; 3, 15 minutes after; 4, 25 minutes; 5, 33 minutes.

If we compare the effects produced on muscle by the same metallic elements with ONO_2 , we note a striking difference. Whilst sodium nitrite is a poison causing the muscle quickly to lose its contractile power and vitality, sodium nitrate is in small proportions quite as favourable to the vitality of muscle as is chloride of sodium itself. A muscle in a 0.6 to 0.75 per cent. solution of sodium nitrate will contract as well and live as long as a muscle in normal saline. Strong solutions of the nitrate, like strong solutions of sodium chloride, will, of course, kill muscle quickly.

The difference between the physical properties of nitrite and nitrate solutions of the same metals of equal strengths will not account for the great differences between their physiological effects on muscle and other tissue. It must be due to the action of the acid molecules ONO and ONO_2 .

The way in which such molecules as ONO and ONO_2 produce their effects it may not be possible to determine with certainty. Yet the matter is worthy of some consideration. The specific changes which a chemical compound produces in tissues must be due to the influence, chemical or molecular, exerted on the ultimate elements of the tissue (1) by the large molecules of the compound itself, or (2) by new mole-

cules resulting from decomposition of these larger molecules. When complex molecules enter the body they not infrequently undergo decomposition, and their elements are broken up into new groups. When, however, metallic nitrites and nitrates act on muscle tissue we have no absolute evidence that any decomposition takes place. Binz, in speaking of the action of nitrites on the nervous system, has suggested that when nitrates are taken nitrous acid, HNO_2 , is set free and decomposed, HNO_3 and NO being formed. He attributes the effects of nitrites on the nerve cells to these products of decomposition, but especially to active oxygen, which he holds is formed.

There is evidence that such changes as he indicates may take place, for in the body HNO_2 is to some extent oxidised and HNO_3 formed, but there is no proof that such oxidation is necessarily the antecedent of the physiological effects on tissue, especially on muscular tissue, which nitrites produce. I have been unable to obtain any indication of the oxidation of nitrites during muscle contraction, and their influence on muscle is in no sense akin to that produced by free acid, the formation of which should precede, or at least accompany, the changes he speaks of.

It seems more probable that the influence exerted by nitrites and nitrates is due directly to the molecules of which they are composed acting on the tissue elements. It is possible for a chemical compound built up of two or more molecules to act in two ways (1) as a whole molecule, the influence being the resultant of the molecules of which it is composed, or (2) each of the molecules may divide partnership, and each may influence the tissues in its own peculiar way, the general result being the outcome, as it were, of two separate actions.

It is probable that in a large number of chemical compounds it is the whole molecule which influences the tissue elements, and it may then produce an effect which is intermediate between the action of each of its constituent molecules and allied to both, or its influence may be quite different to either of them. But there are some grounds, I think, for believing that the constituent molecules of a compound may act separately, and it seems to me the nitrites and nitrates are probably an example of such separate action. The marked individuality and uniformity of the influence of the ONO group in its various compounds, which I have pointed out and shall still further illustrate in other tissues, may, I think, be taken as an indication of this, and the difference I have noted between the action of smaller and larger amounts of some of the metallic nitrites on muscle seems to me to support the view.

If we expose a muscle to 1 in 1,000 of barium nitrite we see, as I have pointed out already, what may be called the barium effect, that is, the prolonged and heightened contraction and contracture; though distinctly less than in the case of barium chloride is still at first well marked, but it gradually disappears, and the curve becomes smaller, and the muscle dies. When, however, instead of 1 in 1,000 of barium nitrite we use a smaller amount, say 1 in 5,000, the distinct barium effect on the form of the curve is at times absent, though the muscle dies almost as quickly.

In the action of calcium nitrite the conditions are reversed.

Fig. 10.—Calcium chloride, 1 in 1,000; 1, normal curve; 2, 1 hour after; 3, 5 hours after.

Fig. 11.—Calcium nitrite, 1 in 1,000; 1, normal tracing; 2, 1 hour after calcium nitrite; 3, 2 hours; 4, 3 hours.

