A STUDY OF THE FATE OF

CURARE,

(dextro-TUBOCURARINE CHLORIDE) IN THE BODY.

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THESIS PRESENTED FOR THE DEGREE OF PH.D.,

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FEBRUARY, 1949.

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INTRODUCTION

A characteristic feature marking the early history of curare, is the confusion about its actions, chiefly resulting from the lack of exact data in the correlation of crude curare with definite botanical sources. The art of curare-making is rapidly becoming lost, and has been displaced, at present, by a rising tide of enthusiasm for its clinical applications.

However, one of the most reliable life-saving measures in curarization, which still maintains its importance in clinical practice, was demonstrated by Brodie in 1811, who showed that artificial respiration was capable of supporting life in fully curarized animals.(20). Brodie's experiments on artificial respiration were extended by Waterton, and applied to larger animals.(116). These experiments were important, because they led, in 1814, to the use of curare in the treatment of tetanus of horses, from which stemmed the clinical use of curare in man.(87).

It was not until 1856, when the chief action of curare on the neuromuscular transmitting

apparatus/

apparatus was shown by Claude Bernard, in his experiments on the muscle-nerve preparation, which are classical examples of pharmacological demonstrations in their simplicity and final implications.(9,10). His first paper on curare appeared in 1850, and was written in conjunction with Pelouze.(8).

In analogy with Claude Bernard findings, were those of Kölliker, in 1856(77), and Kühne, in 1860(80); and lately of Steiman, in 1943, on the nerve single-muscle fibre preparation.(110). The study by Lucas, of the electrical properties of the nerve, nerve endings, and muscle, made it also possible to determine a differential action of curare on the muscle-nerve unit.(84).

It may be owing to the stimulating effects of Claude Bernard researches, that the therapeutics of curare were tried in France at an early date, by Thiercelin (112); followed by Benedict (4); and later by Lionville and Voisin (83); who tried to allay and prevent the convulsions of epilepsy in man, but their project was interrupted by the lack of a continued supply of curare.

Beigel, in 1868, was the first to employ a crystalline/

crystalline curare preparation in man, by using Preyer's "Urarin",(3,99); and his reports are of special interest, as he was among the first to describe the signs of curarization in man. Hunter, in 1878,(65); followed by Hoffmann (61); described the use of curare for the treatment of tetanus; and in the following year Offenberg (92), attempted to use it in the treatment of hydrophobia.

Then the drug fell apparently into disuse until 1931, when interest was again stimulated by West, who, together with Hartridge, were able to remove the muscular spasm of tetany in dogs, without affecting their power of locomotion.(59). It was again hoped that the lissive action of the drug may be helpful in removing the rigidity of tetanus for which condition it was tried by Cole, in 1934; by Mitchell, in 1935; and by West, in 1936; but the results as a whole were not very encouraging.(30,90,118,119). Recently, however, Edwin and Laurence in 1948, reported successful management in two cases of tetanus by intocostrin, where they also noticed that the drug was more effective than codeine in relieving the pain of muscular spasm.(40).

A/

A beneficial effect in some spastic conditions was also reported by Burman in 1939,(24); and by Schlesinger in 1946 (104), to follow the injection of curare preparations.

Current enthusiasm for the clinical application of curare is also largely due to the efforts of Bennett, a psychiatrist in Omaha, who, in 1940, popularized the use of curare to control the convulsions associated with metrazol or electric treatment, in which case the drug is described to be "tailor made" as a shock-absorber.(5). Subsequent reports by Gray, Fechner and Spradling (51); Cummins (37); Cash and Holkestra,(25); Stewart,(111); and others, have provided ample evidence of the great value of curare in convulsive shock-therapy.

The inception of curare in anaesthesia dates back to 1912, when Läwen, the surgeon, used it to produce muscle relaxation during surgical operations, (82). Then apparently nothing was recorded about the use of the drug in this field until 1942, when Griffith and Johnson of Canada described their experience with intocostrin in twenty-five cases on the suggestion of Dr. Lewis H. Wright(55).Then the application of the/

the drug as an adjuvant in anaesthesia for the attainment of profound muscular relaxation, without the use of excessively large doses of the anaesthetic, was more widely investigated, and in the following year, Cullen published careful and clearly detailed observations on a large number of cases in the States.(35). Other reports describing the usefulness of curare in anaesthetic practice include those of Brody, using sodium pentothal-nitrous-oxide oxygen anaesthesia.(21) Cole, using cyclopropane (31); Harroun, Beckert and Hathaway, using nitrous oxide (58); Shane (106); Forrester (43); Gray and Halton (52,53); and many others, using various anaesthetic agents. All workers reported the same general findings: good condition of patients during operation, expedition of the surgeons' work and low incidence of post-operative complications. By 1947, there was a world-wide interest in the clinical use of curare and a bibliography recording its effects is rapidly growing. Thus the death drug of the blowpipes has become a powerful weapon in the treatment of spastic conditions and a great addition to the art of anaesthesia.

CHEMISTRY

As was mentioned before, curare is not a pure substance, and the need for isolating a purified active principle from crude curare started as early as 1828, in South America, when Boussingault and Roulin succeeded in obtaining a bitter principle which they differentiated from strychnine isolated eight years previously by Pelletier and Caventou.(17). But apparently "curarine" or "urarine" was the first alkaloid isolated in a crystalline form by Preyer, in 1865(99).

The chemical problem was somewhat clarified by the work of Boehm, who found that in general, the containers were fairly diagnostic of the curare in them and he accordingly divided curare into three types: calabash curare; pot curare, and tube curare. They all contained active quaternary alkaloidal fraction which he called "curarine"; the pot and tube curares contained, in addition, tertiary alkaloids, which are less active and which he termed "curines". (14,15). Unfortunately, Boehm's curarine, which was not obtained by him in a crystalline form, thus being not a pure substance, has been utilized in much of the biological work performed during the last forty years.

For/

For many years, it was thought that the curare exported in the three types of containers, had been obtained and prepared from different types of plants, until Gill, in 1946, discounted this idea showing that regardless of the plants from which a given batch of curare had been prepared, the batch was simply placed in the most accessible container.(48,49).

It was Harold King, in 1935,(73), who was the first to obtain and work out the formula of the active crystalline alkaloidal salt: dextro-tubocurarine chloride, isolated from a specimen of 25g. of native tube curare provided by the museum of the Pharmaceutical Society. In subsequent publications, King directed the attention to members of the family "Menispermaceae" especially the genus "Chondrodendron" as likely sources of the drug.(74,75).

Another major contribution to the chemistry of curare was made by Pistor and Wieland in 1941,(94) and by Konz and Wieland in 1943,(78) who were able to obtain "toxiferine", the active principle of calabash curare, in a purified form. In 1943, Wintersteiner and Dutcher announced the isolation of dextro-tubocurarine chloride in a good yield from curare obtained - as King predicted - from chondrodendron tomentosum./

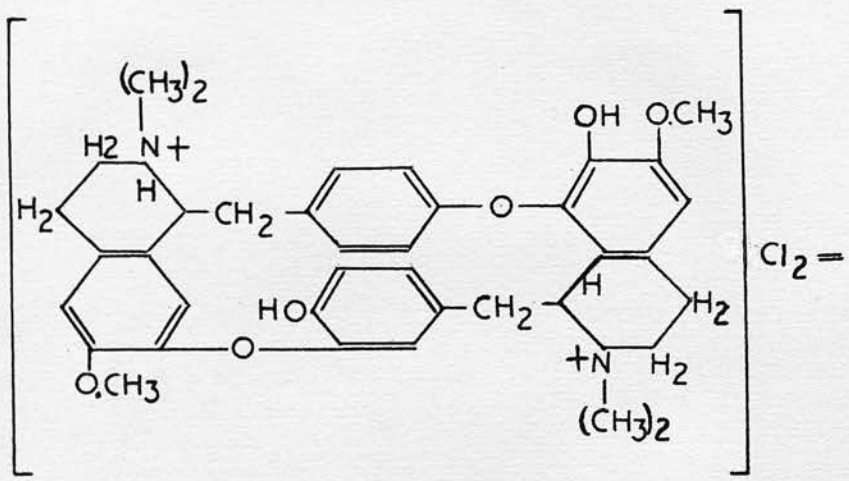

FIG. 1.

Structural formula of d-tubocurarine chloride, isolated in 1943 from chondrodendron tomentosum, by Wintersteiner and Dutcher.

tosum. Thus they obtained the alkaloid for the first time, from a single plant species, which is probably, by far, its chief botanical source. The structural formula of this alkaloidal salt is shown in Fig.I. They also extracted two new tertiary alkaloids which could be converted into physiologically active quaternary bases and noticed that methylation of the phenolic-hydroxy group of the latter compounds resulted in a 3-9 fold increase in their physiological activity.(122).

In 1939-1940, a new reliable and stable preparation of curare "intocostrin", marketed by "Squibb", was introduced in the U.S.A. as a result of the work of Gill,(47) and others. It is a purified standardized extract of crude curare containing a mixture of alkaloids of which tubocurarine is the most important and of which one unit equals the specific activity contained in 1mg. standard crude curare, and this is equivalent to 0.15mg. tubocurarine, by the head-drop method.(34,85). Clinically, however, this ratio is probably in the region of 2.5-3 units intocostrin to each 1mg. tubocurarine.(95,98).

Thus the isolation of an active alkaloid, with clinical importance, from curare would make a systematic study more feasible. Nevertheless, the fate/

fate of the drug in the body has, probably, received less attention than any other aspect of its pharmacology, and the present work is an attempt towards providing some information about this point.

A study of the fate of tubocurarine in the body would necessitate two main requirements:-

I. A suitable method of assay which should be sensitive enough to detect minute amounts of the drug, likely to be encountered.

II. A suitable method for extracting the drug from the various tissues and biological fluids, and the presentation of the final extract in a form suitable for the test.

PART I

METHODS OF ASSAY OF CURARE PREPARATIONS.

The methods of assaying curare in general are either chemical or biological.

Chemical methods:

In 1903, Barbosa published elaborate colour charts depicting the colours obtained with various curare compounds.(2). Qualitative, though non-specific, reactions for the curare alkaloids with potassium ferrocyanide, were described by Cole, in 1923 (29); and with trichloracetic acid by Schoofs in 1927 (105).

Recently, Foster and Turner (1947), have developed a tentative polarimetric method for the assay of dextro-tubocurarine chloride. They also described a colorimetric method for the assay of the alkaloid depending on the use of Folin-Ciocalteu phenol reagent. (44).

All these methods, however, must be of limited application on account of the many materials which yield a colour with the reagent, and the high concentration required to be detectable.

Furthermore, the physical and chemical data about/

about dextro-tubocurarine chloride are not sufficiently well defined to exclude contamination with active compounds and therefore the biological tests remain essential for evaluating its activity.(70).

Biological methods:

These depend either on the use of the whole animal or the isolated muscle preparation.

Whole animal:

(1) One of the early methods was used by Gaddum in 1937, and consisted in determining the quantity of the poison which would prevent the frog's body-righting reflexes from functioning for one minute within fifteen minutes of the injection as shown by the inability of the animal, when laid on its back, to recover its normal posture within one minute. The minimum quantity producing such an effect was defined as the paralysing dose. Injections were made into the ventral lymph sac of the frog, and contained in a volume of solution corresponding to 0.02c.c./g. body weight.(45).

(2) In 1941, the "Head-drop" method was developed by Holaday, which employs muscular relaxation in an intact mammal as the criterion of curare activity.

To/

To reach a head-drop dose, a rabbit is tied, belly downwards, and given an interrupted intravenous injection of a solution containing two units intocostrin/ml, at a rate of 0.1ml. every fifteen seconds, until the muscles supporting the head become sufficiently relaxed to prevent its being raised when the back is stroked.(6). In this respect, it may be noted that the head-drop dose in different rabbits, even of the same weight and sex, varies widely, and therefore it is important to compare the potency of the unknown with that of the standard on the same animal, the assay being applied as a two days cross-over test and the average head-drop dose per kg. body weight (H.D.50) determined.(63).

(3) The intravenous injection of curare in mice, likewise produces a head-drop.(72). This method, described by Kimura and Unna in 1948, is claimed to be more economical and allows the statistically valid determination of the head-drop dose on a uniform population.

In this connection, however, it may be noted that the difference between the H.D.50 and the L.D.50, represents the margin of safety of the drug, and that this margin is so narrow, that the determination of the/

the H.D.50, often results in loss of the animal.

Moreover, in a head-drop method, the results are not recorded objectively.

(4) Skinner and Young described in 1947, a "mouse-method" of assay for curare activity, as being simple and objective.(108). In this method young female mice are used, twenty-five of them are assigned, at random, to each of four dose levels, arranged in geometric progression. Then, immediately after the subcutaneous injection of the dose, in a suitable volume of fluid, the mice are placed in rotating cylinders, and the mice falling away from the cylinders during the first twenty minutes are considered reactors.

Then the proportion of mice reacting at the different dose levels are transformed into probits, and the slope of the logarithmic dosage response relationship computed in the usual manner.

(5) In 1948, Marsh and Pelletier, used a method of assay depending on comparing the paralytic doses for rats, i.e., finding the dose producing paralysis of the hind limbs for a minimum of three minutes in a group of twenty rats.(89).

The main use of any of these methods, at present, is mostly confined to the evaluation of

compounds/

compounds with curare-like activity.

Even there, they all suffer from the disadvantage that they do not establish the site of action of the drug, which is one of its most characteristic features.

