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THE FATE OF TUBOCURARINE IN THE BODY

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The need for isolating a purified active principle from crude curare started at an early date in South America, when Boussingault and Roulin (1828) succeeded in obtaining a bitter principle which they differentiated from strychnine, isolated eight years previously. Although the problem was somewhat clarified by the work of Preyer (1865) and Boehm (1886, 1897), it was not until 1935 that the active alkaloidal salt, *dextro*-tubocurarine chloride, was isolated in a pure crystalline state by King from a sample of native tube-curare. The same alkaloid was obtained in a good yield by Wintersteiner and Dutcher (1943) from a single plant species, *chondrodendron tomentosum*, which is probably its chief botanical source.

The fate of this alkaloid in the body has received little attention; the present work is an attempt towards providing some information on this point.

Choice of a Method

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

Chemical methods.—Barbosa (1903) published elaborate charts depicting the colours obtained with various curare compounds. Qualitative, though non-specific, reactions for the curare alkaloids with potassium ferrocyanide were described by Cole (1923) and with trichloracetic acid by Schoofs (1927). Recently, Foster and Turner (1947) have developed a tentative polarimetric method for the assay of *d*-tubocurarine chloride. They also described a colorimetric method for the assay of the alkaloid depending on the use of Folin-Ciocalteu phenol reagent.

All these methods, however, must be of limited application on account of the many materials which yield colours with the reagents, and the high concentration of the alkaloid required for its detection.

Biological methods.—One of the early methods was described by Gaddum (1937) and consisted in determining the paralysing dose for the frog. Holaday (1941) developed the head-drop method, which employs muscular relaxation in an intact mammal (rabbit) as the criterion of curare activity. The intravenous injection of curare in mice likewise produces a head-drop. This method, described by Kimura and Unna (1948), is claimed to be more economical and allows the statistically valid determination of the head-drop dose on a uniform population. In this connexion it may be noted that the difference between the average head-drop dose and the average lethal dose represents the margin of safety of the drug, and that this margin is so narrow that the determination of the average head-drop dose often results in loss of the animal. Moreover in a head-drop method the results are not recorded objectively.

Skinner and Young (1947) described a "mouse-method" of assay of curare activity as being simple and objective. Marsh and Pelletier (1948) used a method depending on comparing the paralytic doses for rats.

The main use of any of these methods at present is mostly confined to the evaluation of compounds with curare-like activity, and even there they suffer from the disadvantage that they do not establish the site of action of the drug, which is one of its most characteristic features.

The isolated frog's nerve-muscle preparations (gastrocnemius-sciatic or nerve-sartorius) have also been used; most authors (for references, see Ing, 1936) have estimated either concentrations which paralysed the muscle completely or which just failed to cause complete paralysis. After such severe poisoning, recovery is usually slow.

Chou (1947) used the rat's phrenic nerve-diaphragm preparation (Bülbring, 1946) for the estimation of curare-like activity. The use of this method for assay requires the presence of the test substance in relatively large concentrations.

The frog's rectus abdominus muscle method

This 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. It was described by Jalon (1947) and was found suitable for the purpose of the present work. The bath used here for this preparation contains 2 ml. 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 10 and the tension about 3 g. weight. A suitable fixed dose of acetylcholine is added to the bath every 6 min. and allowed to act for exactly 2 min. before it is changed and the preparation 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 90 sec. 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 amount of tubocurarine present in an extract is obtained by comparing it with a standard solution of tubocurarine, given in alternate doses. By this procedure it is possible to detect 0.1 μg. tubocurarine. As it made no difference to the assay whether or not the rectus had been sensitized beforehand by eserine, the uneserinized preparation was preferred.

The Preparation of Extracts

In extracting added or injected tubocurarine from the various tissues and biological fluids, the following methods were tried:

Blood.—Using whole blood *in vitro*, most of the drug added was recovered from the plasma by the use of acid alcohol. Extracts of blood cells gave no evidence that tubocurarine had passed inside them. After an injection, extracts were made in the following way: The blood was mixed with heparin and cooled with ice, and the plasma immediately separated. The acid alcohol was prepared by acidifying absolute ethyl alcohol with a crystal of tartaric acid or with 0.1 ml. *N*.HCl, and 10–15 ml. were used for each 1 ml. plasma. The plasma was added dropwise to the acidified alcohol, which was shaken thoroughly after each addition. The precipitate was separated and washed with acid alcohol, the alcoholic solution taken down to dryness on a water bath, and the residue dissolved in Ringer's solution and filtered.