It will be seen from the curves on the diagram when a small quantity of calcium nitrite (1 in 5,000) is used, the peculiarities of the calcium tracing are preserved, and the vitality of the muscle is scarcely interfered with; in stronger solutions (1 in 500 to 1,000) the nitrite influence overwhelms the calcium influence. The increase in the height of curve which

calcium produces is almost annulled, and we no longer see the effect of calcium in promoting vitality; the muscle dies in four hours, whilst in a similar solution of calcium chloride good contractions can be obtained after twenty-four hours.

It seems to me that the curious difference in kind as well as in degree between the effects of small or large quantities

Fig. 12.—Calcium nitrite, 1 in 5,000; 3, normal; 2, one hour after calcium chloride; 1, 2 hours after; 4, 3 hours after; 5, 4 hours after 6, 5 hours.

of such compounds of barium and calcium on muscle tissue lends some support, though it may be but slight, to the view that the influence which they and similar compounds exert on tissue may be due to the separate and distinct action of the molecules of which the compound is built up. A difficulty in the way of accepting this view is that we have, as a consequence, to allow definite and specific powers to groups of molecules which we cannot prove actually to exist; whether the difficulty will in any way be bridged over by the theory of "ions," which in the last few years has found increasing acceptance in the chemical world remains to be seen.

THE NITRITE OR THE NITROXYL GROUP.

I turn now to the first group on the list of oxygen and nitrogen molecules, the nitrites, or ONO, or, as it is sometimes called, the nitroxyl group. I propose to draw attention to the nitrites of sodium, potassium, calcium, barium, and also to following organic nitrites: Ethyl nitrite C_2H_5ONO , isopropyl nitrite, C_3H_7ONO , isobutyl nitrite, C_4H_9ONO , and amyl nitrite $C_5H_{11}ONO$ —though I shall not of course pretend to give a detailed account of them.

GENERAL CHARACTERS.

Nitrous acid, HNO_2 , itself is not known as a definite compound in a pure state. When nitrogen trioxide, N_2O_3 , is passed into cold water nitrous acid is produced. It is formed, too, when metallic nitrites are acted on by weak acids and the organic nitrites yield it in contact with water or its vapour. It is an unstable compound at ordinary temperatures, and decomposes rapidly, nitric acid and nitric oxide being formed.

Nitrite of sodium and potassium are deliquescent salts, neutral or slightly alkaline, and very soluble in water. It is very easily decomposed by dilute acids.

The nitrites of calcium, barium, and strontium are like the alkaline nitrites in these two points.

The double nitrite of cobalt and potassium, or Fischer's salt, is very slightly soluble in water. According to Atkinson, it is more easily decomposed by acids than the other nitrites, but my experiments on this point have not accorded with his. I find it less easily decomposed than the other nitrites.

Ethyl nitrite is a volatile liquid, boiling at $62.6^\circ F$. It is insoluble in water at 32.0° , but very soluble in alcohol. When an alcoholic solution is mixed with water effervescence takes place, which is due, not to the decomposition of the ethyl nitrite, but to its separation and escape; a portion of the ethyl nitrite remaining dissolved decomposes, and the mixture at once becomes acid owing to the formation of nitrous acid. The amount of ethyl nitrite remaining in solution varies greatly. I find that when a drachm of a $2\frac{1}{2}$ per cent. solution of ethyl nitrite is added to an ounce of water at least 50 per cent. of the nitrite is at once dissipated.

Dilute acids do not immediately decompose ethyl nitrite. It is probable, however, that they hasten decomposition of nitrite in large quantities of water. The vapour of ethyl nitrite is converted into nitrous acid in the presence of watery vapour.

Propyl nitrite is a volatile liquid, boiling at 110° to $115^\circ F$. Isobutyl nitrite has a still higher boiling point— 152.6° . It is a pale yellow liquid, with a fragrant odour.

Nitrite of amyl is a yellow ethereal liquid. It is insoluble in water, and decomposed by contact with water, nitrous acid being set free, but not nearly so rapidly as ethyl nitrite.