Isolated muscle preparation:

1. The frog's nerve-muscle preparation:-

Here the gastrocnemius-sciatic, or the nerve-sartorius preparation of the frog (Rana temporaria), has been used.

Ing and Wright (67), found that the nerve-sartorius preparation was the more suitable, as the muscle is thinner and the time of diffusion of the drug is almost negligible.

In these cases, most authors (for reference, see Ing,1936(68), have estimated either concentrations of curare which paralysed the muscle completely, or concentrations which just failed to cause complete paralysis. After such severe poisoning, recovery is usually slow.

2. The rat's phrenic nerve diaphragm preparation:-

In 1930, Dale and Gaddum described experiments on the denervated kitten's diaphragm in their investigations/

investigations on the reactions of denervated voluntary muscle.(38).

Bülbring in 1946, tried to use the same preparation with the phrenic nerve attached, in investigating the effects of certain autonomic drugs on neuromuscular transmission. The thinner muscle of the rat's diaphragm, however, was found more suitable for this purpose.(23).

This preparation was subsequently used by Chou in 1947, in working out a method for the estimation of curare-like activity.(27). Jalon and West in 1947, found that, in this preparation, if the temperature of the bath was kept at a lower level than that used for mammalian tissue work, namely at room temperature, results were more constant.(71). West in 1948, also noticed that under these conditions, the addition of small quantities of potassium ion to the bath, enhanced recovery.(117). It may be noticed, however, that the use of this method for assay would require the presence of the test substance in relatively large amounts.

3. <u>The frog's rectus-abdominus muscle method</u>:-

The action of acetylcholine on the frog's rectus/

rectus abdominus muscle was first described in 1921, by Riesser.(100). The use of this preparation in the biological assay of acetylcholine, and in the measurement of the acetylcholine-equivalent of tissue extracts provides one of the most sensitive methods for the purpose.(26). The present method depends on a measurement of the antagonism between acetylcholine and tubocurarine, and although it is probably more sensitive than other methods, it is not specific. Antagonism between cholinergic drugs and curare was first reported by Pal in 1900.(93); and further investigated by Rothberger,(101); by Magnus,(88); by Ikeda,(66); and many others. Hori in 1927, attempted to place the eserine-curare antagonism on quantitative bases.(64).

Jacobsohn and Kahlson also demonstrated, in 1938, the anticurare effects of various substances with anticholinesterase activity.(69). In 1944, Koppanyi and Vivino (79), in their work on the decurarizing effects of the anticholinesterases showed that small doses of physostigmine would prevent paralysis and death in rabbits after a usually lethal dose of dextro-tubocurarine chloride.

The acetylcholine and related curare antagonists/

onists were also extensively investigated by Briscoe, who showed that such an antagonism is most probably due to a rise in the threshold of response to the transmitter.(18,19). Furthermore, it has been shown by Brown, Dale and Feldberg (1936), that curarine reduces the ability of intra-arterial injections of acetylcholine to cause contraction in normal muscle. (22).

However, one of the most important facts that came to be known about curare in the present time, is due to the work of Dale, Feldberg and Vogt, in 1936, who showed that when transmission of excitation from the nerve to the perfused muscle is prevented by curarine, stimulation of the motor nerve still caused the usual release of acetylcholine.(39).

Hence, there is a considerable body of evidence that the curarine-acetylcholine antagonism is brought about by curarine causing a rise in the threshold of response of voluntary muscles to acetylcholine. This rise in the threshold-response has been found by Jalon to be directly related to the dose of tubocurarine used. This was the basis of a method, developed in 1947, for the biological assay of curare preparations, using the frog's rectus abdominus muscle.(70). This method was adopted as it was found more suitable for/

for the purpose of the present work.

The bath used here for this preparation contains 2c.c. Ringer's solution at room temperature, aerated by a continuous stream of oxygen bubbles and the contractions are recorded on a slowly moving smoked drum. The magnification is about ten and the tension about 3g. weight.

A suitable fixed dose of acetylcholine is added to the bath every six minutes, and allowed to act for exactly two minutes before it is changed, and the preparation washed and allowed to relax. When the responses of the rectus muscle to acetylcholine have become quite regular, a suitable volume of the solution to be tested is added ninety seconds before the addition of acetylcholine, and its effect on subsequent responses to the latter observed.

When doses are thus added at a constant time interval, the effect produced in the given constant time is regularly related to the dose and can be taken as an index of the potency of the solution. Thus a quantitative estimate of the curariform activity of an extract is obtained by comparing it with a solution of tubocurarine, given in alternate doses.

By this procedure it was possible to detect activities corresponding to 0.1µg. tubocurarine. As it/

it made no difference to the assay whether or not the rectus had been sensitized beforehand by eserine, so the uneserinized preparation was preferred.

THE PREPARATION OF EXTRACTS

Tubocurarine is soluble in alcohol and is not inactivated by desiccation or by alkali.(34). In extracting added or injected tubocurarine from the various tissues and biological fluids, the following methods were tried.

Extraction from the blood:

In preliminary experiments on whole blood in vitro, most of the drug added, was recovered from the plasma, by the use of acid alcohol and none from the blood cells. After injection, extraction of the drug was tried in the following way:

The blood was drawn on heparin over ice and the plasma immediately separated. The acid alcohol - prepared by acidifying absolute ethyl alcohol with a crystal of tartaric acid or with 0.1c.c. normal hydrochloric acid - is used in a dose of 10-15ml. acidified alcohol for each 1ml. plasma.

The plasma was then added to the acidified alcohol dropwise, shaking thoroughly after each addition. The precipitate was separated and washed with acid alcohol and the alcoholic solution taken down to dryness, and the residue dissolved in Ringer's solution/

ion and filtered.

Extraction from the urine:

A certain volume of the urine was evaporated to dryness on the water bath. The solid residue was then thoroughly mixed with absolute ethyl alcohol, 5ml. for each 1ml. urine, and the precipitate separated by centrifuge and washed with same absolute alcohol.

The alcoholic solution was then evaporated to dryness, and the final residue taken up in Ringer's solution, 1-10ml. for each 10ml. urine; thus the tubocurarine in the urine could be concentrated 1-10 times.

Extraction from the tissue:

In extracting the drug from the tissues, several methods were tried. A convenient one was found to depend on extraction with acid-alcohol, and here the use of sulphuric acid as an acidifying agent was found, in most cases, superior to hydrochloric acid in providing a final clear extract. This was observed by Chang and Gaddum in 1933, when they were estimating the acetylcholine equivalent of tissue-extracts.(26). This method extracted the acetylcholine equivalent of the tissues, along with their tubocurarine content, and acetylcholine was present in such quantities as to interfere with the test and mask the curariform activity/

activity of the extract; but it was finally removed from the extract by hydrolysis.

It may also be noticed that extracts prepared in this way may contain other pharmacologically active substances, and are only therefore suitable for use in a biological test when the physiological reaction used in this test is one which is relatively little affected by these substances. This is another advantage of the frog's rectus abdominus muscle in this respect, since such substances are without apparent effects on this preparation.(26).

Extraction of the drug from the tissues was then tried in the following way:

The tissue was weighed, cut up with scissors and mixed with acidified alcohol,(15-20c.c. per g.tissue) where its cutting up and mixing was completed. This acidified alcohol was prepared by adding 1.2ml. 2N. H_2SO_4 to each 100ml, of absolute ethyl alcohol. The deposit was then separated by centrifuge and washed with acid alcohol, and the alcoholic solutions evaporated to dryness and taken up in Ringer's solution and filtered. The extract was then made slightly alkaline (pH 8.3) and boiled for one to two minutes to destroy its acetylcholine, but not tubocurarine. It was neutralised and concentrated until 1c.c. corresponded/

sponded to 1-5g. tissue.

Extraction from other biological materials:

Faeces: In some animal experiments, it was desired to look for the presence of the drug in the faeces. Here the masses were powdered and a weighed quantity transferred to a dry clean mortar and absolute ethyl alcohol - 10ml. per g. - added to the contents of the mortar and mixed thoroughly. Then the deposit was separated and washed with alcohol, and the alcoholic solutions finally taken down to dryness and the residue dissolved in Ringer's solution and filtered.

Gastric juice: In the conscious human subject, excretion of the drug was sought for in the saliva and gastric juice. The fasting gastric juice aspirated through a Ryle's tube was well shaken and filtered. The filtrate was heated on the flame and the coagulum separated. Then the clear fluid neutralised and used for the test.

Saliva: This was collected after the mouth had been thoroughly cleaned and rinsed. To each 9ml. absolute alcohol, acidified by a few drops of dilute HCl. 1c.c. saliva was added, drop by drop, shaking thoroughly after each addition and for sufficient time/

time at the end. The thin precipitate was separated and washed with acid alcohol. The alcoholic solutions were evaporated to dryness and the residue taken up in Ringer's solution and filtered.

It may also be mentioned that, in the case of biological fluids, a control sample of the fluid was always obtained before the injection of the drug was made and was extracted and examined in the same way as the post injection samples.

When the samples, obtained after the injection, showed measurable curariform activities, this usually decreased as the time interval after which the sample had been obtained, became longer until it faded away approaching the blank control. Such an activity was assumed to be most probably due to the presence of the drug - in some form - in the corresponding fluid. As for the tissues, a control extract was prepared from the corresponding tissue of a control animal, chosen preferably of the same sex, and as near as possible, of the same weight. The control biological fluids and tissue extracts thus obtained were devoid of curariform activity. All extracts were, if necessary, made neutral before they were used in the test.

RESULTS

In order to test the accuracy of the methods used, the recovery of known amounts of dextro-tubocurarine chloride added to the various tissues and biological fluids, was tried. The drug is often referred to here as "tubocurarine" or abbreviated as "d.T.C". Table I, shows that the recoveries of known amounts added to blood, urine and saliva, were satisfactory.

Table I

Recoveries of tubocurarine added to biological fluids.

Biological fluid.	Tubocurarine concentration: µg./ml.		Per cent loss
	Added	Recovered	
Human blood	2.0	1.80	+10
	1.0	0.90	+10
	1.0	1.0	00
Rabbit's blood	2.0	1.9	+5
	1.0	0.85	+15
	1.0	1.12	-12
			Mean+4.6
Human urine	2.0	1.9	+5
	1.0	1.0	00
Rabbit's urine	2.0	1.90	+5
	1.0	1.15	-15
Rat's urine	1.0	1.0	00
	1.0	0.90	+10
			Mean+0.83
Human saliva	2.0	2.10	-5
	1.0	0.85	+15
	1.0	0.85	+15
			Mean+8.3

In/

In Table II, the recoveries of known amounts added to the various tissues are represented.

Table II

Recoveries of tubocurarine added to tissues.

Tissue	Tubocurarine concentration: µg./g.		Per cent loss
	Added	Recovered	
Minced mouse	1.0	1.10	-10
	1.0	1.05	-5
	0.5	0.45	+10
Rabbit's liver	1.0	1.07	-7
	1.0	0.9	+10
Rabbit's muscle	0.5	0.45	+10
	0.5	0.4	+20
Rabbit's kidney	0.5	0.45	+10
	0.4	0.35	+12.5
			Mean+5.6

Blood levels and volumes of distribution of tubocurarine in man.

In these experiments, samples were obtained from anaesthetized patients undergoing surgical operations, and from the conscious unanaesthetized subject.

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Blood levels and volumes of distribution of tubocurarine in man.

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In the first case, the patient of choice was one/

one undergoing a surgical operation of a relatively short duration, thus requiring not more than a single dose of the drug. Intravenous anaesthesia was avoided, and the patients were kept under cyclopropane-oxygen anaesthesia.

A study of the fate of the drug in man was made by determining the blood levels of the drug at various intervals following its intravenous administration; by determining the amount excreted in the urine and, in the conscious subject, by detecting and estimating the drug in some other accessible biological fluids: i.e., the saliva, gastric juice and cerebro-spinal fluid.

Experiments on patients:

In the surgical cases, control samples of blood and urine were always drawn, on the operation table, during the second plane of anaesthesia, and just before the administration of tubocurarine. Then the drug: "Tubarine,"B.W"., 0.2mg./kg. was injected intravenously.

Blood samples were drawn on the third, fifteenth and thirtieth minute following the injection. Whole urine specimens were collected at hourly intervals after the injection, the pre-injection and first post/

post injection samples were usually drawn by a sterile rubber catheter. All samples were extracted and examined for their curariform activity and the concentrations of the drug estimated.

The various blood concentrations and volumes of distribution of the drug in the human subjects, (4 anaesthetized patients:A,B,C,D; and 1 conscious subject:E) at the specified intervals are given in Table III.

TABLE III

The blood concentrations and volumes of distribution of tubocurarine in human subjects, following the intravenous injection of 0.2mg./kg.

Subject	Case	Tubocurarine concentration µg./ml. of plasma.		
		3 min.	15 min.	30 min.
A	Appendicectomy	4.2	2.6	0.8
B	Ulnar nerve suture	3.5	2.8	1.0
C	Laparotomy	4.6	2.4	1.2
D	Laparotomy	3.2	2.2	0.9
E	(Conscious)	4.5	3.0	1.1
Mean concentration		4.0	2.6	1.0
Log. $\frac{\text{mean concentration}}{\text{Dose (mg./kg.)}}$		1.30	1.114	0.70
Volume of distribution, l/100kg.		5	7.7	20.