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 (5 ml. for each 1 ml. urine) and the precipitate separated by centrifuging and washed with absolute alcohol. The alcoholic solution was then evaporated to dryness, and the final residue taken up in Ringer's solution (1–10 ml. for each 10 ml. urine); thus the tubocurarine in the urine could be concentrated 1–10 times.

Tissue.—A convenient method was found to depend on extraction with acid alcohol, and here the use of sulphuric acid as an acidifying agent was usually found to be superior to hydrochloric acid in providing a final clear extract; this was observed by Chang and Gaddum (1933) when they were estimating the acetylcholine equivalent of tissue extracts. Since the acetylcholine in these extracts would interfere with the test for tubocurarine it was inactivated by hydrolysis. Extracts prepared in this way may contain other pharmacologically active substances, and are therefore only suitable for use in a biological test, which is relatively little affected by these substances; this is another advantage of the frog's rectus muscle which is insensitive to most substances in extracts except acetylcholine.

The tissue was weighed, cut up with scissors, and mixed with acidified alcohol (15–20 ml. per g. tissue), where its cutting up and mixing were completed. This acidified alcohol was prepared by adding 1.2 ml. $2N.H_2SO_4$ to each 100 ml. of absolute ethyl alcohol. The deposit was then separated by centrifuging and washed with acid alcohol; the alcoholic solutions were evaporated to dryness and the residue taken up in Ringer's solution and filtered. The extract was then made slightly alkaline and boiled for 1–2 min. to destroy acetylcholine but not tubocurarine; finally it was neutralized and concentrated until 1 ml. corresponded to 1–5 g. tissue.

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 thoroughly mixed with absolute ethyl alcohol (10 ml. per g.); the deposit was separated and washed with alcohol, and the alcoholic solutions finally evaporated 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; the clear fluid was neutralized and used for the test.

Saliva.—To each 9 ml. absolute alcohol, acidified by few drops of dilute HCl, 1 ml. saliva was added drop-by-drop, the alcoholic solution being shaken thoroughly after each addition and for sufficient 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 extracting 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 later 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 due to the presence of the drug in the corresponding fluid. As for the tissues, a control extract was prepared from the corresponding

tissue of a control animal. 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 *d*-tubocurarine chloride added to the various tissues and biological fluids was tried. Table I shows that the recoveries of known amounts added to blood, urine, and saliva were satisfactory. In Table II the recoveries of known amounts added to the various tissues are recorded.

TABLE I

RECOVERY 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	0
Rabbit's ,,	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	0
Rabbit's ,,	2.0	1.90	+5
	1.0	1.15	−15
Rat's ,,	1.0	1.0	0
	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

TABLE II

RECOVERY 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

The fate of tubocurarine in man

This was studied in five subjects, one conscious man and four patients undergoing surgical operations and kept under cyclopropane-oxygen anaesthesia. The study was made by determining the blood levels of the drug at various intervals after 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 biological fluids: i.e., the saliva, gastric juice, and cerebrospinal fluid. All control samples were collected before the injection, then the drug, tubarine "B.W.," was injected intravenously in a dose of 0.2 mg./kg. The patients were in the second plane of anaesthesia. Blood samples were drawn on the third, fifteenth, and thirtieth minutes after the injection. Urine specimens were collected at hourly intervals after the injection. Furthermore, in the conscious subject, a sample of saliva was collected on the twenty-first minute. The spinal canal was tapped and a sample of cerebrospinal fluid drawn thirty-three minutes after the injection, and ten minutes later a specimen of the fasting gastric juice was aspirated.

In Fig. 1 the average levels of the drug in the blood are presented. From this curve it may be noticed that: (*a*) an average concentration of about 4 μg. per ml. plasma, occurring three minutes after