ABSORPTION, COURSE, AND EXCRETION.

Nitrous acid decomposes very rapidly, but it is not so quickly destroyed that absorption from the stomach cannot take place when it is taken internally. When absorbed it is no doubt at once converted into an alkaline nitrite, for it can replace carbonic acid in its sodium compounds and convert normal sodium phosphate into acid phosphate, sodium nitrite being at the same time formed.

Sodium Nitrite.—When mixed with artificial gastric juice and put in an incubator at $30^\circ C$, solutions of sodium nitrite are rapidly decomposed and nitrous fumes given off, and all nitrite disappears in about twenty minutes. The same change takes place in the stomach, as is testified by the nitrous eructations which at times follow the administration of sodium nitrite. Probably some of the nitrite of sodium is absorbed as well as some of the nitrous acid produced by its partial decomposition, the relative amounts depending on the acidity of the stomach at the time or administration. Manifestly it very quickly passes from the stomach into the system, for its effects on the circulation are usually distinctly visible in from two to five minutes. A portion of the nitrite taken is excreted in the urine unchanged. I have found it after a dose of 6 grains of sodium nitrite. After smaller doses I have been unable to detect it, but the test for nitrites in the urine is not so delicate as in water, and minute quantities will in acid urine be rapidly destroyed. Whether nitrites are eliminated in the saliva and perspiration, it is not easy to say, since they are sometimes found there normally, but they can certainly be excreted by the stomach after injection into the circulation. Dr. Gamgee informs me that in a dog poisoned by the injection first of small then of large doses of sodium nitrite into the jugular vein, the contents of the stomach examined immediately after death were found by him to give clearly the nitrite reaction with the metaphenylenediamine test. The contents of the duodenum and jejunum likewise gave perceptible nitrite reaction. Probably only a small amount is thus usually excreted; the urine in the dog seemed to contain ten times as much nitrite as the stomach contents. Not a trace of nitrite could be found in the bile, which was also carefully examined. In another dog the contents of the stomach gave a strong nitrite reaction. There can be no doubt, therefore, that the stomach is capable of excreting as well as absorbing nitrites.

It is certain, however, that a considerable portion of the nitrite taken is oxydised into nitrate. Röhmann¹ recovered from the urine as nitrate 46 per cent. of the nitrite of sodium he injected into a rabbit, and in the case of a dog 36 per cent. In what manner and when nitrates are formed from nitrites we can of course only conjecture. Binz suggests that the nitrite is thus broken up.

It is possible that such a change takes place in the tissues. Yet, as I have previously said, we have no proofs that it leads to the phenomena observed when muscles are poisoned by nitrites. It certainly seems likely that the contact of unstable molecules like ONO with the ultimate elements of tissue may account for the marked and rapid effects of nitrites on the functions of some tissues. But even if the breaking up of nitrites is connected with activity of function it does not follow that the products of this decomposition are the cause of altered functions.

There seems reason for believing that the amount of nitrate and nitrite excreted does not account for all the nitrate taken, and it has been suggested that reducing agents in the body may convert part of the nitrite taken into ammonia. No proof of this has yet been given, but I find that in blood left to decompose nitrites are destroyed by the reducing agents developed during decomposition, but no trace of nitrate can be found when the nitrite has disappeared.

Ethyl Nitrite.—Experiments with an incubator and artificial gastric juice render it improbable that ethyl nitrite itself is absorbed from the stomach. If a drachm of a 3 per cent. solution of ethyl nitrite is mixed with artificial gastric juice and HCl at $38^\circ C$, nearly all the ethyl nitrite is at once dissipated, but some nitrous acid is formed. In fifteen minutes, if the flask remains corked, there is still a slight nitrite reaction, but this is due entirely to free nitrous acid. It is

¹ Zeit. f. phys. Chem., v, p. 233.

possible that some of the ethyl nitrite which is set free when a 3 per cent. solution is taken into the stomach may be absorbed, but it seems more likely that the whole of the nitrite element of the ethyl nitrite ingested is taken into the circulation as nitrous acid, and combines with sodium of the blood. Nitrous acid acts like ethyl nitrite.