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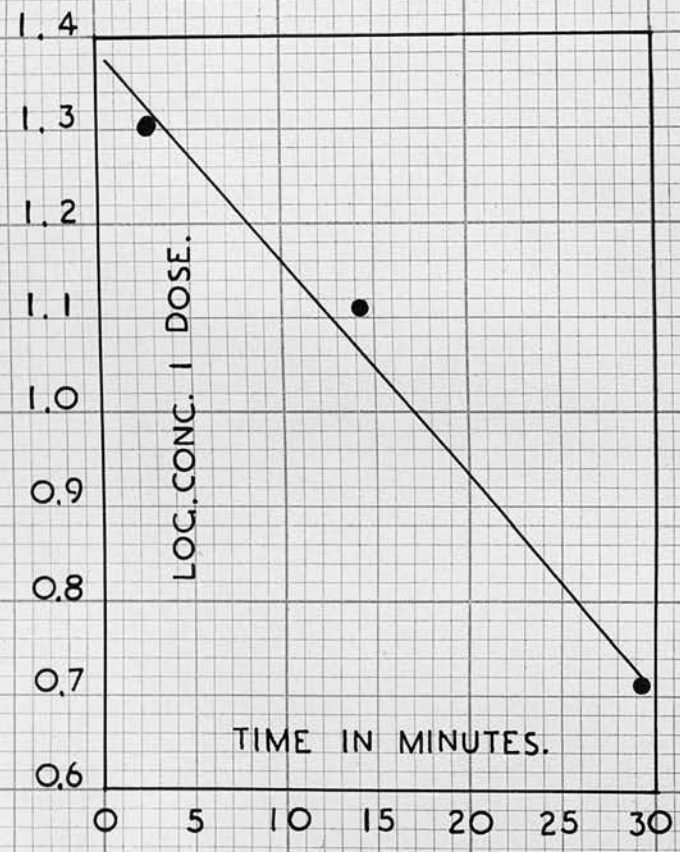

FIGURE - 2.

THE CONCENTRATION DOSE RELATIONSHIP AT VARIOUS INTERVALS FOLLOWING THE I.V. INJECTION OF TUBOCURARINE IN MAN.

The relation between the dose of the drug and its average concentrations in the blood, at various times following the intravenous administration is shown in Fig.II.

The ordinate corresponding to zero time is 1.38 or log.24. The volume of immediate distribution is thus estimated as 100/24 or 4.2% which is probably about equal to the plasma volume. This may also show that the drug probably does not pass into the blood corpuscles.

Experiments on the conscious subject:

Experiments were conducted on a 75kg. healthy medical man (self), not undergoing operation and with no concurrent pathological history with the object of:

1. Observing the onset and nature of the clinical symptoms of curarization in man.
2. Attempting to correlate this clinical symptomatology with the blood levels of the drug and its urinary excretion.

Two experiments were performed:

Experiment I:

Here the drug: Tubarine, "B.W." was injected in a small single dose, 7.5mg. slowly intravenously over a period of two minutes. Within one minute from the beginning of the injection, i.e., after/

after about 3.5 mg. were injected. There were definite myasthenic symptoms and descending paralysis involving the eyelids, face, neck muscles and the extremities. The movements of the eyes became sluggish and the gaze tended to be fixed. There was also some heaviness of the tongue, with difficulty of speech and harshness of voice. Although these symptoms were increasing in severity, and towards the end of the injection were actually alarming, no respiratory difficulty was complained of. In this connection it may be noted that Gray and Halton, in 1948 (54), noticed the development of such severe symptoms in a non myasthenic case following the injection of a small dose, 2mg. of tubocurarine. Thus it appears that the occurrence of alarming myasthenic symptoms following the injection of small doses of the drug, is not necessarily a specific reaction of the myasthenic, and that the advocated use of small doses of the drug as a specific diagnostic test for myasthenia gravis (7), should be regarded with caution.

Experiment II:

On a later occasion, the drug (15mg. Tubarine,"B.W".) was injected into the same subject intravenously in a few seconds. The various steps/

steps of this experiment are illustrated in Table IV.

TABLE IV

Exp.II. On the conscious subject

TIME	10a.m.	10.20	10.25	10.27 Omin.	0.5	3.0	8.0	10.0	15.0	21.0	23.0	27.0	30	33	43	11.27 60	15 27
PULSE			78			90	88	88	86		86	80			80		
RESP.				N	A.	A.	A.	A.	A.	N.							
B.P. S/D			145/90			165/100	165/100	160/95	156/90		147/90				147/90		
REMARKS	I	II		III	IV	V	VI		VII	VIII			IX	X	XI	XII	XIII

I. Ryle's tube swallowed and fasting juice aspirated.
II. Control blood and urine samples collected.
III. d-Tubocurarine chloride 15mg. I.V. in 5 seconds.
IV. Oxygen assisted respiration, (subject V. distressed and convulsing.)
V. Blood sample collected.
VI. Sweating of brow.
VII. Blood sample collected.
VIII. Specimen of saliva.
IX. Blood sample collected.
X. Lumbar puncture: cerebro-spinal-fluid drawn.
XI. Ryle's tube swallowed and gastric juice aspirated.
XII.-XIII. Urine samples collected at hourly intervals.
N: Normal respiration. A: Artificial, with O_2 under positive pressure.

A means of communication had been established beforehand between the subject and the operators, since paralysis of the tongue muscles was almost certainly expected.

For a period of not less than half a minute after the injection, muscular power was almost full and no symptoms were at all experienced, then the subject was suddenly caught by a severe asthmatic attack.

At this stage, muscular power was apparently still full, because the subject was able to move his arm pointing with his palm to the face, asking for the oxygen mask. The mask was put on, and artificial respiration conducted with oxygen under positive pressure.

Despite this artificial aid to the respiration, the respiratory embarrassment maintained its severity for about three minutes during which the subject was thrown into occasional convulsions, probably anoxaemic. The condition, however, was not painful but was most distressing (see the anaesthetist report: Remark IV, Table IV: subject very distressed and convulsing).

Shortly afterwards, he was lying flaccid with full ptosis, and muscular paralysis. The tongue did not fall to the back of the throat and the upper airways were not plugged with mucous.

Consciousness/

Consciousness was not lost, and the memory was not clouded.

The colour of the skin was normal or slightly pinkish, and there were no evident changes in the sensations. There was profuse sweating of the brow.

The pulse was accelerated and the blood pressure slightly raised during the fifteen minutes following the injection, then gradually came down to the original level.

The subject was starved for seventeen hours before the experiment; and all the control samples were collected before the administration of the drug. Following the injection, blood samples were drawn on the third, fifteenth, and thirtieth minute.

The saliva sample was collected on the twenty-first minute.

The spinal canal was tapped and a sample of cerebro-spinal-fluid drawn thirty-three minutes after the injection; and ten minutes later, a specimen of the fasting gastric juice was aspirated.

Result of assay:

The drug was detected in the saliva in a concentration of 1.2µg. per ml.

Thirty-three minutes after the injection, the concentration/

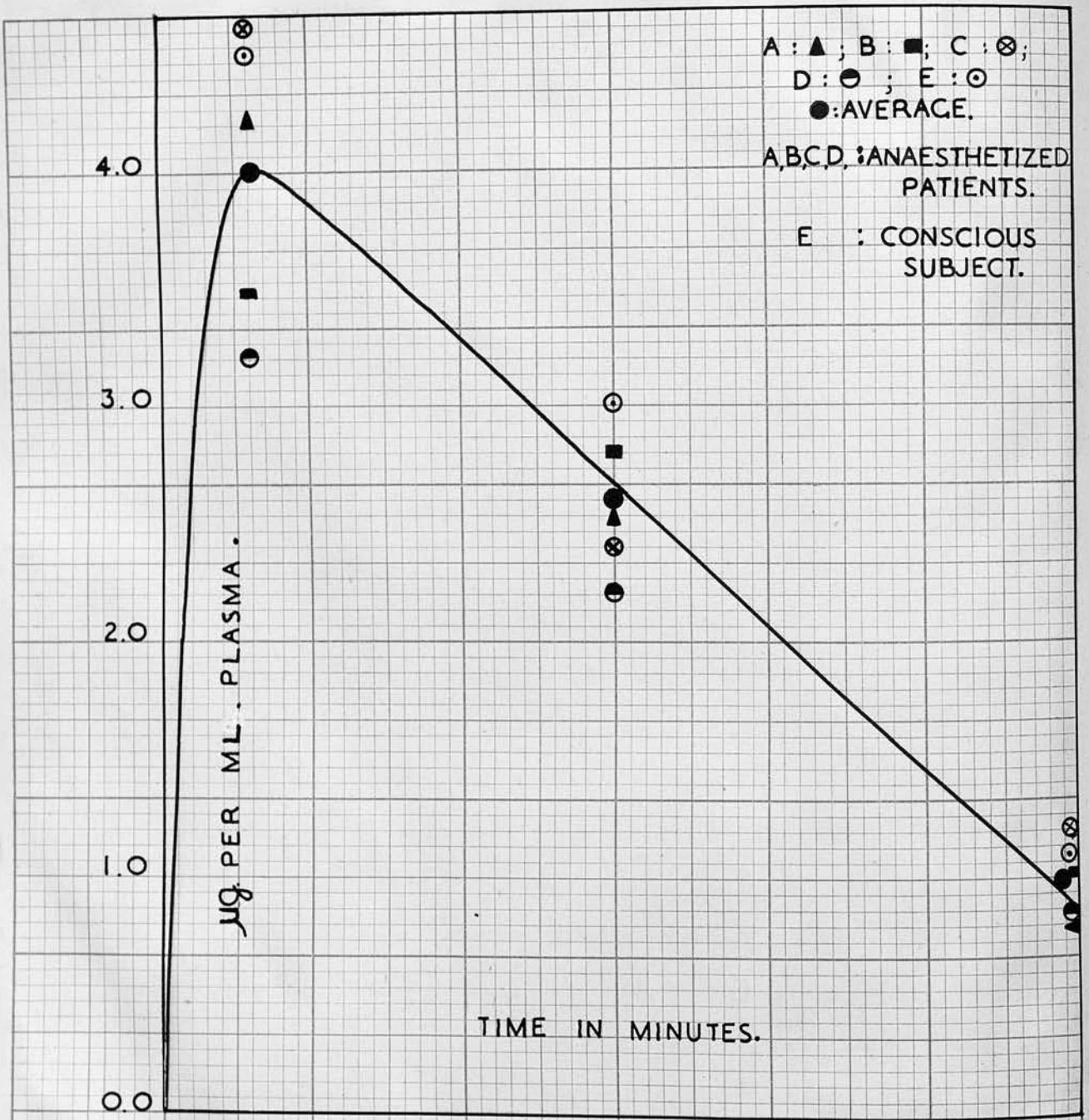

FIGURE-3:-

AVERAGE BLOOD CONCENTRATIONS OF TUBOCURARINE IN MAN FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 0·2 MG./KG.

concentration of the drug in the C.S.F. was 2.5µg./ml.

About 12% of the injected dose was recovered from the gastric juice. This amount is probably going to the waste, as it was shown, in later experiments, that about 25mg./kg. given by stomach tube to rats is generally ineffective.

In Fig.III, the average levels of the drug in the blood of anaesthetized and conscious human subjects, following the intravenous administration of 0.2mg./kg. are presented.

From this curve, it may be noticed that:

1. A concentration of about 4µg. per ml. of plasma, occurring three minutes after the intravenous injection, seems desirable for the production of full muscular paralysis, providing adequate relaxation for surgical procedure.

   This corresponds in the conscious subject to the complete classical picture of curarization.

2. Fifteen minutes after the injection, when the muscles begin to regain their tone, and in the conscious subject their power as evidenced by the improvement of ptosis and the ability to move a limb slightly and use the tongue, the corresponding concentration is about 2.6µg. per ml. plasma.

3. Half/

3. Half an hour after the injection, where there was apparent recovery, the conscious subject being able to sit up and talk - though still feeling weak - a level of about 1.0μg. per ml. plasma was reached.

It may be interesting to note that at this stage, or even one hour after the injection, where the freedom from the symptoms was almost complete, the drug was still being excreted and continued to be excreted in the urine during the following three hours.

The renal excretion of intravenously administered tubocurarine in man.

The tubocurarine-equivalents of human urine following the intravenous injection of the drug, (0.2mg./kg.) in five subjects are shown in Table V.

TABLE V/

TABLE V

The renal excretion of intravenous tubocurarine (0.2mg./kg.) in human subjects.

Subject	Dose mg.	Tubocurarine equivalent of Urine: mg.					Total excretion as % of dose given
		1st hour	2nd hour	3rd hour	Next 3 hours	Total	
A	14	2.82	1.24	0.50	-	4.56	32.6
B	15	2.48	1.82	0.72	0.3	5.32	35.5
C	12	2.20	1.24	0.62	-	4.06	33.8
D	15	2.6	2.02	1.50	-	6.12	40.8
E	15	3.0	1.4	1.0	-	5.4	36.0

From the examination of the human urinary findings, it may be seen that:

1. The renal excretion of the drug is relatively "slow". It could be detected three hours and sometimes four hours after its intravenous administration.