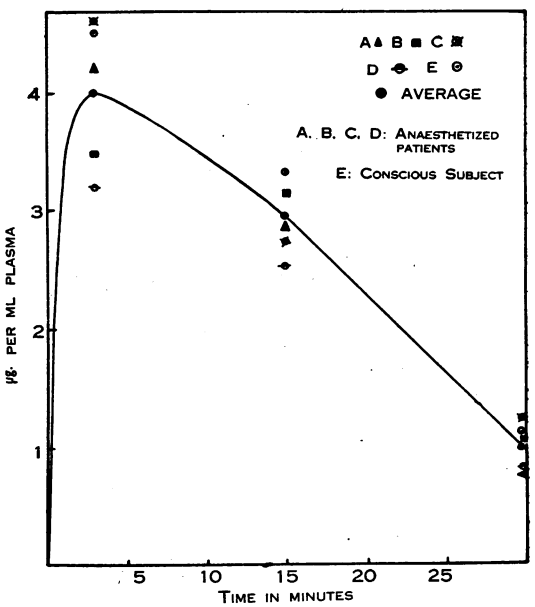

FIG. 1.—Plasma concentrations of tubocurarine in man after the intravenous administration of 0.2 mg./kg.

the injection, seems desirable for the production of full muscular paralysis, providing adequate relaxation for surgical procedures. This corresponds in the conscious subject to the complete classical picture of curarization; (*b*) fifteen minutes after the injection, when the muscles begin to regain their tone, and in the conscious subject their power, the corresponding average concentration is about 2.6 μg. per ml. plasma; and (*c*) half an hour after the injection, when there was apparent recovery from the drug effects, a level of about 1 μg. per ml. plasma was reached.

The various volumes of distribution of the drug at the specified intervals are given in Table III.

TABLE III

VOLUMES OF DISTRIBUTION OF TUBOCURARINE IN MAN

Dose: 0.2 mg./kg. i.v.

Time after dose (min.)	Mean concentration in plasma (mg./ .)	$\text{Log}\left(\frac{\text{mean concen.}}{\text{dose (mg./kg.)}}\right)$	Volume of distribution l./100 kg.
3	4.0	1.30	5
15	2.6	1.11	7.7
30	1.0	0.7	20

In the conscious subject the drug was detected in the saliva in a concentration of 1.2 μg. per ml. twenty-one minutes after the injection; and in the C.S.F. in a concentration of 2.5 μg. per ml. thirty-three minutes after the injection. About 12 per cent of the dose injected was recovered from this subject's gastric juice.

The gastric excretion of tubocurarine was also demonstrated in cats in the following way:

In spinal cats, previously starved for 15–18 hours, the stomach was washed with warm saline solution, tied at the cardia, and filled with 80 ml. saline through a cannula tied into the pylorus. Adequate artificial ventilation was maintained. The drug was injected in a dose of 0.2 mg./kg. intravenously through a cannula connected to the femoral vein. One hour after the injection a sample of saline was withdrawn from the stomach and examined for its tubocurarine content. Two such experiments were performed; in one, the total excretion was equivalent to 19.4 per cent of the dose injected (cat ♂ 2 kg.) and in the other (cat ♀ 2 kg.) to 14 per cent.

Renal excretion.—The tubocurarine equivalents of the hourly samples of urine obtained from the five human subjects are shown in Table IV.

TABLE IV

RENAL EXCRETION OF TUBOCURARINE IN MAN

Dose: 0.2 mg./kg. i.v.

Subject	Dose mg.	Tubocurarine equivalent of urine: mg.					Total excretion as % of dose given
		1st hr.	2nd hr.	3rd hr.	Next 3 hrs.	Total	
A.	14	2.82	1.24	0.50	0	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	0	4.06	33.8
D.	15	2.6	2.02	1.50	0	6.12	40.8
E.	15	3.0	1.4	1.0	0	5.4	36.0

TABLE V

BLOOD CONCENTRATIONS AND VOLUMES OF DISTRIBUTION OF TUBOCURARINE IN RABBITS

Dose: 0.12 mg./kg. i.v.

Rabbit		Tubocurarine concentration mg./l. of plasma at times stated after dose		
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
$\text{Log}\left(\frac{\text{mean concen.}}{\text{dose (mg./kg.)}}\right)$		1.262	1.097	0.92
Volume of distribution l./100 kg.		5.4	8.0	12.0

The fate of tubocurarine in the rabbit

The drug was injected intravenously in a single dose of 0.12 mg./kg. body weight. At the 2nd, 10th, and 15th min. after the injection blood samples were collected and the plasma separated as usual. Urine samples were usually collected by a sterile catheter at the end of the 2nd, 4th, and 7th hours after the injection. All samples were extracted and examined for their curariform activity. Six rabbits of both sexes were used.