I gave nitrous acid to two men, and by means of the sphygmograph recorded the effect on the circulation. The next day doses of nitrous acid, containing the same molecular equivalent of NO, were administered to them. The influence on the pulse tracing, both as regards extent and duration, was the same after the nitrous acid as after the ethyl nitrite.

When ethyl nitrite is taken internally in ordinary doses the alcohol set free is probably not in sufficient amount to exert any physiological influence.

Amyl Nitrite.—When taken into the stomach amyl nitrite is probably broken up, but more slowly than ethyl nitrite. How far any undecomposed amyl nitrite is taken into the blood we do not know. From the comparatively slight effects produced by amyl nitrite when taken internally, I am inclined to think but little is absorbed. When organic nitrites are inhaled they act much more rapidly and powerfully. They quickly reach the systemic vessels in an undecomposed state, and the fatty molecule is probably effective, as well as the nitrite molecule.

PHARMACOLOGY OF THE NITRITES.

Influence of Nitrites on Blood.—It is probable that nitrites affect more or less all the tissues of the body, but the most distinctive influence is seen on the blood and on the muscles. Dr. Gamgee, in 1868, pointed out the important fact that on the addition of nitrites to blood the colour became brown, and the spectrum altered. He found that these changes were due to the conversion of oxyhæmoglobin into what is now known as methæmoglobin, and showed that this conversion was connected with a locking up of oxygen in the new compound.

Since the presence of methæmoglobin in the blood tends to unfit this fluid for its functions, it is important to consider the conditions under which it is formed by nitrites and destroyed.

In blood outside the body, 1 part of nitrite to 500 of blood causes methæmoglobin to appear at once. With 1 in 1,000 I have found it in three or four minutes, with 1 in 10,000 after a few hours, but it is not in sufficient quantity to be detected by the spectroscope with so small an amount of nitrite as 1 in 20,000.

According to Henocque,² injection of a few centigrammes of a nitrite of sodium solution into the peritoneal cavity of an animal causes methæmoglobin to appear almost immediately, and its effects are well marked in a minute, but after subcutaneous injection not for three to twenty minutes. Given by the stomach, the blood changes are still more slowly produced.

In man, 10 to 12 grains caused in one case blueness of the lips, doubtless due to the production of methæmoglobin, but I find no record of cyanosis caused by a small single dose. To many healthy people I have given 2 to 4 grains of sodium nitrite, to a few 5 to 6 grains; to many, too, I have given 1 to 3 drachms of a 3 per cent. solution of ethyl nitrite, but I have never seen any indication that the production of methæmoglobin has followed, nor have I seen it produced by similar doses in cardiac or pulmonary dyspnoea or other ailments in which I have used the nitrites, notwithstanding that I have given 2 to 3 grains of sodium nitrite every two or three hours, and as much as 5 drachms of ethyl nitrite solution within an hour. I find, however, a case recorded in which after a drachm of nitrite of ethyl solution given every three hours in an asthmatic subject, there was an appearance of cyanosis after the fourth dose, which is said to have passed away after the drug was discontinued. It is by no means certain that the cyanosis in this case was due to the nitrite given.

Methæmoglobin in the circulating blood of living animals, unless in large quantities, is quickly disposed of. Henocque produced methæmoglobin in rabbits by injecting various quantities of sodium nitrite, and by frequent examinations of blood from the ear determined the time of its disappearance from the blood. He finds that the methæmoglobin, once produced, continues for a time vary-

ing from fifteen minutes to an hour and a-half. Doubtless, however, it often exists a much longer time. After large quantities of nitro-glycerine have been introduced into the stomach of a dog, methæmoglobin was found in the blood for two days.

