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# THE ACTION OF D-TUBOCURARINE CHLORIDE ON FOETAL NEUROMUSCULAR TRANSMISSION AND THE PLACENTAL TRANSFER OF THIS DRUG IN THE RABBIT

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The employment of curare to procure muscular relaxation during anaesthesia is no longer a novelty, and there are many clinical reports of its widespread use in surgery and the treatment of some spastic conditions. However, before curare may be used, as an adjunct to anaesthesia during Caesarian section, definite information is desirable concerning the action of curare on foetal neuromuscular transmission and the permeability of the placental barrier to curare.

Preyer (1885) believed that the foetal neuromuscular junction did not show adult characteristics until the end of the gestation period. His crude curarine paralysed newborn guinea-pigs and post-mature foetuses, but doses adequate to curarize an adult guinea-pig failed to paralyse full-term or more immature foetuses. Other workers have assumed that curare blocks transmission at the foetal neuromuscular junction in a manner exactly comparable to the effect of the drug on voluntary muscle in the adult animal. Coghill (1934) studying the development of somatic movements in the killi fish (*Fundulus heteroclitus*), Gonzalez (1935) investigating the innervation of skeletal muscle in rat foetuses, and Kuo (1939) in a study of the development of the nervous system in chick embryos, all used crude curarine preparations to distinguish between the myogenic and neurogenic origin of motor activity. The paralyses observed might have been due to handling of the material and the subsequent development of anoxia, for in mammalian experiments the integrity of the placental circulation is readily impaired by handling of the uterus.

Preyer was the first to record observations upon the placental transfer of curare. In guinea-pigs the crude drug passed very readily from the foetal to the maternal circulation, but as his embryos were not susceptible to curare he possessed no means of testing whether it would cross the placental barrier in the

reverse direction. However, following the introduction of curare into anaesthesia by Griffiths & Johnson (1942), its use in Caesarian section revives this question of the transfer of the drug from the maternal to the foetal circulation, together with the problem of the susceptibility of the human foetus to curare. In two recent clinical accounts, standard preparations of curare have been used. Whitacre & Fisher (1945) published observations on one hundred Caesarian sections, anaesthetized with cyclopropane in which muscular relaxation was obtained with Squibb's Intocostrin. As a 'slight depression' of the infant was encountered more frequently than in babies from Caesarian sections where a spinal or local anaesthetic alone was used, they concluded that the use of curare in these cases needed further investigation to assess its safety. Gray (1947) recorded a further thirty cases in which he induced anaesthesia in the mother with Kemithal and maintained it with cyclopropane. Muscular relaxation was produced by 15 mg. D-tubocurarine chloride (Burroughs Wellcome 'Tubarine'). He found that the infants cried lustily as soon as the head was delivered and therefore considered the full-term foetus insusceptible to curare.

Existing evidence concerning the action of curare on foetal neuromuscular transmission is therefore limited and discordant; these discrepancies may be ascribed to the use of crude curare preparations of varying composition and potency and to the existence of foetal anoxia. The present observations were made to determine the action of a standardized preparation, D-tubocurarine chloride, on foetal neuromuscular transmission and to investigate the passage of this drug across the placental barrier from the maternal to the foetal circulation and in the reverse direction.

## METHODS

### *Action of D-tubocurarine chloride on foetal neuromuscular transmission*

This was investigated by two methods; first, by intravascular injection of lively foetuses having a good placental circulation; and secondly, by observations on isolated phrenic-nerve-diaphragm preparations. The experimental material consisted of 28–31-day rabbit foetuses, 50–60-day guinea-pig foetuses and 20–22-week human foetuses.