   This might explain the common observation of the anaesthetist, that if he has to give a second dose of tubocurarine during a lengthy operation, he usually requires a smaller dose to produce a full/

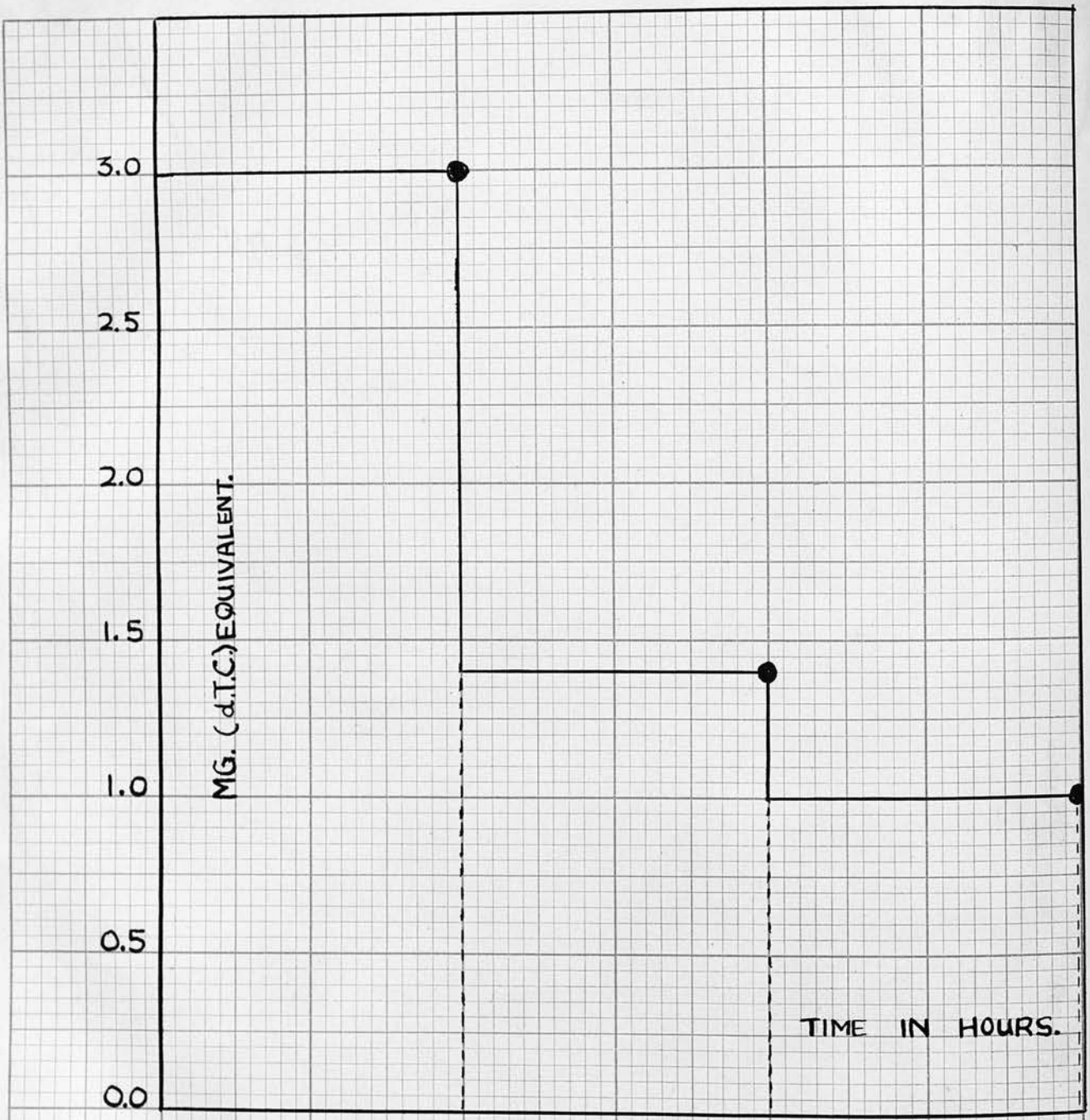

FIGURE-4-

THE URINARY EXCRETION OF 15 MG. d-TUBOCURARINE CHLORIDE (d.T.C.) GIVEN INTRAVENOUSLY TO NORMAL SUBJECT. (EXP. II.)

full effect; some of the previous dose is probably still in the system.

In the conscious subject, the symptoms of curarization practically disappeared about two hours before the renal excretion of the drug was over.

2. The total amount excreted by the kidneys varied between 32%-40% of the intravenous dose given.

Fig.IV is a graphic representation showing the renal excretion of the intravenously administered tubocurarine in the normal conscious subject.

Urine-histamine following the intravenous injection of tubocurarine.

The purpose of the following experiment, done on an asthmatic patient, was:

1. To find out whether the histamine, known to be liberated as a result of the intravenous injection of tubocurarine (56,81), would precipitate an attack of asthma in a sensitive individual.
2. To test if there would be any increase in the histamine content of the urine, following upon such an injection.

The patient, a male of 45 years old, has been suffering from asthma since 1942. His attacks are readily removed by adrenaline.

Here, the drug: Tubarine "B.W". was injected very slowly intravenously, 10mg. given over a period of three minutes. Although there was definite ptosis, difficulty of speech with sweating and flushing of the cheeks, no subjective attack of asthma was complained of.

Result of assays:

Urine-/

Result of assays:

Urine-tubocurarine: Total excretion was equivalent to 40% of the dose given.

Urine-histamine: No increase in the histamine content of the urine was detectable in the first three hourly specimens, or in the twenty-four hours pooled sample.

The fate of tubocurarine in the rabbit.

The fate of tubocurarine in the rabbit was studied by determining the blood concentrations at various intervals after the injection. The renal excretion of the drug was also estimated, and the distribution of the drug between the various organs examined.

Blood levels:

The drug was administered intravenously in a single dose of 0.12mg./kg. body weight. A control blood sample was usually collected before the injection. On the second, tenth and fifteenth minutes after the injection samples were collected, as usual, in heparin over ice, the plasma separated and extracted, and its activity estimated. Six rabbits of both sexes were used. Fig.V represents the average concentrations of the drug in the rabbits' blood following such an injection.

From this curve, it may be noticed that:

1. Two minutes after the injection, and while the animal was usually still in a head-drop position, the corresponding blood level was about 2.2µg. per/

per ml. of plasma.

2. Ten minutes after the injection, when there was apparent recovery, the concentration of the drug in the blood was about 1.5µg. per ml. of plasma.

3. Fifteen minutes after the injection, the corresponding blood level was about 1µg. per ml. of plasma.

In Table VI, the blood concentrations and volumes of distribution, at the stated intervals following the intravenous administration of the drug are presented.

TABLE VI./

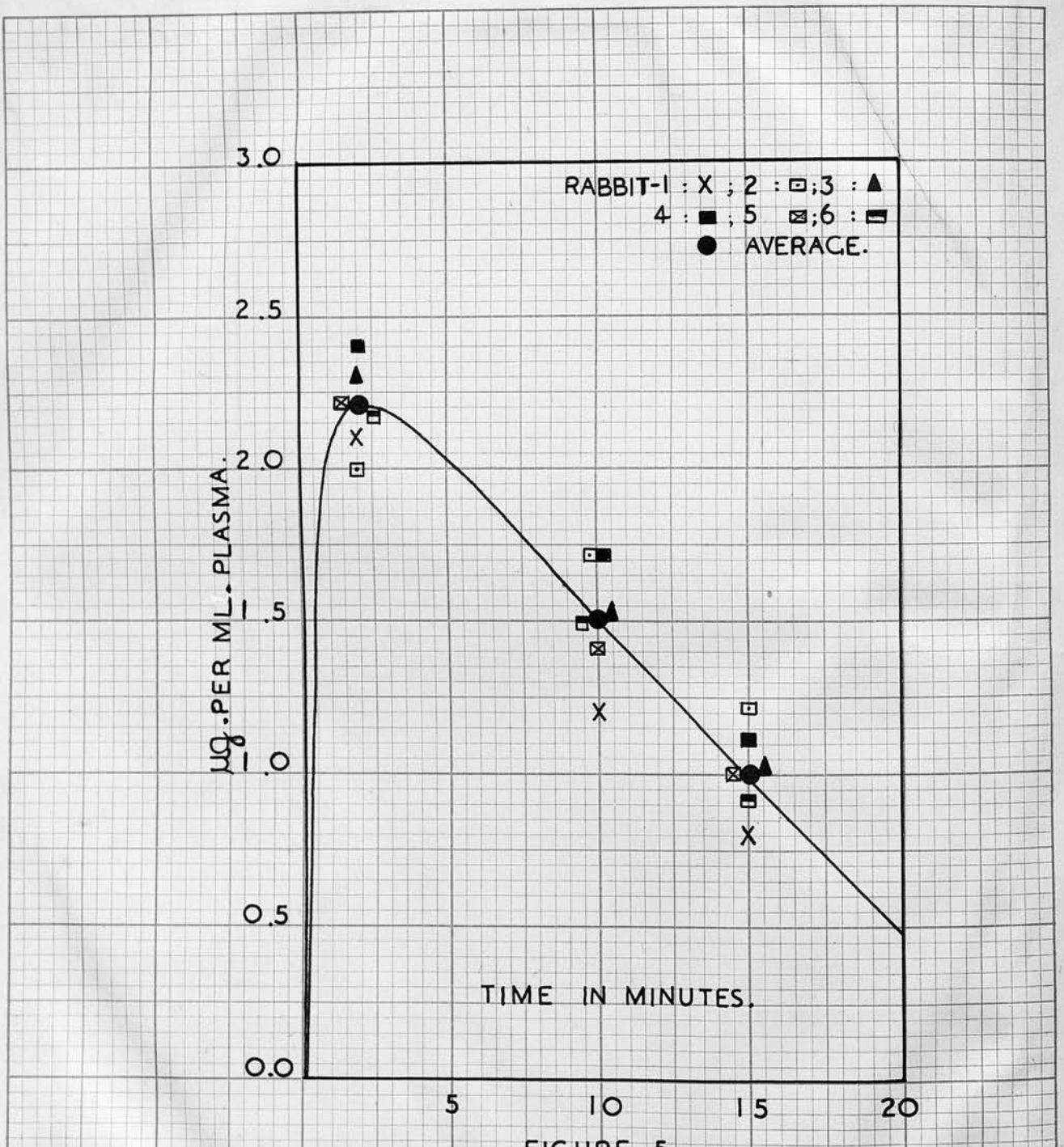

FIGURE-5.

THE AVERAGE CONCENTRATIONS

OF TUBOCURARINE IN RABBITS' BLOOD

AFTER THE INTRAVENOUS ADMINISTRATION OF 0·12 MG./KG.

TABLE VI.

The blood concentrations and volumes of distribution of tubocurarine in rabbits following the intravenous injection of 0.12mg./kg.

Rabbit		Tubocurarine concentration µg./ml. of plasma.		
No.	Weight Kg.	2 min.	10 min.	15 min.
1	2	2.1	1.2	0.8
2	2.4	2.0	1.7	1.2
3	2.	2.3	1.5	1.0
4	2.5	2.4	1.7	1.1
5	2.8	2.2	1.4	1.0
6	2.8	2.2	1.5	0.9
Mean concentration		2.2	1.5	1.0
Log. $\frac{\text{mean concentration}}{\text{Dose (mg./kg.)}}$		1.262	1.097	0.92
Volume of distribution. ℓ/100kg.		5.4	8.0	12.0

Fig.VI, represents the relationship between time and the log. of the ratio: $\frac{\text{mean conc. in plasma}}{\text{dose: mg./kg.}}$, following the intravenous administration of the drug.

It/

It may also be noticed that, as in the case of man, the volume of distribution - theoretically calculated from the curve at zero time - corresponds approximately to the normal volume of plasma. (1.33=log. 21.4, and $\frac{100}{21.4}$ = 4.7). The drug disappears from the plasma exporentially, with a halving time of about thirteen minutes.

Renal excretion:

The urine samples were usually collected by a sterile rubber catheter, at the end of the second, fourth and seventh hour following the intravenous administration of the drug in a dose of 0.12 mg./kg. body weight.

All samples were extracted and examined for their curariform activity. It was noticed that samples, collected at the end of the seventh hour, as well as the control samples, were devoid of such an activity.

It was also noticed that the amount excreted was usually greatest in the specimen from the first two hours and that the total amount excreted by the kidneys was between 29%-39% of the dose administered.

In Table VII, the tubocurarine equivalents

of/

of rabbits' urine, following the intravenous administration of the drug, are given.

TABLE VII/

TABLE VII

The renal excretion of intravenous tubocurarine (0.12mg./kg.) in the rabbit.

Rabbits		Tubocurarine injected	2 hours		4 hours		7 hours		Total amount excreted	% of total amount administered
No.	Weight kg.	mg.	Urine volume c.c.	Tubocurarine equiv. mg.	Tubocurarine equiv. mg.	Urine volume c.c.	Urine volume c.c.	tubocurarine equiv. mg.	mg.	
1	2	0.24	5.2	0.057	.027	3.7	2.9	-	0.084	35
2	2.4	0.288	7.5	.08	.034	3.1	-	-	.114	39.5
3	2	0.24	4.5	.043	.027	5.1	-	-	0.07	29.2
4	2.5	0.30	3.5	.097	.009	3.0	6.2	-	0.106	35.3

The distribution of injected tubocurarine in rabbits' tissues:-

The distribution of the drug in the rabbit's tissues was examined in two rabbits of different sex and of equal body weight.

Ten minutes following the intravenous administration of 0.17mg. per kg. body weight, the animals were killed by stunning and bleeding and their organs removed and extracted by the method mentioned previously.

Extracts of brain, kidneys, liver and voluntary muscles were examined for their curariform activities and the results are shown in Table VIII.

TABLE VIII.

The tubocurarine-equivalent of rabbits' tissue extracts ten minutes following the intravenous administration of 0.17mg./kg.