Henocque thinks that the methæmoglobin may be transformed into oxyhæmoglobin in the respiratory processes. In blood outside the body methæmoglobin is not transformed into oxyhæmoglobin till putrefaction sets in, but it seems probable that the contact of methæmoglobin with living tissues causes this transference. Dr. Gamgee has sent me an interesting record of an experiment he has made which bears on this subject. He tied one aorta of a frog, and inserted into the other a cannula connected with a reservoir containing 1 part of defibrinated calves' blood and 10 of normal salt solution in which methæmoglobin had been produced by the addition of $\frac{1}{2}$ th of its volume of a 1 per cent. solution of nitrite of amyl. Into the sinus venosus he fixed a cannula, which was connected with a flattened glass tube allowing of spectroscopic examination of blood flowing through it. The heart pumped blood by which it was fed from the reservoir through this glass tube, and hence, by an india-rubber tube back into the reservoir, continuous circulation of the methæmoglobin blood through the heart thus taking place. Within an hour he found that the blood seemed brighter and the methæmoglobin band less distinct. In twelve hours these changes were still more marked. In twenty-four hours the methæmoglobin band could not be detected, only the oxyhæmoglobin bands could be seen. The heart continued to work vigorously, the beats, which under the influence of methæmoglobin blood had fallen from 8 to 5.5 in 15", having again risen to the original number. This experiment seems to show that in contact with living tissues methæmoglobin is easily converted into oxyhæmoglobin, the nitrite doubtless disappearing at the same time, and it is evident that methæmoglobin does not interfere seriously with the power of blood to nourish the heart muscle.

Outside the body sodium nitrite seems to have no effect on the form and size of the red corpuscles. From what has been stated it is manifest that we have no cause to fear the production of methæmoglobin or injury to the blood from the medicinal administration of nitrites.

INFLUENCE OF NITRITES ON SKELETAL MUSCLE.

With regard to the skeletal muscle tissue, I have already indicated the nature of this action. The presence of ONO molecules in striated muscular tissue tends to decrease the contractile power and the duration of vitality. A solution containing 0.75 of sodium nitrite kills a muscle in fifteen minutes, but at first it powerfully irritates it, causing spontaneous contractions. A weaker solution, 1 in 1,000, quickly diminishes the contractile power of muscle, and causes it to die in thirty to forty minutes. The same strength of potassium nitrite kills muscle even more quickly, for the potassium element is also injurious. Sometimes it appears as if a primary stimulation effect is produced at least in the muscle tissue of the frog. In a series of observations made in June, July, and August, this effect was a rare occurrence, whilst in another series, in April and May, it was almost always seen, and was found to commence within a few minutes after exposure of the muscle to the nitrite solution. It lasted about fifteen minutes, after which the muscle rapidly died.

A muscle exposed to 1 in 5,000 of sodium nitrite can only be made to contract for about two hours, and is so injured by a strength of 1 in 6,000 that it dies in four hours; but 1 in 20,000 seems to have hardly any toxic effect, its contractile power continuing after exposure to this strength for twenty-four hours. It is worthy of remark, however, that the alteration in the function and vitality of muscle caused by sodium nitrite decreases and passes away with great rapidity, as the nitrite in contact with the fibre is lessened in quantity or removed.

I do not intend to notice in detail the influence of the organic nitrites, for saline solution containing these compounds at once becomes acid from their decomposition. When, therefore, muscle is immersed in normal saline solution containing them, it is impossible to say how far the phenomena observed are due to the free acid or to the nitrite itself. I may point out, however, that 1 in 1,000 of amyl

² *Comp. Rendu de Biol.* v. 3.

nitrite in saline solution is fatal to a muscle in forty minutes, or in about the same length of time as sodium nitrite, but it produces a certain amount of contracture, and I found the

Fig. 13.—Effect of sodium nitrite on the separated frog's ventricle. contracture still present, even when, by the addition of a minute quantity of soda, the fluid was kept as nearly as possible neutral.

ACTION ON INVOLUNTARY MUSCLE.