*Intravascular injection.* Pregnant rabbits were anaesthetized with urethane given intravenously, 2 g./kg. body wt.; the arterial blood pressure was recorded with a mercury manometer throughout the observations, since it had been noticed previously that if the blood pressure fell below 80–70 mm. Hg the foetuses soon became moribund. The pregnant guinea-pigs were also anaesthetized with urethane given intraperitoneally (2 g./kg. body wt.); the arterial blood pressure was not recorded. A foetus was located and carefully exposed through a longitudinal incision made in the uterine wall from crown to rump of the foetus; in this way there is very little disturbance of the rest of the uterus and little bleeding, as the uterine wall contracts down around the incision. The placenta was examined quickly and judged to have an adequate circulation on both the maternal and the foetal side if the colour was a characteristic purple red and the umbilical vein noticeably more red than the umbilical arteries. During the examination of the foetus it was held so that its attachment with the placenta was in no way strained. A few of the experiments were performed in a saline bath and these results were in complete agreement with the others. A total of twelve rabbit foetuses and six guinea-pig foetuses received doses varying from 10 to 100 $\mu$g. D-tubocurarine chloride, in 0·1 ml. saline, injected into the umbilical vein. When the foetuses became quiescent the phrenic and sciatic nerves were exposed and pinched with fine forceps. This test for neuromuscular

transmission was chosen to obviate any spread of stimulus to the muscle. Finally, the corresponding muscles were stimulated directly with weak induction shocks.

*Isolated phrenic nerve diaphragm preparations.* These preparations from rabbit and guinea-pig foetuses are about two-thirds the size of the average rat diaphragm preparation, the muscle is thinner and the nerve very delicate. Observations were made at 37° C. with the muscle immersed in Tyrode containing 0·2 g.% glucose, adjusted to pH 7·4–7·6 with N/10 HCl, and aerated with oxygen containing 5% $CO_2$. The adjustment of pH, together with aeration by oxygen and carbon dioxide, was essential to the success of the preparation, which under these conditions was extremely robust, lasting 4–6 hr. The method of Holmes, Jenden & Taylor (1947) was used to suspend the muscle so that it could be stimulated directly while immersed. A 2 mfd. condenser charged to 36–120 V. was used for stimulation. The nerve lay over electrodes held just above the surface of the Tyrode and was stimulated from 0·01 mfd. condenser charged to 12 V. The muscle and nerve were stimulated alternately every 10 sec. D-Tubocurarine chloride was added in concentrations varying from 1 to 10 $\mu$g. per ml. of bath and the time noted for the onset of neuromuscular block and the completion of the block. These times were compared with those for the action of the same concentration of the drug upon the adult rat phrenic nerve diaphragm preparation. The activities of prostigmine and potassium ions as antagonists to the action of curare were also investigated.

Four 20–22-week human foetuses were obtained from termination of pregnancies. Phrenic-nerve-diaphragm preparations are readily made, and the muscle is found to be about four times as thick as the adult rat preparation, and the nerve about double in diameter. The preparations were set up in the bath at intervals ranging from 1½ to 3½ hr. after removal of the foetus from the uterus. Initially, in each case, the muscle responded poorly to direct stimulation and not at all to stimulation of the phrenic nerve. A full response was not obtained by either method of stimulation until the preparation had been in Tyrode for 1 hr., thereafter it responded well for 17–20 hr.

*Placental transfer of curare*

The passage of D-tubocurarine from the maternal to the foetal blood and from the foetal to the maternal blood stream was investigated in does 28–31 days pregnant. The general experimental procedure was similar to that described above with the following differences:

*Transfer from maternal to foetal circulation.* Before the initial injection of curare into the mother, one foetus was examined as a control and the simple pinch test for neuromuscular transmission performed. This foetus was then discarded and the placenta gently pulled away from the uterine wall which contracted down in this area with very little bleeding. The doe received an initial dose of 0·5–0·75 mg. D-tubocurarine intravenously, which was just sufficient to inhibit respiratory movements completely in the average 3 kg. rabbit for ¾ hr. Subsequently, 0·5 mg. D-tubocurarine was given intravenously at half-hourly intervals after the first injection. The success of the experiment depended upon keeping the maternal blood fully oxygenated: therefore oxygen in a closed circuit was used in maintaining artificial respiration. Foetuses were examined at half-hourly or hourly intervals, throughout the average 3 hr. experimental period, after the first injection of curare.