Tissue	Voluntary muscle	Brain	Kidneys	Liver	Rabbit
Tubocurarine equivalent (µg./g.)	0.16 0.15	0.12 0.15	1.6 2.5	0.10 0.13	(2Kg) I ♂ II ♀ "
Total equivalent of organ(µg.)	144 135	1.27 1.50	25.6 35.0	9.5 11.44	I II
% of dose in organ.	42.35 39.70	0.37 0.44	7.52 10.29	2.79 3.36	I II
Average % of dose in organ	41.03	0.41	8.91	3.08	

It was noticed that the distribution of the drug/

drug in the organs seemed to be uniform except for the kidneys. These showed the highest concentration of the drug-equivalent, per gramme tissue. In one case this was 1.6µg. per g., and in the other 2.5µg./g.

The average total tubocurarine-equivalent of the kidneys was about 8.9% of the dose given.

In one rabbit (I), the muscle extract was prepared from a specimen of voluntary muscles taken from the neck muscles; and in the other rabbit (II) it was prepared from a specimen taken from the thigh muscles. No appreciable difference, between the concentrations of the drug in the two extracts, was noticed.

The calculation of the total tubocurarine-equivalent of voluntary muscles was made on the assumption that they constitute 45% of the total body weight. The high percentage of the dose-equivalent present in the voluntary muscles, about 41%, is therefore largely due to the total weight of the whole muscular system. It is possible however, that the amounts present in the organs are less than the amounts shown, due to the inevitable presence of plasma in these organs.

Similar extracts of the same organs of the control/

control, non injected animal did not show any curariform activity.

The absorption and fate of ingested tubocurarine in the rat.

It has long been believed that curare taken by mouth is innocuous, either because it is not absorbed from the gastro intestinal tract, or because it is destroyed there, or because it is excreted as quickly as it is absorbed, so that an effective blood level is not easily reached.

However, as far as tubocurarine is concerned, it was found that:

1. In the rat, the administration of a dose of less than 30mg. per kg. body weight, is usually without any apparent effects on the animal, and the presence of the drug in the urine or faeces of such animals could not be demonstrated.
2. The ingestion of 30-40 mg. per kg. body weight resulted in obvious paralytic signs of variable severity.

   While the faeces of such animals were free from the drug, the urine (24hours) contained only about 0.1-0.2% of the dose given.
3. The administration of a dose over 40mg. per kg. body/

body weight, resulted invariably in a typical picture of curare paralysis of progressive severity, ending in death.

The drug in these cases was administered by a stomach tube, after the animal had been starved over a night. Animals of both sexes, weighing between 200-280g. were used and the results are in Table IX.

TABLE IX.

The fate of tubocurarine given by stomach tube to rats.

Dose mg./kg.	No. of rats	Effects	Urine	Faeces
10	5	None seen.	Nil	Nil
25	5	" "	"	"
30	2	variable paralysis.	0.15%	Nil
35	5	" "	0.1%	"
40	3	severe paralysis.	0.2%	"
42	5	severe paralysis and death.	-	-
45	3	" "	-	-

From these experiments, it became clear that the/

the drug is not innocuous when given by mouth, and that absorption does occur from the gastro-intestinal tract.

In an attempt to localise the site of absorption of the drug from the gastro-intestinal tract, the following experiments were arranged:-

Ligation of the duodeno-pyloric junction:

Group I Rats:

a. Here, the animal was starved over night, and in the morning a median laparotomy incision was made under ether anaesthesia and the duodeno-pyloric junction secured and tied.

b. Then a stomach tube was passed through the mouth - with the animal still under the anaesthesia - and tubocurarine injected through the tube into the stomach.

c. The abdominal incision was then quickly stitched up, and the animal allowed to recover from the anaesthesia.

It was noticed then that the presence of large amounts of the drug (50-100-120mg. per kg. body weight) introduced into the stomach in this way, was without any obvious effects on the animal for a period of two hours, after which the animal was painlessly killed./

killed. (After such an operation, animals were not allowed to live more than two hours: condition attached to grant of Cert.B). Two animals were put on each of the lower doses and four on the higher dose. The weight of these rats ranged between 200-260g. The observation that the presence of large amounts of the drug in the stomach, was without any visible effects would be due to the fact that the drug is either not absorbed from the stomach or that it is destroyed there.

When the animal was killed, two hours after the operation, and the stomach contents examined, practically all the amount introduced was recovered from there. The drug was not absorbed by the gastric mucosa.

Group II Rats:

a. The animal here was also starved over night, and in the morning a median abdominal incision made under ether anaesthesia and the duodeno-pyloric junction secured.

b. Then a stomach tube was passed through the mouth, and manipulated from the abdominal wound into the duodenum.

The tube was then kept in position by a loose/

loose loop placed around the duodeno-pyloric junction.

c. The drug was then introduced into the small intestine by injecting it through the stomach tube. Then the latter was carefully withdrawn while the loose loop was tightened around the duodeno-pyloric junction.

d. The laparotomy incision was then quickly stitched up and the animal allowed to recover from the anaesthesia.

The injection of the drug by a needle directly into the stomach or intestinal lumen - through the abdominal wound - was avoided lest the drug got absorbed through the puncture caused by the needle.

On recovery from the anaesthesia these rats were observed to pass quickly into a typical condition of curare paralysis of variable severity. In six rats, weighing between 200-250g. when the dose of tubocurarine left inside the intestine was over 3mg./kg. body weight, (i.e., 3.5mg./kg. in four rats and 4mg./kg. in two rats), this curare paralysis was very severe, and progressed to complete respiratory arrest. Death in these cases usually followed in 3-8 minutes after/

after administration.

Doses of tubocurarine (2-2.5-3mg.per kg.body weight), produced invariably the following picture:

1. A certain degree of curare paralysis of variable severity starting about 4-6 minutes after the internal administration.
2. This paralysis extended over a period of 15-25 minutes, after which the animal recovered. It was severer when the dose left inside the intestine was 3mg.per kg.

Four rats, weighing between 200-260g., were used for each dose level. The results of these experiments are summarised in Table X.

TABLE X

The site of absorption of tubocurarine introduced into the gastro-intestinal tract of rats.

Introduced proximal to duodeno-pyloric ligature.			Introduced distal to duodeno-pyloric ligature.		
Dose mg./kg.	No. of rats	Effects	Dose mg./kg.	No. of rats	Effects
50 100	2 2	None seen in 2hours	2 2.5 3	4 4 4	Variable paralysis in 4-6min
120	4	ditto	3.5 4	4 2	Severe paralysis and death.

It/

It became obvious, therefore, that tubocurarine was absorbed from the mucous membrane of the small intestine, and that by this method of introduction, the size of the effective dose by mouth was reduced more than tenfold.

It was also noticed that the duration of action of the drug was rather short, although the absorption started fairly soon. That renal excretion could be keeping pace with that rate did not seem very probable as it has been shown to be a relatively slow process.

This suggested that this process of intestinal absorption may be one of "limited absorption", limitation being probably due to another process taking place inside the intestine, thereby preventing any further absorption.

It was suspected that the pancreatic juice might be causing inactivation of the drug. To investigate this, the following procedure was followed:

The pancreatic juice of a cat was incubated with tubocurarine, at 37°C. and the curariform activity of the mixgure evaluated at the end of two hours.

Collection of pancreatic juice:

The juice was collected from a 2kg. decerebrate cat, by a cannula introduced into the pancreatic duct/

duct. The flow of the juice was stimulated by the injection of 'secretin' prepared from the cat's own small intestine by the method recommended by Sherrington.(107). The juice was then divided into two portions "A" and "B" of 0.3c.c. each. After inactivating "B" by heating it to about 90°C. 0.1c.c. tubocurarine solution containing 10µg. was added to each portion and both portions then incubated at 37°C. for two hours.

No loss of the tubocurarine content of either mixture, could be detected. The pancreatic juice thus does not appear to catalyse the destruction of tubocurarine.

In this connection it may be mentioned that Clement and Pistorio, in 1928, showed that bile and bile salts could precipitate the alkaloid from curare. (28).

So it is possible that when the drug reaches the small intestine, and while it is being absorbed, some of it may be thrown out of action by a process of precipitation, and then destroyed along its course in the intestines.

The effect of water diuresis on the urinary excretion of tubocurarine in the rat.

In these experiments the tubocurarine-equivalent in the urine of a group of rats was determined following the intramuscular administration of 0.3mg. tubocurarine per kg. body weight.

These rats were starved over night and on the following day, they were injected with the same dose of the drug, just after they had received 50 c.c. water per kg. body weight by a stomach tube, and the curarine equivalent of their urine again determined.

Three groups of rats, A. B. and C. each containing three male rats of body weights ranging between 230g. and 260g. were used. The total weight of group A. was: 745g.; of B: 750g. and of C: 730g.

In all cases, urine specimens were collected at the end of the fifth hour and the ninth hour following the injection. All specimens were extracted and examined in the usual way.

The second samples (6th-9th hour), as well as the control preinjection samples, were devoid of curariform activity.

The extracts of the five hours' samples

showed/

showed curariform activity; and when the total tubocurarine-equivalent of the urine was calculated, in each case, before and fater the water diuresis, an increase in the total equivalent was noticed to have followed upon the water-diuresis produced.

The results are shown in Table XI.

The effects of removal of both kidneys on curarized rats:

In these experiments, the effects of double total nephrectomy on the degree of paralysis and duration of action of tubocurarine was examined.

The extent of the degree of paralysis in an intact animal is not easy to determine. However, since the way of spreading of the curare paralysis is such that the respiratory muscles are the last to be involved, and when the respiration is sufficiently affected, the animal is usually thrown into anoxaemic convulsions; the occurrence of convulsions among the various rats was taken as a crude measure of the extent of spread or degree of severity of the curare paralysis.

TABLE XI/

TABLE XI.

The urinary excretion of tubocurarine (0.3mg./kg.) given intramuscularly to rats, with and without water-diuresis.

Rats	Urine volume (5hours)	Total amount excreted. (d.T.c.equiv.)	% of amount administered
Group A.	5ml.	44.7µg.	20
Group B.	3.8ml.	52.8µg.	23.5
Group C.	4.5ml.	39.4µg.	18
After 50c.c. water/kg. body weight by stomach tube:			
Group A.	12.5ml.	68.2µg.	30.6
Group B.	15ml.	63µg.	28
Group C.	18ml.	67.8µg.	31

From/

From the course of spreading of this paralysis, the inability of the animal to lift up his head in response to a stimulus, i.e., head-drop (H.D.), was taken as the starting point of the paralysis.

And as recovery from the paralysis occurs in the opposite way, the ability of the animal to lift up his head in response to the same stimulus, i.e., head-lift (H.L.) was taken as the finishing point of this paralysis.

The time elapsing between the occurrence of head-drop and head-lift being taken to represent roughly the duration of paralysis, and give some idea about the duration of action of the drug.

To minimise the individual variation of the various animals in their reaction to tubocurarine, each animal in the group served as its own control. The period of time elapsing between the first control and the second experiment was not less than twenty-four hours.

Thus the animal was injected on one day and its paralysis time determined, and on the next day both its kidneys removed. Three to four hours after the surgical interference, when the animal had recovered from the operation, it was similarly injected and its reactions recorded.

In/

In a group of five rats, ranging in weight between 200g.-250g. these injections were made intravenously in a dose of 0.08mg. per kg. body weight. One member of the group showed convulsions when it was injected after recovery from the operation. The average paralysis time for the group was increased from 12 minutes to 15.5 minutes. These results are shown in Tabe XII.

TABLE XII

Effects of double nephrectomy on the rats' reaction to tubocurarine given intravenously, (0.08mg./kg.).

Exp.	Control		After double nephrectomy.	
	Duration of paralysis: min.	Occurrence of convulsions.	Duration of paralysis: min.	Occurrence of convulsions.
1	12	-	15	+
2	10	-	14	-
3	15	-	17	-
4	11	-	15	-
5	12	-	16	-

In another group of ten rats, weighing between 200g.-280g. tubocurarine was injected intramuscularly in a dose of 0.3mg./kg. body weight. Thirty per cent of the animals convulsed when injected after recovery from/

from the operation as compared with ten per cent convulsing when injected before the operation.

The increase in the duration of action of the drug became more obvious,(from an average of 29.5min. to 40min.). These results are shown in Table XIII.

TABLE XIII

Effect of double nephrectomy on the rats' reaction to tubocurarine given intramuscularly.(0.3mg/kg.).

Exp.	Control		After double nephrectomy.	
	Duration of paralysis. min.	Occurrence of convulsions.	Duration of paralysis: min.	Occurrence of convulsions.
1	32	-	44	+
2	29	-	40	-
3	26	-	34	-
4	35	-	47	+
5	24	+	39	-
6	35	-	44	-
7	26	-	35	-
8	29	-	39	-
9	31	-	38	+
10	28	-	40	-

It/

It was also observed that although the duration of action of the drug was increased in the doubly nephrectomized series, the recovery of the animals, from the tubocurarine paralysis, by the end of this period was apparently complete.

Thus, although the kidneys seem to be an important organ in the elimination of the drug, renal damage - in these cases amounting to total nephrectomy- does not prevent the full recovery of the animal.

Effects of hepatectomy on curarized rats:

In a group of five rats, (weighing between 200g.-250g.) also acting as their own controls, the reactions of each rat to tubocurarine was determined before and after the removal of about 75% of the liver. While the duration of paralysis in the control period was: 32,35,32, 29 and 36 min.; after the subtotal hepatectomy, it became: 32,38,34, 30 and 35 min. respectively. No convulsions were observed to occur in either case and there was no appreciable difference between the average duration of paralysis following the injection, before and after hepatectomy.