On involuntary muscle, both in coldblooded and warm-blooded animals, nitrites have a powerful paralysing effect. This can be shown by perfusing alternately a pure saline solution, and one containing a small quantity (1 in 1,000) of a nitrite through the vascular system of a tortoise after decapitation. When saline is perfused a fairly equal flow is soon established, when the saline containing nitrite is passed through, the flow is considerably increased, often to the extent of 100 to 200 per cent. This increase can only be due to relaxation of the vessel walls. When the nitrite saline is replaced by pure saline, the vessels contract again, and the flow becomes less. The further perfusion of nitrite saline again increases the flow, which will again decrease with normal saline.

What part the peripheral ganglia play in the dilatation of the vessels cannot be ascertained, but there are grounds for believing that the nitrite influence exercised is largely on the muscle tissue itself. I have found 1 in 10,000 quickly double the flow through the vessels of the tortoise, and Atkinson³ says he has seen 1 in 100,000 increase the flow 16 to 18 per cent.

One in 1,000 of nitrite of sodium will usually increase the flow by from 50 to 200 per cent.

The dilatation of the vessels in warmblooded animals may be shown by passing through excised portions first pure blood and then blood containing nitrites.

When a solution containing 1 in 1,000 of sodium nitrite is passed through the kidney vessels, the increased blood flow is sometimes enormous, amounting to 200 or 300 per cent., and the same is the case when the similar solution is passed through the hind quarters of an animal recently killed. In both cases the vessels contract, and the flow diminishes when the nitrite is replaced by normal blood, and is increased again when nitrite blood is perfused.

The group ONO then seems to have a very powerful effect in causing temporary paresis of the muscular structure contained in the arterial walls, and hence dilatation of the walls. As is the case with the skeletal muscles, the effect of the nitrite ceases at once when it is removed from contact with the tissue by washing through with pure blood, and no evil effect is left behind. I believe this to be an important point to bear in mind in the therapeutic use of the nitrites. I pointed out a preliminary stimulation was not infrequently observed when the gastrocnemius of a frog was exposed to a very weak solution of sodium nitrite. I have not seen evi-

dence of such primary stimulation of the contractile tissue of the vessels under smaller quantities of the nitrites, but I have seen contractions occur in the vessels of a tortoise with a 0.75 per cent. solution, and here the subsequent perfusion of normal saline raised the flow perhaps by diluting the nitrite poison in the vessel walls.

The influence of the nitrites of potassium, barium, calcium, and strontium I have only tested on coldblooded animals. Potassium itself acts in the same direction as the nitrite group, that is, it dilates the vessels; chloride of potassium, for example, in dilute solutions always causes an increased flow through the vessels. It would be expected, therefore, that nitrite of potassium would, other things being equal, dilate the vessels of an animal more than sodium nitrite, and this, on the whole, I have found to be the case; but in barium and calcium we have metals which cause contraction of the vessels, and antagonise the influence of the group ONO. The result of this antagonism resembles somewhat that which has been described as taking place in the gastrocnemius of the frog. When relatively strong solutions of barium nitrite are used marked contraction occurs; with weaker solutions it is slight, or there may even be dilatation.

The calcium element almost antagonises the ONO group, relatively strong calcium nitrite solutions showing temporary dilatation like the barium, though it is less marked. Nitrite of ethyl is so rapidly converted into nitrous acid and alcohol when it comes in contact with water that it is impossible to determine its influence on vessels. It only dissolves in saline solution with the aid of a little alcohol, and so much acid is produced by its decomposition that 1 in 10,000 or any stronger solution causes distinct contraction of the vessels. Dilatation follows the circulation of 1 in 30,000, but this may be due to the faint acidity of the solution, since very dilute acids dilate vessels, though stronger acids contract them.

Amyl nitrite is only soluble in saline solution when a small quantity of alcohol is added, but the presence of a quantity of alcohol sufficient to dissolve 1 in 10,000 of amyl nitrite does not influence the vessels. I find that whilst 1 in 10,000 sometimes contracts and sometimes dilates vessels, a stronger solution always contracts them. A weaker solution, 1 in 20,000 to 1 in 30,000, dilates them temporarily. It is impossible to eliminate the disturbing influence of the acid which is developed when amyl nitrite comes in contact with water.