*Transfer from the foetal to the maternal circulation.* The maternal respiratory movements were recorded from the diaphragm slip; contractions of the gastrocnemius muscle, elicited by stimulation of the peripheral end of the sciatic nerve, were recorded at 10 sec. intervals. (The discharge from a 2 mfd. condenser charged to 36–120 V. was used for stimulation.) A foetus was exposed and 0·5–4 mg. D-tubocurarine in 0·1 ml. was injected into one umbilical artery towards the placenta. As the needle was withdrawn the artery was ligatured above and below the puncture to prevent leak of either blood or curare. The foetus was carefully replaced and the abdominal wound closed. After about half an hour the placenta was inspected to make sure that it was still adherent to the uterine wall and that there was no leak of either maternal or foetal blood.

RESULTS.

*Action of* D-*tubocurarine chloride on foetal neuromuscular transmission*

*Intravascular injection.* 10–100 μg. D-tubocurarine chloride intravenously caused complete paralysis in 2–3 min. both in rabbit and guinea-pig foetuses. This is slower than the onset of paralysis in the adult animal, and is possibly due to slowing in the cardiac rhythm and a relatively slower circulation in the foetus caused by haemorrhage from the umbilical vein which occurred, however sucesssful the injection. D-Tubocurarine (10 μg.) injected intravenously in a 50 g. foetus is weight for weight equivalent to the 0·5–0·75 mg. D-tubocurarine chloride which is just sufficient to inhibit respiration in the average 3 kg. rabbit.

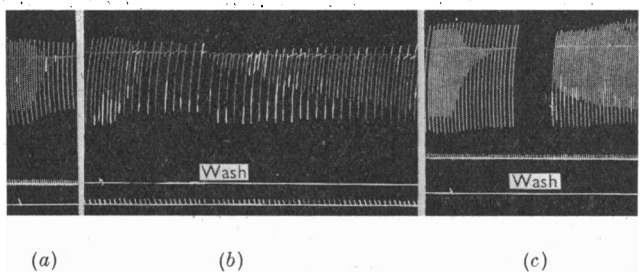


Fig. 1. Phrenic nerve and diaphragmatic muscle stimulated alternately every 10 sec. with condenser discharges. (*a*) 57-day guinea-pig foetus; D-tubocurarine 2 μg./ml. in bath. Complete paralysis in 3½ min. (*b*) 31-day rabbit foetus; D-tubocurarine 2 μg./ml. in bath. Complete paralysis in 2½ min. (*c*) 28-day rabbit foetus; D-tubocurarine 1 μg./ml. in bath. Complete paralysis in 5 min. In all figures 10 sec. time is marker in every experiment.

Ten minutes after delivery by Caesarian section, one 22-week human foetus, weighing 550 g., received an injection of 2 mg. D-tubocurarine chloride into the umbilical vein, which was followed by 1 mg. into the jugular vein 5 min. later. Initially, the cord was pulsating 92/min. but otherwise the foetus was not very lively, responding only by sluggish twitches to tapping of the skin. Pinching the freed sciatic produced a sustained contraction from the gastrocnemius which was abolished 2 min. after the intrajugular injection of D-tubocurarine. The pulse rate at this time had fallen to 76/min. This foetus received weight for weight about thirty times the threshold dose required to inhibit respiration in rabbit and guinea-pig foetuses.

*Isolated diaphragm phrenic nerve preparations.* Typical results for rabbit and guinea-pig foetuses are shown in Fig. 1. The height of the maximal response to direct stimulation of the muscle was always slightly greater than the maximal response to indirect stimulation. The average time for complete neuromuscular block was 5 min. with a concentration of 1 μg. D-tubocurarine/ml. in the bath and 2½ min. with a concentration of 2 μg./ml. These times are reproducible and identical with those for the adult rat diaphragm-phrenic-nerve preparation.

In all preparations, however, complete paralysis is always slower in onset following the first dose of D-tubocurarine.