All injections were made intramuscularly in a dose of 0.3mg./kg. body weight.

Experiments on mice.

A balance sheet for tubocurarine.

The balance sheet for tubocurarine was constructed from experiments on mice, in which the drug was injected intravenously in a dose of 0.2mg./kg. Male animals weighing between 20g.-25g. were used and the excreta were collected at variable intervals following the injection.

The animals were killed, minced and the tubocurarine equivalent of the extracts of the mince and of the excreta determined. This was done at: 0, 1 and 4 hours following the injection. In the first two cases it was not possible to collect any excreta.

TABLE XIV/

TABLE XIV

Balance sheet, showing recovery of tubocurarine (0.2mg./kg.) injected in mice.

Time in hours	Mice weight (g.)	Dose per mouse (mg.)	Per cent recovery		Mean percentage recovery.	
			mice	excreta	mice	excreta
0	20 20	.004	92	-	92.5	-
	22 22	.0044	93	-		
1	20 20	.004	76	-	78	-
	25 25	.005	80	-		
4	22 22	.0044	20	30	15	26
	25 25	.005	10	22		

Table XIV, shows the relationship between the amounts in the mice and in the excreta, and the percentage recovery of the dose. These results show that during the first four hours 26% of the dose was excreted/

excreted, and 59% of the dose disappeared in some other way, presumably by destruction.

PART II.

BRONCHOCONSTRICTION FOLLOWING RAPID INTRAVENOUS INJECTION OF TUBOCURARINE IN THE GUINEA-PIG.

During the course of this work, it was noted that sometimes a rapid intravenous injection of tubocurarine produced effects not produced by slower injections. In the conscious subject, Exp.II, the sudden severe respiratory embarrassment which occurred during the early part of the experiment had followed upon such an injection, and was believed to be probably due to a condition of bronchoconstriction.

The occurrence of bronchospasm was reported in 1936, by West, to follow the injection of curarine in some cases of Parkinsonian rigidity.(119). It was believed that this effect was due to the presence of an impurity contaminating the sample used.(120).

Numerous clinical reports, however, continued to mention the occurrence of an undesirable and dangerous side reaction with a sudden onset, following upon the administration of curare-preparations to patients./

patients.(36,60, 62,121). The reaction was described as respiratory difficulty accompanied by cyanosis. Increased resistance to inflation of the lungs by manual compression on the breathing bag has been reported by Whitacre and Fisher (121), and by Holaday(62), to occur in some anaesthetized patients given intocostrin. This respiratory spasm was observed by the same workers to be relieved by another injection of the drug.

In reports on animal experimentation, a reaction to curare administration, similar to that occurring in man has been described. Cole(32), reported the occurrence of cyanosis in dogs following the administration of intocostrin, despite vigorous artificial respiration through an endotracheal tube, and he attributed this condition to bronchospasm.

Since Alam, Anrep, Barsoum, Talaat and Weininger, in 1939, demonstrated the release of histamine as a result of curarine injection (1), evidence has been accumulating of the occurrence of histamine reactions following injection of even the most purified preparations, intocostrin and tubocurarine.

Comroe and Dripps, in 1946, showed that certain/

certain vascular effects of intocostrin in man, i.e., the wheal and flare response, were similar to histamine reactions.(33).

Grob, Harvey and Lilienthal, in 1947, reported similar reactions to occur with dextro-tubocurarine chloride.(56).

Feldberg and Holmes, reported that the injection of curarine caused - like that of histamine - gastric secretion of free HCl.(42).

Landmesser has recently shown that bronchoconstriction occurred in spinal dogs as a result of the injection of curarizing doses of the pure alkaloidal salt d-tubocurarine chloride and that that was due to the histamine liberated.(81). He used the Drinker-Murphy infant resuscitator adapted to record bronchial calibre by the plethysmographic method of Jackson.

Since probably the absolute contra-indication to the clinical use of tubocurarine may be the inability to perform artificial respiration and since such a position might develop if bronchoconstriction occurs even if the means of artificial respiration were at hand, its occurrence should be avoided.

Experimental/

Experimental procedure.

In the experiments to be described, the broncho-constrictor power of tubocurarine is demonstrated. As it was probable that this effect is due to the histamine mobilised as a result of the injection, the modification of such an effect by an anti-histamine - notable for its efficiency in antagonising histamine bronchospasm - namely, neoantergan, was also observed.

Guinea-pigs were chosen for these experiments as they have been shown to be most susceptible to the action of tubocurarine (41).

Furthermore, if the bronchoconstrictor properties of tubocurarine are due to the liberation of histamine, the effects of this substance may be more easily reflected on the guinea-pig bronchi.

Decerebrate animals of both sexes, weighing 500g.-650g. were used. The animal was anaesthetized with ethyl ether and a tracheal glass cannula - with a side tube attached - introduced into the trachea, and the anaesthesia continued through a Wolff's bottle, until the brain was destroyed.

Then artificial respiration was installed by

a/

a pump, delivering air at a constant pressure. The chest cavity was opened by severing the junction between the xyphoid process and the body of the sternum, then cutting the latter upwards in the middle line for about 2-2.5 inches from its lower margin.

The two edges of the chest wall were fixed moderately apart by rigid clamps. The opening thus made in the chest was well covered by warm, moist gauze.

The side tube in the tracheal cannula was then connected - through a piece of pressure tubing - to a recording tambour, moving a lever which amplified its movements about twenty times and recorded them on a smoked drum.

A sensitive tambour of about 4 inches diameter covered with thin rubber at a suitable initial tension was used.(46).

The air delivered by the pump will be distributed between the bronchi and the tambour, and the state of balance recorded by the lever on the drum. The occurrence of constriction in the bronchial calibre will result in accommodation of less air in the bronchi, thus more air will be deflected along the side tube to the tambour, causing an increase in the amplitude of the record.

Better/

Better records were obtained with the chest opened than when it was closed.

The drugs were injected intravenously through a cannula connected to a superficial neck vein. Tubocurarine was used in curarizing doses of 0.03mg. in 1c.c. saline per kg. body weight; histamine as 0.01mg. of the base in 1c.c. saline per kg. body weight; unless otherwise indicated.

For protection with neoantergan, this was made by giving the drug in doses of 1mg. per kg. body weight subcutaneously 0.5-1 hour before the operation, and for the treatment of an attack it was given as 0.1-0.2mg. per kg. in 1c.c. saline intravenously. All intravenous injections were given rapidly (i.e. in 2-3 sec.) unless otherwise mentioned.

The slow intravenous injection of tubocurarine was made after passing a capillary glass tube into the drug solution filled into a burette connected to the vein cannula. The drug was infused at the rate at which the air bubbles were leaving the lower end of the capillary tube, and this was adjusted so that the corresponding dose would flow over a period of one minute or slightly more.

With the above arrangement of experiments the following results were obtained:

Results/

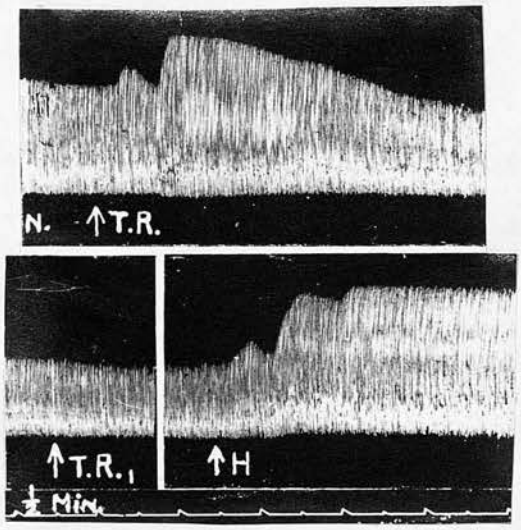

FIGURE VII

T.R. Tubocurarine injected rapidly (15µg); $T.R_1$: same dose similarly injected 6 mins. later; H=Histamine (5µg): G.Pig=500g.

RESULTS

Bronchoconstriction caused by tubocurarine:

The intravenous injection of tubocurarine in the unprotected animal caused the development of a condition of bronchoconstriction reaching its maximum in about half a minute and passing off gradually in about four minutes.

When the constriction was apparently relieved, a second dose of the drug, similarly injected about six minutes after the first, was without any effect. The subsequent injection of histamine, however, produced a bronchospasm reaching its maximum also in about half a minute, but it stayed longer and was more sustained.

Fig.VII, is an example of such effects produced in a guinea-pig weighing 500g. Larger initial doses of tubocurarine did not appreciably increase the degree of constriction.

This refractoriness of the animal to a second dose of tubocurarine - when the effects of the first dose have passed off - was still there, when this second dose was greater than the first one and given after a longer interval. Therefore, no animal could be used more than once to show these responses.

Fig.VIII/,

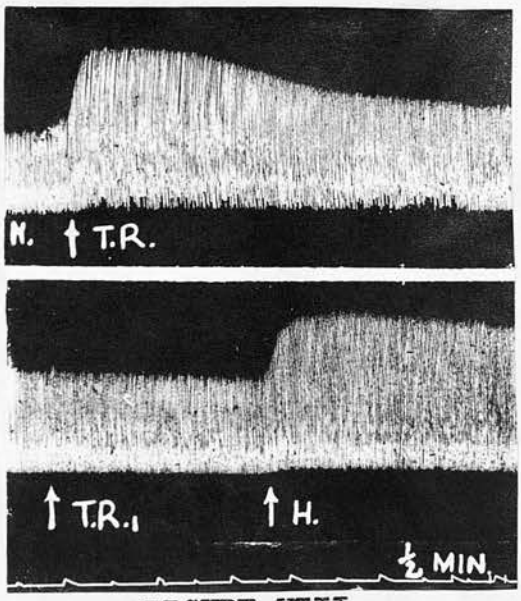

FIGURE VIII

T.R. Tubocurarine injected rapidly (16.5µg); $T.R_1$: Tubocurarine (66µg) injected rapidly 90 min. later.
H=Histamine (5.5µg) G.Pig=550g.

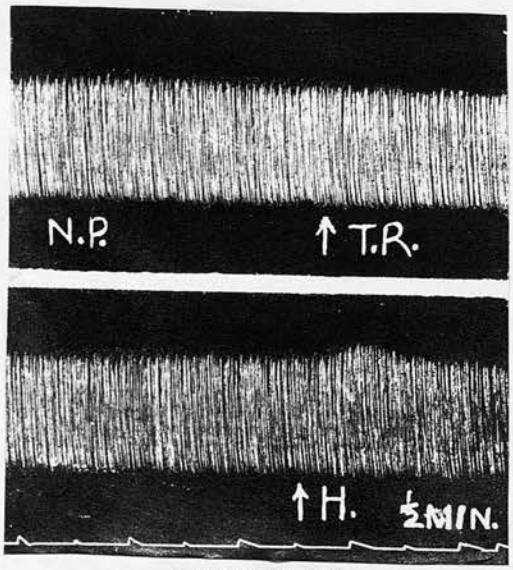

FIGURE IX

N.P.=normal record of an animal protected with neoantergan (1mg./kg.) subcutaneously 1 hour previously.
T.R.=tubocurarine injected rapidly (60µg.).
H=Histamine (30µg). G.Pig 520g.

Fig.VIII, is an example of such an experiment where a second dose four times larger than the first and rapidly injected ninety minutes after the first dose was without any effect. This, however, was followed by the usual histamine responses. Of eighteen guinea-pigs however, seven were refractory to the first rapid dose of tubocurarine but not to histamine.

The prevention of bronchoconstriction caused by tubocurarine:

a. By protection with neoantergan:

In these experiments, done on seven guinea pigs, the animal was protected ·5-1 hour before the operation by the subcutaneous injection of neoantergan 1mg./kg. body weight.

In these cases, the rapid injection of tubocurarine did not precipitate an attack of bronchoconstriction.

The efficiency of such a protection is illustrated in Fig.IX.

In this case, tubocurarine was rapidly injected (about four times the usual dose in a 520g. guinea-pig), fully sixty minutes after the animal had received its subcutaneous protection. No constriction resulted. When the histamine injection of 30µg. followed half/

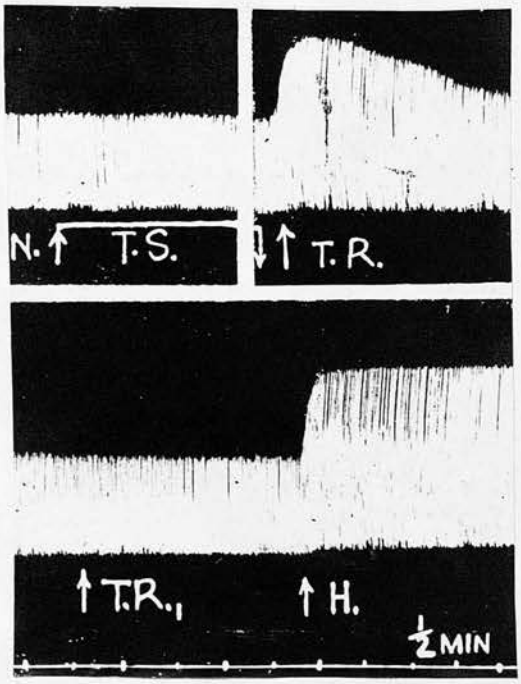

FIGURE X

N=normal; T.S.=tubocurarine (225µg) injected slowly at a rate of 15µg./min. T.R.=tubocurarine (15µg) injected rapidly. $T.R_1$=same dose similarly injected 7min. later.