We have some evidence that nitrites act on involuntary muscular fibre in other parts in the same manner as they act on that which surrounds the vessel walls.

Mr. Cook, in some experiments made under Professor Stirling's directions in the Physiological Laboratory of the Owens College, has shown that 1 part of sodium nitrite to 1,000 of saline causes diminution of the spontaneous contraction, which naturally takes place in the circular fibres of the frog's stomach, and rapidly paralyses the muscle. Atkinson, too, finds that a 1 per cent. solution destroys the contractility of the intestinal muscle to electric current in thirty to forty minutes, but after exposure to 1 in 1,000 for three or four hours contractions could be obtained.

Atkinson has also proved that muscular tissue in the ureter dilates under the influences of nitrites of sodium. If a rabbit be killed, and placed in a hot chamber, and normal saline run through the ureter, dilatation of a marked character occurs.

ACTION ON THE HEART MUSCLE.

It can be shown that the action of nitrites on the heart muscle is much the same as on the contractile tissues of the vessels.

In the apparatus devised by Professor Roy it is possible to determine the action of a drug on the muscular tissue of the heart alone. On feeding the ventricle with a saline solution from a reservoir placed above it, it contracts after a while regularly, and continues to do so for a long time; on allowing a saline solution⁴ containing a definite quantity of a substance to be tested to pass through the ventricle, modifications in the frequency and form of the heart's contractions will indicate the pharmacological activity of the substance, so far as the

³ The Pharmacology of the Nitrites and Nitroglycerine, *Journ. of Anat. and Phys.*, vol. xxii. I desire to express my indebtedness to this most valuable paper for many of the facts set forth in these lectures. With one or two slight exceptions my experiments have entirely confirmed those of Dr. Atkinson.

⁴ By saline solution, I mean in the case of the heart a 0.6 per cent. solution of chloride of sodium, containing also a minute amount of potassium and calcium, as recommended by Dr. Ringer.

muscle wall is concerned. The tracings I give represent the average effects produced.

Now it is found that liquid containing 1 part of sodium nitrite in 1,000 of saline solution first quickens the action of the heart for two or three minutes, decreasing, however, the force of each contraction, but afterwards the heart beats more slowly as well as less powerfully, and in ten minutes stops in diastole. One in 5,000 also quickens the heart's beat for a few minutes, and slightly weakens it; then it slows it, but the heart will continue to live for two hours. One in 10,000 quickened and weakened the heart for twenty minutes, but in other ways it had very little effect.

When the nitrite is replaced by a saline solution the heart begins beating anew, and soon regains entirely, or almost entirely, its previous vigour. It is possible to start and stop the heart two or three times by alternately using a saline solution and a saline solution containing 1 part in 1,000 of nitrite. The following diagram illustrates the influence of sodium nitrite on the muscular tissue of the heart:—

Fig. 15.—Effect of barium nitrite on frog's heart, 1 in 2,000 and 1 in 1,000; 1, normal saline; 2, barium nitrite 1 in 2,000; 3, 16 minutes after; 4, 25 minutes after; 5, barium nitrite replaced by normal saline 45 minutes before; 6, barium nitrite 1 in 1,000; 7, 3 minutes after.

In no case have I found any actual increase in the force of the individual heart beats. Potassium nitrite acts like sodium nitrite, only the weakening effect is more marked.

When a molecule of calcium instead of a molecule of sodium is combined with the ONO group, a slightly different effect is produced. With 1 in 1,000 the action is much the same as with sodium nitrite of the same strength, except that the quickening is not so great. With 1 in 10,000 there was also very little quickening; the beats are squarer at the top. Calcium, as Professor Ringer has shown, prolongs the systole, and thus renders the top of the beats squarer—that is, as Professor Ringer has shown, it tends to prolong the heart's action. Here, as is usual in the lime compound with ONO, the effect of the calcium is best seen when a very delicate solution is used. One in 1,000 of barium nitrite at once shortens the height of contraction, and kills the heart rapidly.