In Table V the blood concentrations and volumes of distribution at the stated intervals after the administration of the drug are shown. Fig. 2

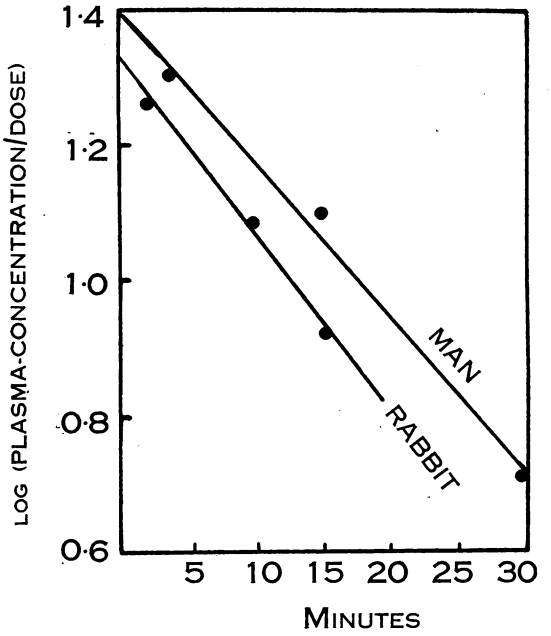

FIG. 2.—The concentration-dose relationship after intravenous administration of tubocurarine (0.12 mg./kg.) in the rabbit and (0.2 mg./kg.) in man.

represents the relationship between time and the logarithm of the ratio: $\left(\frac{\text{mean conc. in plasma}}{\text{dose: mg./kg.}}\right)$ for man and the rabbit. In this Fig. it may be noted that the ordinate corresponding to zero time for man is 1.38 or log 24; the volume of immediate distribution is thus estimated as 100/24 or 4.2 per cent, which is probably about equal to the plasma volume. For the rabbit the volume of immediate distribution calculated in the same way was 4.7 per cent. This is consistent with the finding that the drug does not pass into the blood cells. It disappears from the plasma exponentially, with a halving time of about 13 min.

The average total excretion of the drug in the rabbit's urine was found to be 35 per cent of the dose given; of this about 23 per cent was excreted in the first two hours and 12 per cent in the second two hours. Samples collected at the end of the seventh hour were free from the drug.

Distribution in rabbit's 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 after the intravenous injection of 0.17 mg./kg. the animals were killed by stunning and bleeding, and their organs removed and extracted. Extracts of brain, kidneys, liver, and voluntary muscles were examined, and the results are shown in Table VI.

TABLE VI

TUBOCURARINE-EQUIVALENTS OF RABBIT'S TISSUE EXTRACTS

Rabbits killed 10 min. after the intravenous administration of 1.17 mg./kg.

	Voluntary muscle	Brain	Kidneys	Liver
Tubocurarine equivalent (μg./g.):	0.16 0.15	0.12 0.15	1.6 2.5	0.10 0.13
Total equivalent of organ (μg.):	144 135	1.27 1.50	25.6 35.0	9.5 11.44
Percentage of dose in organ:	42 40	0.37 0.44	7.5 10.3	2.8 3.4
Average % of dose in organ:	41	0.41	8.9	3.1

In one rabbit the muscle extract was prepared from neck muscles and in the other rabbit 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 per cent of the total body weight.

Oral administration

Rats of both sexes weighing between 200 and 280 g. were used, and tubocurarine was administered by a stomach tube after the animal had been starved over-night. The results are shown in Table VII.

TABLE VII

FATE OF TUBOCURARINE GIVEN BY STOMACH TUBE TO RATS
(No drug detected in faeces)

Dose mg./kg.	Number of rats	Effects	Urine % of dose
10	5	None seen	Nil
25	5	,, ,,	,,
30	2	Variable paralysis	0.15
35	5	,, ,,	0.1
40	3	Severe paralysis	0.2
42	5	Severe paralysis and death	
45	3	do.	—

From these experiments it became clear that the drug is absorbed from the gastro-intestinal tract. In an attempt to localize the site of absorption from this tract the following experiments were performed.

Group I rats.—Each animal in this group 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. Then a stomach tube was passed through the mouth, with the animal still under anaesthesia, and tubocurarine injected through the tube into the stomach. The abdominal incision was then stitched up quickly and the animal allowed to recover from the anaesthesia. The presence of large amounts of the drug (50, 100, and 120 mg./kg. body weight) introduced into the stomach in this way was without any obvious effects for a period of two hours, after which the animal was painlessly killed. Two animals were given each of the lower doses and four the higher dose. The weight of these rats ranged between 200 and 260 g. When the animal was killed 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.—Each animal was starved over-night and in the morning a median abdominal incision made under ether anaesthesia and the duodeno-pyloric junction secured. 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 loop placed around the duodeno-pyloric junction. The drug was 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. The laparotomy incision was then quickly stitched up and the animal allowed to recover from the anaesthesia.