H=Histamine (5µg). G.Pig 500g.

half an hour later, this showed a very slight transient effect.

b. By slow intravenous infusion:

When the dose of tubocurarine was injected slowly, over a period of one minute or more, this procedure in itself seemed to have modified the reaction of the animal to tubocurarine. No bronchoconstrictor effects were noticed to follow upon such an administration, in seven guinea-pigs. When the slow intravenous infusion was stopped and a dose of the drug injected rapidly, the usual bronchoconstrictor effects were produced. Then when a second rapid injection of the drug was given, after the effects of the first dose had passed off, the animal showed again the usual refractoriness; but was not refractory to the injection of histamine that followed.

Fig.X, is an example of such experiments, where the slow infusion of 225µg. tubocurarine over a period of fifteen minutes, at a rate of 15µg./min. in a 500g. guinea-pig, did not produce any bronchial spasm.

When the infusion was over, the injection of 15µg. in the usual rapid way produced bronchconstriction. No obvious effects were produced when another dose/

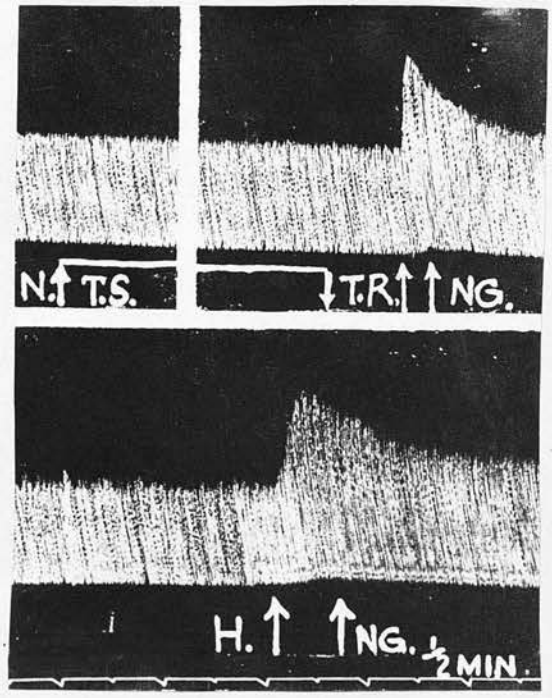

FIGURE XI

T.S.=tubocurarine (150µg) infused slowly over a period of 10min; T.R.=tubocurarine (15µg) injected rapidly; NG=neoantergan 0.1mg; H=Histamine (70µg), given 1 hr. after T.R. G.Pig=500g.

dose of 15µg. tubocurarine was injected rapidly about seven minutes later.

The injection however, of 5µg. histamine after that produced a definite spasm.

Treatment of bronchoconstriction caused by tubocurarine:

In these experiments, it was decided not to give any degree of protection to the animals beforehand, but to develop in them an attack of tubocurarine bronchoconstriction and then see how far such an attack could be modified, by the administration of neo antergan, at various stages of its development.

Fig.XI, shows the production of such an attack in a 500g. guinea-pig by the rapid intravenous administration of 15µg. tubocurarine.

When the attack was at its peak, the rapid injection of neoantergan (0.1mg. intravenously) apparently relieved this attack and restored the bronchial calibre, to some degree.

About one hour later, the effects of the antihistamine seemed to have weakened, and when a large dose of histamine (70µg.) was given, this caused a bronchoconstriction which was again effectively treated at its peak by intravenous neoantergan.

The/

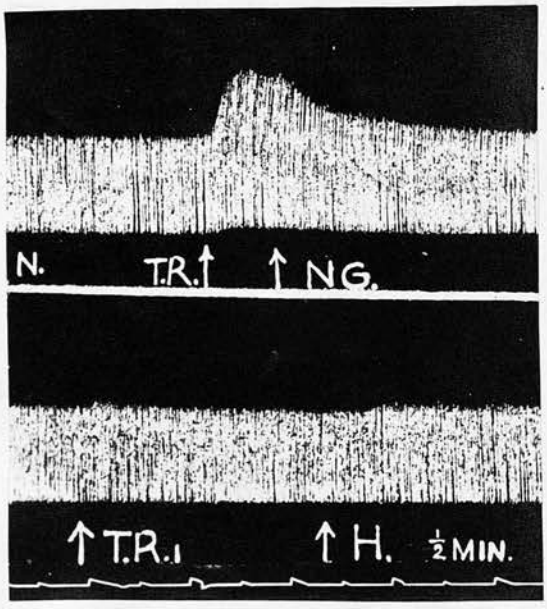

FIGURE XII

T.R.=tubocurarine (18µg) injected rapidly; NG=neoantergan 0.12mg; $T.R_1$=tubocurarine (36µg) injected rapidly. H=Histamine (10µg) given half an hour after T.R. G.Pig=600g.

The first part of this curve shows the harmlessness of the slow injection of tubocurarine on the bronchi, where 150µg. were infused slowly at a rate of 15µg./min.

When an attack was left to pass its peak and get fully developed the neoantergan treatment seemed to relieve it, to some degree.

Fig.XII, is an example of such a condition where the bronchoconstrictor attack in a 600g. guinea-pig, was left to become fully developed in about one minute, when the intravenous injection of 0.12mg. neoantergan accelerated recovery. After five minutes, a second larger dose of tubocurarine, 36µg. rapidly injected, did not produce any effect, nor did 10µg. histamine injected half an hour later.

If an attack is dealt with early enough by the prompt administration of neoantergan, its full development is apparently suppressed.

Fig.XIII, shows such an effect, where in a 600g. guinea-pig, when an attack was starting to develop as a result of a rapid intravenous injection of 18µg. tubocurarine, it was possible to abort it prematurely by the prompt intravenous administration of .06mg. neoantergan. The animal was completely refractory to a second larger dose of tubocurarine similarly/

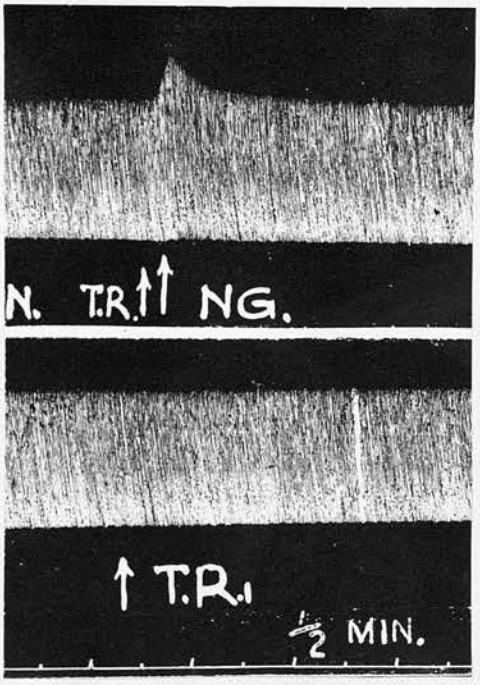

FIGURE XIII

T.R.=tubocurarine (18µg) injected rapidly; NG=neoantergan 0.06mg; $T.R_1$=tubocurarine (36µg) injected rapidly 1 hour later. G.Pig=600g.

ly injected one hour later.

It appears therefore, that the rapid intravenous injection of tubocurarine in the guinea-pig, under these conditions, produced a state of bronchoconstriction. The animal later became refractory to a second dose of tubocurarine but not to histamine.

A condition thus produced could be conveniently treated by the prompt administration of intravenous neoantergan given at the peak of the attack or when it was fully developed. If this treatment was carried out early enough - just at the beginning of the spasm - the attack could seemingly be aborted and its further development checked.

Neoantergan, given subcutaneously 0.5-1 hour before a rapid injection of tubocurarine has apparently prevented such an attack. If such a protection was not provided, but the drug injected slowly intravenously no such attack developed.

THE INHALATION OF TUBOCURARINE

In these experiments, guinea-pigs were exposed to an aerosol of aqueous solution of tubocurarine, with and without preliminary protection by neoantergan. The inhalation was meant to obviate, at least during the early part of the experiment, the general action of the drug, in order to get the effect of its direct application upon the bronchial system of the living animal.

Method:

A Collison spray atomiser was used after connecting it to an animal glass-chamber, and modifying its container so that it was possible to economise in the use of the drug. The glass bottle container was filled to threequarters of its capacity, with a mould of plaster of Paris, "P" (Fig.XIV), in the middle of which a hole was drilled to hold about 25-30c.c. of water. This hole acts as the new container of the apparatus through which the central spraying piston, "S" passes to a short distance from its bottom. The sides and edges of the hole were made sloping and they, as well as the upper surface of the plaster mould, were coated/

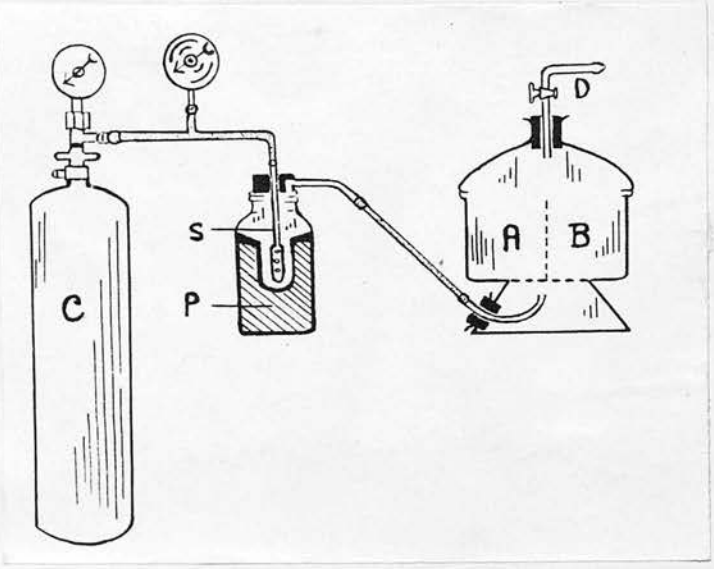

FIGURE XIV

DIAGRAM OF A MODIFIED COLLISON SPRAY ATOMISER.

coated with a water-proof layer of varnish.

The inner sides of the remaining part of the glass bottle were covered with a thin layer of wax, to prevent water particles from forming large drops on the glass during spraying. These particles will instead, fall on the upper sloping surface of the plaster mould and run down into the hole from whence they will be resprayed.

The cloud was produced by passing a continuous stream of compressed air into the aqueous solution in the container. The air was delivered from a compressed air cylinder, "C", at a pressure of 20lbs. per square inch, and maintained constant by an adjusting screw. The cloud was then taken by a curved glass tube to just below the centre of a wire mesh platform fitted horizontally inside a large glass desiccator which acted as an animal chamber. The upper part of the desiccator was divided by a vertical wire mesh screen into two compartments of equal size, "A" and "B", each holding one animal. The cloud escaped by a tube "D" fitted into a hole, drilled in the centre of the desiccator dome.

The output of the atomiser was about ten litres air and 0.7c.c.watery solution per minute.

Tubocurarine/

Tubocurarine was used as 3% watery solution. Two guinea-pigs of the same sex and weight were used at a time, one in each compartment of the animal chamber. One of the animals usually received a subcutaneous injection of 1mg./kg. body weight neoantergan, 0.5-1 hour before starting the spray. Ten such pairs of animals were used in these experiments. The duration of spray was 15-20 minutes.

Results:

No signs of bronchoconstriction were observed in any of the animals. It made no difference to the animal whether it had or had not been protected before hand with neoantergan.

At the end of the period of spray both animals, when taken out, exhibited some signs of the general action of the drug in the form of a moderate degree of paralysis from which they recovered in about seven to ten minutes.

When these animals were exposed, the following day, to an aerosol of an aqueous solution of 2% histamine base - after giving a fresh protective injection to the control - the unprotected animal showed the usual signs of severe histamine bronchospasm, which sometimes resulted in loss of the animal.

In/

In the control animal protection was complete.

It may be concluded that these results are in support of the evidence that the bronchoconstrictor power of tubocurarine is probably not due to the drug itself, but to its histamine releasing properties.

It is, further, probable that tubocurarine may not be able to release histamine from the lungs.

This may also be compatible with the observation of Alam et al (1), that the lungs are not participating in the histamine mobilisation following upon the injection of the drug.

DISCUSSION

The various stages of tubocurarine paralysis could be correlated with the concentrations of the drug in the plasma, though the concentrations at the neuro-muscular junction must be more intimately related to these effects. The degree of this paralysis seems to depend on the sensitivity of the individual to tubocurarine. Gray and Halton, in 1948, reported the development of severe paralytic reactions, amounting to complete respiratory arrest, to follow upon the injection of a small dose (2mg.) of tubocurarine in an adult non-myasthenic patient.(54). As the use of the ordinary dose of tubocurarine may prove hazardous in a myasthenic patient,(7,31), the use of a preliminary small dose, to test the reaction of an individual may be worth trying to obviate unpleasant consequences in an otherwise idiosyncrotic individual.

In the conscious human subject (Exp.II), tubocurarine was detected in his saliva, in a concentration of 1.2µg./ml. occurring twenty-one minutes after the injection. The drug was not concentrated by the salivary glands, as the plasma level at this time was about 2µg./ml.

About 12% of the dose injected was present in his fasting gastric juice, forty-three minutes after the/

the intravenous injection. The excretion of curarine along these channels was reported by Koch, in 1870, and von Huber drew attention to this fact in 1922(76). In this respect, tubocurarine is behaving in a similar way to some heavy metals, and alkaloids, e.g., morphine.