In all four human preparations paralysis was slow in onset and in reaching completion (Fig. 2); complete recovery when the bath was changed took 1–1½ hr. To support the view that these results were due to delay in penetration through

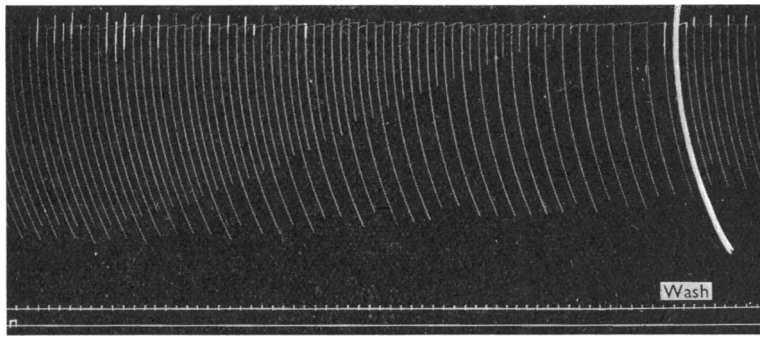


Fig. 2. Phrenic nerve and diaphragmatic muscle stimulated alternately every 10 sec. with condenser discharges. 22-week human foetus; D-tubocurarine 5 μg./ml. in bath; complete inhibition in 10 min.

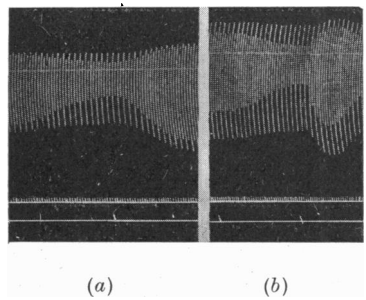


Fig. 3. Phrenic nerve and diaphragmatic muscle stimulated alternately every 10 sec. with condenser discharges. 55-day guinea-pig foetus. Reversal of paralysis due to 20 μg. D-tubocurarine in 40 ml. bath, (*a*) 5 μg. prostigmine, (*b*) 28 mg. potassium chloride.

the thicker connective tissue and muscle, preparations of approximately the same size were made from 1 kg. rabbits; even when freshly prepared these diaphragms did not give any regular response to either direct or indirect stimulation, and after 1 hr. they ceased to respond.

Each record shows that D-tubocurarine chloride apparently causes a small decrease in the response of the muscle to direct stimulation, but this is of doubtful significance since direct stimulation of the muscle immersed in Tyrode is not perfectly satisfactory; when the diaphragm is stimulated the stimulus must also excite the phrenic nerve as it enters the muscle and there is frequently summation of these two stimuli. The optimal stimulus for the muscle had always

to be found in the presence of curare. The neuromuscular block caused by D-tubocurarine was reversed by prostigmine and potassium ions in diaphragms from rabbit, guinea-pig and human foetuses. Fig. 3 shows the reversal of paralysis, due to 20 $\mu$g. D-tubocurarine in a guinea-pig preparation, by 5 $\mu$g. prostigmine and 28 mg. potassium chloride. Here again there is complete agreement with the results in the mature animal.

*Placental transfer of curare*

*Transfer from maternal to foetal circulation.* Transfer of curare from mother to foetus was never demonstrated; foetuses were very lively when exposed, provided the maternal blood pressure did not fall below 80 mm. Hg, but they became quiescent or moribund if the maternal blood pressure fell below 70 mm. Hg for so short an interval as 10 min.