On recovery from anaesthesia, these rats were observed to pass quickly into a typical condition of curare paralysis of variable severity. In six rats weighing between 200 and 250 g. when the dose of tubocurarine left inside the intestine was over 3 mg./kg. (3.5 mg./kg. in four rats and 4 mg./kg. in two rats), paralysis was very severe and progressed to complete respiratory arrest and death in 3–8 min. Doses of 2–3 mg. per kg. invariably produced a certain degree of paralysis of variable severity, starting about 4–6 min. after the internal administration. This paralysis extended over a period of 15–25 min. and was severer when the dose left inside the intestine was 3 mg./kg. Four rats weighing between 200 and 260 g. were used for each dose level.

It was also noticed that the duration of action of the drug was rather short, although absorption started fairly soon. It was suspected that the pancreatic juice might be causing inactivation of the drug. In order to investigate this the pancreatic juice of a cat, prepared by the method recommended by Sherrington (1919), was incubated with tubocurarine at 37° C. and the curariform activity of the mixture evaluated at the end of two hours. No loss of the tubocurarine content of the mixture was detected. The pancreatic juice thus does not appear to catalyse the destruction of tubocurarine.

The effect of water diuresis

In these experiments the tubocurarine-equivalent in the urine of a group of rats was determined after the intramuscular administration of 0.3 mg. tubocurarine per kg. These rats were starved over-night, and on the following day they were injected with the same doses of the drug, just after they had received 50 ml. water per kg. body weight by a stomach tube, and the tubocurarine equivalent of their urine was again determined. Three groups of rats, A, B, and C, each containing three male rats, were used. The total weight of group A was 745 g., of B 750 g., and of C 730 g. In all groups urine specimens were collected at the end of the fifth hour and the ninth hour after the injection; the second samples were inactive, but the fifth hour samples showed curariform activity. When the total tubocurarine-equivalents of the urine were calculated, in each case, before and after the water diuresis, an increase in the total equivalent was noticed to have occurred during the water diuresis. These results are shown in Table VIII.

TABLE VIII

URINARY EXCRETION OF TUBOCURARINE IN RATS, WITH AND WITHOUT WATER DIURESIS

Dose: 0.3 mg./kg. intramuscularly

Rats	Urine volume (ml. in 5 hr.)	Total tubocurarine-equivalent excreted (μg.)	% of amount administered
Group A.	5	44.7	20
Group B.	3.8	52.8	23.5
Group C.	4.5	39.4	18
After 50 ml. water/kg. body wt. by stomach tube:			
Group A.	12.5	68.2	30.6
Group B.	15	63	28
Group C.	18	67.8	31

It was also noticed that although the kidneys seem to be an important organ in the elimination of the drug, renal damage did not prevent full recovery of the animal from the paralytic effects of the drug. This was illustrated by a series of experiments on doubly nephrectomized rats, where it was observed that the removal of both kidneys did not seriously affect the reactions of the animals to tubocurarine. Such animals were apparently able to cope with paralytic doses of the drug (0.3 mg./kg. body weight intramuscularly), and, although the average duration of action of the drug was increased by about 30 per cent, the recovery of the animals by the end of this period was almost complete. Hepatectomy (about 75 per cent of the liver) did not appreciably affect the sensitivity of rats to the drug.

TABLE IX

BALANCE SHEET, SHOWING RECOVERY OF TUBOCURARINE IN MICE

Dose: 0.2 mg./kg. i.v.

Time in hours	Wt. of mice (g.)	Dose per mouse (mg.)	Per cent recovery		
			Mice	Excreta	Total
0	20, 20	0.004	92	0	92
	22, 22	0.0044	93	0	93
1	20, 20	0.004	76	0	76
	25, 25	0.005	80	0	80
4	22, 22	0.0044	20	30	50
	25, 25	0.005	10	22	32

A balance sheet for tubocurarine.—This was constructed from experiments on mice, in which the drug was injected intravenously in a dose of 0.2 mg./kg. Pairs of male animals weighing between 20 and 25 g. were used and the excreta were collected at variable intervals after the injection. The animals were killed and minced, and the tubocurarine equivalent of the extracts of the mince and of the excreta determined. Table IX shows the relationship between the amounts in the mice and in the excreta and the percentage recovery of the dose.