The presence in the C.S.F. of this subject of curariform activity equivalent to 2.5µg. tubocurarine per ml. may be of some clinical interest. It occurred at a time when the concentration of the drug in the plasma was about 1µg. per ml. Everett has shown that when the drug is brought into direct contact with the central nervous system in a sufficient concentration (0.1mg./kg. intracysternally in the rabbit), it is liable to set up convulsions of central origin, and he warned against the use of tubocurarine in cases of suspected pathological changes in the blood vessels of the C.N.S.(41). The occurrence of violent convulsions following the intravenous administration of tubocurarine in a case of schizophrenia was reported by Morrison in 1948(91). This may be due to a greater leakage of the drug from the vessels of a central nervous system set at fault, and

Everett's/

Everett's warning may justifiably be emphasised.

Tillie, in 1890, was among the early workers who clearly demonstrated that the direct application of curarine to the C.N.S. induced prompt convulsions. (113). Similar results were obtained in the frog and in mammals by other workers,(86,103). There is at present, however, a controversy about the action of the drug on the spontaneous resting potentials, of which the electroencephalograph is an indicator. McIntyre noticed that tubocurarine causes increased electroencephalographic activity in dogs followed by depression.(87); while Smith, Brown, Toman and Goodman (109); and Girden,(50), did not observe such changes to occur in mammals.

No apparent changes in the sensations or any detectable anaesthetic effects were observed in the experiments described in this work, to follow the injection of tubocurarine in man. This was also observed by Prescott, Organe and Rowbotham,(97); and by Smith et al (109); although it has been reported by Whitacre and Fisher that intocostrin could produce general anaesthesia.(121).

It has been known that the kidneys play an important part in the elimination of the drug. The urinary/

urinary excretion of crude curare was demonstrated by Voisin and Lionville (114), who poisoned rabbits in series by taking the urine of the first and injecting it into a second rabbit. Similar experiments were conducted by Bidder, who was likewise able to poison frogs in succession (13). Boehm was able to demonstrate the presence of the drug in the urine of curarized animals by the development of a purple ring at the zone of its contact with concentrated sulphuric acid.(16).

Claude Bernard also showed that obstruction to the urine outflow increased the rate of poisoning in the rabbit,(12).

Here, however, it has been found that the renal excretion of tubocurarine following an intravenous injection is a relatively slow process. In hourly samples taken from man it was possible to detect it three hours and sometimes four hours, after such an administration. The greater part of the excretion, however, occurred during the first hour. In the rabbit, as early as ten minutes after the intravenous administration of the drug, the apparent tubocurarine content of the kidneys per g. tissue weight was already higher than that of other organs where the/

the drug seemed to be uniformly distributed.

The beneficial effect of water-diuresis in the elimination of the drug by the kidneys was illustrated in the rat, in which the total excretion of the drug following its intramuscular administration was increased under water diuresis. The ancient use, by the Indians, of large amounts of water by mouth as an antidote to arrow-poisoning, may be of interest!(87).

Although the kidneys appeared to be playing an important part in the elimination of the drug, yet the relief from the obvious effects of curarization did not seem to depend entirely upon renal excretion. This was illustrated by a series of experiments on doubly nephrectomized rats. In these animals, although the total removal of both kidneys caused a slight increase in the duration of action of the drug, yet the recovery of the animals by the end of this period was apparently complete.

From the results of the hepatectomy experiments, it did not seem likely that the liver was providing an appreciable degree of protection against the drug effects. It was also concluded by Rothberger and Winterberg,1905,(102); and later by Polimanti in 1914(96), that the liver plays no part in detoxifying/

detoxifying the drug.

These findings may be in agreement with recent clinical observations by Wall in 1947,(115), that renal or hepatic damage does not necessarily constitute a serious contraindication to the clinical use of the drug. There must be some other mechanism by which tubocurarine disappears from the body. The experiments on mice show that the drug is actually inactivated in the body, since 60% of the dose injected disappeared in four hours. The site of this inactivation is unknown. It may perhaps occur in voluntary muscle, which was found to contain 40% of the dose in the experiments on rabbits.

Gros,(57) obtained evidence that the absorption from mucous surfaces is poor, but absorption from serous cavities is fairly rapid. Bernard showed that the drug given by mouth was not destroyed by the gastric juice, as he was able to poison frogs by the injection of the stomach contents of dogs previously given curare by mouth.(11). It is almost a popular belief that the drug is ineffective when given by mouth, either because it is not absorbed from the gastro intestinal tract or because it is destroyed there or because it is excreted as quickly as it is absorbed, so that an effective blood level is not easily/

easily reached.(36).

However, from experiments conducted in this work, and as far as tubocurarine is concerned, it was noticed that in the rat, and within certain range of dosage (30-40mg./kg.), typical paralytic effects were produced, when the drug was given by stomach tube, indicating absorption. That absorption had taken place from the gastro-intestinal tract of these animals was also shown by the presence in their urine of a part of the dose administered.

In an attempt to localise the site of absorption of the drug from the alimentary tract, the drug was introduced directly into the stomach or small intestine respectively after tying the duodeno-pyloric junction through an abdominal incision. In this case the presence of large amounts of the drug in the stomach, over 100mg./kg. body weight, was without any obvious effects on the animal. That was probably due to lack of effective absorption from this organ.

But, when the drug was introduced directly into the small intestine, in a much smaller dose (2-3mg./kg.), signs of absorption developed rather rapidly, in about 4-6 minutes, and progressed fatally with an increase of this dose.

It/

It is possible that with such drugs, producing obvious characteristic signs within a short interval after administration, the widely different absorbing properties of these neighbouring mucous membranes could be demonstrated pharmacologically. This may be another instance of the use of tubocurarine as a pharmacological tool.

The effects of the drug (2-3mg./kg.) thus absorbed were, however, of short duration, since in 15-25 minutes, the animal seemed to have recovered from the obvious drug effects. It seemed unlikely that the relatively slow renal excretion could be keeping pace with such a rapid absorption, to an extent which would prevent the development of a dangerous blood level.

It is possible that the continuation of absorption from the small intestine was limited by a process of precipitation and that the drug may be further destroyed along its course in the intestine. Clement and Pistorio showed in 1928, that bile and bile salts could precipitate the alkaloid from crude curare.(28).

The pancreatic juice was shown to be not taking part in the destruction of the drug.

It was also noticed that this method of administration/

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The pancreatic juice was shown to be not taking part in the destruction of the drug.

It was also noticed that this method of administration/

administration of the drug directly into the intestine has reduced the size of the effective dose by mouth more than tenfold.

It was proposed - at sometime during this work - to imitate this clinically, as by giving the drug in keratin coated capsules in the hope of getting desirable effects in spastic paralytic conditions. But probably the limited absorption of the drug from the intestine, and the short duration of action of the drug thus absorbed, may limit the clinical value of the drug administered that way.

The sphere of usefulness of tubocurarine in clinical practice, however, is getting wider as the various conditions known to stand as contraindications to its application are being reconsidered. Curarization is believed to occur with little or no manifestations of haemodynamic disturbances; and it is only when secondary effects are allowed to develop that the cardio vascular indices are subsequently disturbed.(109).

Probably the vital system whose responses provide the most important contraindications to the use of tubocurarine is the respiratory system. Therefore one must be definitely convinced before administering the drug of one's ability to care for the

respiratory/

respiratory depression that might ensue. Artificial respiration applied by the Schaefer or similar methods may completely fail to resuscitate and thus means of artificial respiration under positive pressure should always be available(87). This latter method, however, may prove inadequate if the bronchial calibre is seriously interfered with.

It appears that a certain degree of such interference may be caused,in the form of bronchoconstriction, by the use of the drug itself.

This was observed many years ago by West, using the crude drug (119); but recently similar reports followed describing such a condition to occur after the use of the most purified preparations.(32, 81).

When Alam et al (1), demonstrated that histamine was released, from pre-existing stores in the tissues, after the intravenous injection of curarine and that the histamine thus released circulated in a physiologically active form, reports followed that such a release could occur following upon the administration of intocostrin and tubocurarine.(33,56). This was mostly shown clinically to occur in the form of vascular reactions similar to those of histamine.

Clinically/

Clinically, it was also observed that occasionally some degree of bronchial spasm occurred, following the injection, as evidenced by increased resistance to pressure on the breathing bag.(36, 60, 121).

In the conscious human subject, Exp.II, described here, a similar condition had resulted which is believed to be due to the same cause.

Experimentally, Landmesser has clearly shown recently, that active bronchoconstriction occurred in spinal dogs as a result of the release of histamine following upon the injection of tubocurarine.(81).

The exposure of the living guinea-pig to an aqueous tubocurarine aerosol, in experiments described here, was without obvious harmful effects on their bronchi. These animals showed at the end of the inhalation period, some degree of the general action of the drug. It is possible that tubocurarine applied this way may not be causing bronchoconstriction by itself nor able to release sufficient histamine to cause it.

Similar results to those of Landmesser were obtained here using the spinal guinea-pig. That the bronchoconstriction produced in these animals as a result/

result of the rapid intravenous injection of tubocurarine was probably due to the histamine liberated, may be seen from the fact that repetition of the injection - after the effects of the first dose had apparently disappeared - did not produce obvious effects. The assumption is that the first injection had probably depleted most of the histamine stores in the tissues.

The clinical observation that in the event of such bronchoconstriction, a second injection of the drug seemed to relieve it, may be explained by the observation that the second injection was probably acting on exhausted histamine stores and might have thus been unable to produce bronchoconstriction, while the constrictor effects of the first dose were gradually passing off.

Furthermore, premedication of the animals by an antihistamine, notable for its efficiency in counteracting histamine bronchospasm, namely, neoantergan was effective in protecting the guinea-pigs against bronchospasm produced by the rapid intravenous injection of tubocurarine.

It was also possible to prevent the bronchial spasm in the guinea-pigs by injecting the curarizing/

curarizing dose of the drug slowly intravenously, over a period of one minute or more. In this way, it was possible to infuse ten to fifteen times this dose, without producing signs of bronchoconstriction. It may be that the histamine mobilising power of the drug was reduced by giving it that way, or that whatever small amounts of histamine were released at a time, were rapidly taken up by the tissues.

However, it seemed that a histamine level sufficient to precipitate such an attack was not easily built up under such conditions.

It may be remembered that in the event of bronchoconstriction, artificial respiration under positive pressure - which may be the only reliable life-saving measure in curarization - may be rendered difficult. Such a condition will probably be aggravated by an additional reduction in the size of the chest cavity, resulting from weakness and loss of tone of the respiratory muscles with subsequent rise of the intrapleural pressure. All possible precautions should therefore be taken to obviate such conditions. Should respiratory depression ensue from the paralytic actions of the drug on the respiratory muscles, there will then be no difficulty in artificially inflating and deflating the lungs.

SUMMARY

1. The method described by Jalon for estimating tubocurarine by its action on the frog's rectus abdominus, was adopted for determining the drug equivalent of tissue extracts and biological fluids. This method was used to follow the fate of the drug in man and animals.

2. The immediate volume of distribution on intravenous injection corresponds to the plasma volume. The drug does not enter the blood cells. It disappears from the plasma exporentially with a halving time of about thirteen minutes. These conclusions apply both to man and to rabbits.

3. In the conscious human subject, a concentration of 4µg. per ml. in the plasma causes complete paralysis. A concentration of 1µg. per ml. has very little effect.

4. About 20-40% of the drug appears in the urine. This percentage may be increased by water diuresis. Excretion continues for several hours, even when the paralysis only lasts about half an hour.

5. The main route of disappearance of the drug from the/

the body does not depend on the kidneys, since double nephrectomy in rats did no more than slightly delay full recovery from paralysis.

By extracting whole mice, it was shown that about 60% of the dose was inactivated in the body within four hours.

Since subtotal hepatectomy in rats did not appreciably affect the duration of paralysis, the liver is probably not the main site of inactivation. It is possible that inactivation occurs in voluntary muscles, which were found to contain 40% of the dose in an experiment on rabbits.

6. The effective dose by oral administration in rats is about 100 times the effective dose by intramuscular administration. Absorption occurs in the small intestine but not in the stomach. On intravenous injection, appreciable quantities (12% of the dose) may be excreted in the stomch.

7. A rapid intravenous injection of tubocurarine usually causes bronchoconstriction in guinea-pigs. A second injection has no such effect.

The effect may be prevented or interrupted by neoantergan and is probably due to the release of histamine./

histamine. The best way to avoid this effect in the clinical use of tubocurarine is to give the injection slowly. Slow injections of large doses in guinea-pigs had no effect on the bronchi, although a dose ten to fifteen times smaller caused bronchoconstriction when given rapidly.

ACKNOWLEDGMENTS

It is a pleasure to express my indebtedness to Professor J.H. Gaddum for suggesting this problem, and for continued guidance and stimulating interest.

I am deeply grateful to Dr. M. Vogt for the valuable help and criticism she has given throughout this work, and in the preparation of this paper.

I am also thankful to Dr. J. Gillies, Chief Anaesthetist to the Royal Infirmary, and Dr. H.W. Griffith of the Anaesthetic Department, for the facilities they provided in the collection of samples from patients, and for their help in the experiment on the conscious subject.

The d-tubocurarine chloride used in the animal experiments, was kindly supplied by Dr. J.W. Trevan, Director of the Wellcome Physiological Research Laboratories.

My thanks extend to Dr. H. Adam for estimating the histamine content of the urine of the asthmatic patient; and to the Moray Fund of Edinburgh University for defraying some of the expenses of this work.

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