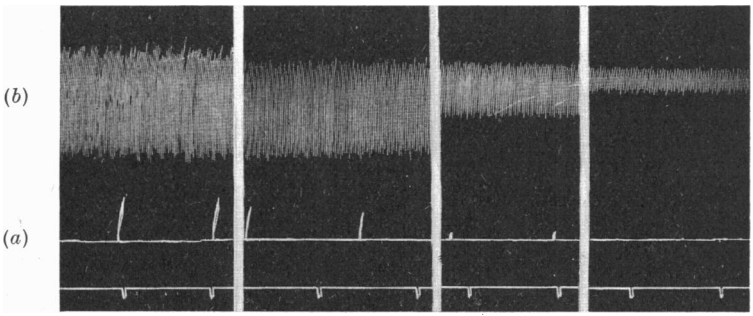


Fig. 4. Effect of 1 mg. D-tubocurarine injected into umbilical artery in the rabbit. (*a*) Response of maternal gastrocnemius muscle to stimulation of sciatic nerve, inhibited in 11 min.; (*b*) movements of diaphragm slip. Maternal blood pressure 110–120 mm. Hg. Intervals represent 5, 2 and 2 min.

*Transfer from the foetal to the maternal circulation.* The passage of D-tubocurarine from the foetal blood to the maternal blood stream was shown in seven pregnant rabbits and a typical result is shown in Fig. 4, where 0·5 mg. D-tubocurarine injected into the umbilical artery of one of the foetuses was sufficient to inhibit the response of the maternal gastrocnemius muscle to stimulation of the sciatic nerve; 1·0 mg. D-tubocurarine was necessary to decrease her respiratory movements. Inhibition of these responses was complete in times varying from 5 to 30 min. after the foetal injection. Transfer of the drug took place equally well whether the maternal blood pressure was 40 or 100 mm. Hg, but the excitability of the uterine muscle was very important since transfer took longer in two rabbits, 31 days' pregnant, in which very active contraction of the uterus accompanied any handling, and in one experiment no demonstratable quantity of D-tubocurarine crossed into the maternal blood stream during 1 hr.

## DISCUSSION

In the present series of experiments there was no demonstrable difference between the sensitivity of the adult and that of the late-term rabbit or guinea-pig foetus to D-tubocurarine injected intravenously. Moreover, the sensitivity of both these isolated foetal rabbit and guinea-pig diaphragm-phrenic-nerve preparations is very similar to those of the adult rat to D-tubocurarine, which confirms Wien's (1948) results that so far as this substance is concerned there is little species difference. Indeed, since these late term foetuses are capable of complicated and co-ordinated reflex movements, the pathways for which are laid down early in development, it would be expected that the motor end-plate should possess properties similar to those of the adult. The increased excitability of the muscle produced by both prostigmine and potassium and the antagonism of prostigmine and potassium ions to the paralysis caused by curare in these foetal muscle-nerve preparations, further bears this out.

Although the 20–22-week human foetuses studied were, developmentally, very much younger than the animal material, similar blocking of the myoneural junction by curare was observed, together with the reversal of this paralysis by prostigmine and potasssium ions. The human diaphragm-phrenic-nerve preparation had a lower sensitivity to the drug than any of the others studied; histological preparations of this human muscle show that it is about four times as thick as the foetal rabbit and adult rat diaphragms examined and surrounded by a very much thicker connective tissue sheath, both of which would delay penetration of the drug. The one observation on a 22-week human foetus also showed that neuromuscular transmission was abolished by intravenous D-tubocurarine, though the total dose, 2 mg. into the umbilical vein plus 1 mg. into the jugular vein, was thirty times that required to paralyse rabbit and guinea-pig foetuses. In contrast, Bourne (1947) reported that 2·5 mg. D-tubocurarine injected into the umbilical vein in one 19-week human foetus produced tetanic spasm of the flexor muscles of the limbs which was followed by death of the foetus a few minutes later. Convulsions have been observed in the cat after intracisternal and intrathecal injection of D-tubocurarine (von Euler & Wahlund, 1941).