When the heart is fed with 1 of barium nitrate in 2,000 of saline, the duration of systole is so much increased that full diastole does not occur, but soon the action of the heart is quickened and weakened. Eventually the barium nitrite acts as a poison to the heart. A tracing I show illustrates this:—

Fig. 16.—Influence of 1 in 4,000 of amyl nitrite in blood in the heart; 1, beats with normal blood; 2, blood containing 1 in 4,000 of amyl nitrite used for 48 seconds; 3, normal blood used 3 minutes.

The rapid decomposition which ethyl nitrite undergoes prevents its effects on the heart being determined, and in saline solutions even small quantities cause the fluid to

become acid. When blood is used, however, the fluid does not become acid, and the influence of amyl nitrite on the heart muscle can be determined. Fig. 16, taken with Kronecker's apparatus, shows well the influence of amyl nitrite on the heart muscle.

It will be seen that 1 in 4,000 of blood the beat of the heart is quickened; and though the height of the contractions is less, there can be little doubt that the heart does more work. When a larger quantity of amyl (1 in 2,000) is present, the contractile power of the heart is decreased, and the heart beat becomes slower. A still larger amount stops the heart in diastole.

The outcome of experiments on the heart muscle is as follows. The molecules of the ONO group in whatever combination, when present in even small quantities, tend to quicken the contractions but to weaken them. If the solutions coming in contact with the heart are very dilute, the quickening may make up for the weakened action, and the heart probably does as much, it may be more, work for a short time. Stronger solutions containing compounds of ONO, after quickening, weaken, slow, and arrest the heart's contractions. But susceptible though the heart is to the influence of nitrites, the evil influence is very evanescent if the nitrite molecules be removed.

TYPHOID FEVER AND ITS TREATMENT BY THE CARBOLIC ACID METHOD.

By R. COYLE, M.B., C.M.

On May 6th I was called to see a boy, aged 10 years, suffering from severe frontal headache, some abdominal uneasiness, and dull, aching pain in the limbs. The temperature was 100° F. I ordered the usual mineral acid mixture, with milk diet. On May 8th the symptoms were as before, only accentuated, the temperature being 102.5°. From this on I ordered antifebrin, 3 gr., every eight hours. On May 11th his temperature was 101.5° in the morning, in the evening 102.8°. The eruption was fairly well developed on the chest and abdomen; there was tympanites and marked gurgling over the right iliac region; the tongue was brown and furred. On May 12th the morning temperature was 102°, the evening 104°; there was low muttering delirium, and an appearance of complete helplessness. Previous to this I had a practitioner of wide experience to see the case; he agreed with my diagnosis and approved of my treatment.

On May 12th I began to be alarmed for my patient, and determined to try Professor Charteris's carbolie acid method. I took the temperature in the evening, and found it 104° F., and the boy was passing into what seemed to me a semi-moribund condition; the tongue was heavily coated and filthy. I gave him one of the pills¹ (2½ grs.) on that night, and one morning and evening for eight days after. On the morning of May 13th, the improvement was palpable; the lad was brighter, the tongue considerably cleaner, and the temperature 101°; the boy had slept well. In the evening the temperature was 101.5°, and there was marked general improvement. For the following days the temperature was as follows:—

	A.M.	P.M.
14th	100.0	100.5
15th	98.7	100.0
16th	98.6	99.0
17th	98.8	99.0
18th	98.0	98.0
19th	98.0	98.0
20th	98.0	97.5
21st	98.8	98.0
22nd	98.8	98.0

And so on up till present date, when the lad is convalescent and, with the exception of the weakness consequent on so severe an illness, as well as ever he was.

Indeed, I may say that from the very first the improvement was so rapid and so continuous that I was beginning to be doubtful of my diagnosis, and on May 15th I waited on my old teacher, Professor Charteris, and gave him a complete history of the case. He very kindly volunteered to visit the boy with me, and confirmed my diagnosis.

¹ The pills can now be obtained in two strengths, namely, 2½ grs. and 1 gr. of carbolie acid.