Discussion

The various stages of tubocurarine paralysis could be correlated with the concentrations of the drug in the plasma, though the concentrations at the neuromuscular junction must be more intimately related to these effects. The degree of this paralysis varies widely in different individuals (Gray and Halton, 1948). In the conscious human subject no apparent changes in the sensations were observed to follow the injection of tubocurarine; this was also observed by Prescott *et al.* (1946) and by Smith *et al.* (1947), although it has been reported by Whitacre and Fisher (1945) that intocostrin produces general anaesthesia. The presence in the C.S.F. of this subject of curariform activity equivalent to 2.5 μg. tubocurarine per ml. may be of clinical interest. It occurred at a time when the concentration of the drug in the plasma was about 1 μg. per ml. Everett (1948) has shown that when the drug is brought into direct contact with the central nervous system in a sufficient concentration, it is liable to set up convulsions of central origin. The occurrence of violent convulsions after the intravenous administration of tubocurarine in a case of schizophrenia was reported by Morrison (1948); this may have been due to a greater leakage of the drug from the vessels of a pathological central nervous system. The drug is excreted by the salivary glands and gastric mucosa. The excretion of curarine along these channels was reported by Koch (1870), and von Huber (1922) drew attention to this fact. In this respect, tubocurarine is behaving in a similar way to some heavy metals and alkaloids, e.g., morphine. The amounts of tubocurarine excreted this way, however, are insufficient to produce poisoning after reabsorption.

The renal excretion of tubocurarine after an intravenous injection is a relatively slow process. In hourly samples taken from man it was possible to detect it in the urine three hours, and sometimes four hours, after such an 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 effect; some of the previous dose is probably still in the system. In the rabbit, as early as ten minutes after the intravenous administration of the drug, the apparent tubocurarine content of the kidneys per gramme of tissue weight was already higher than that of other organs, where the drug seemed to be uniformly distributed.

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. In rats, 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 complete. Partial hepatectomy

did not appreciably increase the sensitivity of these animals to tubocurarine. It was also concluded by Rothberger and Winterberg (1905), and later by Polimanti (1914), that the liver plays no part in detoxicating the drug.

These findings agree with recent clinical observations by Wall (1947) that renal and hepatic damage do not necessarily constitute a serious contradiction 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 inactivated in the body, since 60 per cent of the dose injected disappeared in 4 hours. The site of this inactivation is unknown. It may perhaps occur in voluntary muscle which was found to contain 40 per cent of the dose in the experiments on rabbits.

It is almost a popular belief that curare 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 reached. Bernard (1857) showed that the drug given by mouth to dogs was not destroyed by the gastric juice.

Here it was noticed that, within a certain range of dosage, typical paralytic effects were produced when tubocurarine was given by stomach tube to rats, thus indicating absorption. However, the presence of large amounts of the drug in the stomach alone (over 100 mg./kg. body weight) was without any obvious effects on the animal; this was probably due to lack of effective absorption from this organ. When the drug was introduced directly into the small intestine, in a much smaller dose (2–3 mg./kg.), signs of absorption developed rather rapidly and progressed fatally with a slight increase of this dose.

It is possible that with such drugs, producing obvious characteristic signs within a short period 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 (2–3 mg./kg.) thus absorbed were, however, of short duration, since in 15–25 min. the animal 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 intestines. Clement and Pistorio (1928) showed that bile and bile salts could precipitate the alkaloid from curare.

The direct administration of the drug into the intestine reduced the size of the effective dose by mouth more than tenfold. It might be possible to imitate this clinically 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 its action when so absorbed may limit the clinical value of the drug administered that way.

Summary

1. The method described by Jalon for estimating tubocurarine by its action on the frog's rectus abdominis 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 exponentially with a halving time of about 13 min. These conclusions apply both to man and to rabbits.

3. About 20–40 per cent 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.

4. The main route of disappearance of the drug from the body does not depend on the kidneys. By extracting whole mice it was shown that about 60 per cent of the dose was inactivated in the body within four hours. 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 per cent of the dose in an experiment on rabbits.

5. 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 (12–19 per cent of the dose) may be excreted into the stomach.

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