The absence of transfer of D-tubocurarine from the maternal to the foetal blood stream in rabbits in sufficient concentration to cause any paralysis of the foetal muscles is in accordance with the clinical experience of Gray (1947) who used D-tubocurarine for abdominal relaxation during Caesarian section, and the observation made by Harroun, Beckert & Fisher (1947) who performed a Caesarian section in a bitch, of unspecified weight, that had received 60 mg. curare. (0·5–0·75 mg. D-tubocurarine given intravenously in a 3 kg. rabbit is equivalent weight for weight to the 15 mg. injected intravenously in the average 60 kg. woman prior to Caesarian section, and represents a concentration of

3 $\mu$g./ml. circulating blood; this dose is sufficient to inhibit respiratory movements in the urethanized rabbit rapidly and completely, but in the anaesthetized human there is only slight depression of respiration accompanying the immediate muscular relaxation.) The duration of the average Caesarian section up to the time when the child is delivered is 20–30 min., and the placental circulation must be good until the uterus is incised. In our experiments, it might be objected that the placental circulation was poor and that no curare reached the foetal villi. However, this view may be discounted on several grounds. First, the placentae were a good purple red in colour with the umbilical vein markedly more red than the umbilical arteries; secondly, the foetuses, when exposed, were lively throughout the experimental period. Finally, in some experiments radioactive phosphorus was given intravenously, as the disodium hydrogen salt, with the first injection of D-tubocurarine to the mother and followed by a further dose after 1 hr. (a total of 25 $\mu$c. $P_{32}$ equivalent to not more than 0·8 mg. total phosphorus). Radioactive phosphate was transferred across the placental barrier in every case and the foetal blood levels during the experimental period averaged 88% of the maternal concentration. This evidence indicates that the placental circulation was intact during the experimental period and it thus appears that a circulating drug would have ample opportunity to come into contact with the foetal placenta. The absence of curarization of the foetuses, does show that this structure is an effective barrier against the passage of D-tubocurarine from the maternal to the foetal blood stream. No estimation of the circulating D-tubocurarine, nor that present in other body fluids in the mother or foetus has yet been attempted. The rapid disappearance of the drug from the maternal circulation, the presence of an inhibitor in the blood stream or at the placental barrier might all account for the absence of its appearance in the foetal blood stream. Again, there may be a very powerful inhibitor present in the foetal blood stream, or the drug may pass the barrier so slowly that it is excreted by the foetal kidney before an effective circulating concentration can accumulate.

The ready passage of D-tubocurarine from the foetal to the maternal blood stream amply confirms Preyer's results in guinea-pigs towards the end of the gestation period, but our observations differ from his in one respect. Very high concentrations of curare must of necessity be present in the foetal blood for sufficient to cross the barrier to cause any demonstratable block on the maternal side; in our experiments these large doses of curare injected into the umbilical artery always caused instantaneous and complete paralysis of the rabbit or guinea-pig foetus together with a bradycardia which was followed by complete cessation of the heart beat within 20–30 min. Preyer, on the other hand, found that his foetuses remained very active after large doses of crude curare. This passage of D-tubocurarine chloride from the foetal to the maternal blood stream has only been observed with a calculated concentration of 140 $\mu$g./ml. circulating

foetal blood (assuming that the average 50 g. foetus has a blood volume of approximately 3·5 ml.). This concentration is forty-seven times that calculated to be present in the maternal circulation with a curarizing dose, i.e. 3 $\mu$g./ml. It would be interesting to perfuse the placenta artificially on the foetal side with blood containing 3 $\mu$g./ml. D-tubocurarine in sufficient volume so that 0·5–0·75 mg. D-tubocurarine might cross the placental barrier to produce paralysis in the mother.

SUMMARY

1. The neuromuscular mechanism of 28–31-day foetal rabbits and 50–60-day foetal guinea-pigs is blocked by D-tubocurarine chloride; isolated diaphragm-phrenic-nerve preparations from these embryos are as sensitive to the drug as a similar isolated preparation from the adult rat.

2. The neuromuscular mechanism in the 20–22-week human foetus is also blocked by D-tobucurarine chloride; the isolated diaphragm phrenic nerve preparation is, however, less sensitive to the drug than the adult rat diaphragm.

3. The neuromuscular block caused by D-tubocurarine chloride is reversed by prostigmine and potassium ions in rabbit, guinea-pig and human foetal preparations.

4. D-Tubocurarine chloride given intravenously over a maximum 3 hr. period to does 28–31 days' pregnant, in a dose equivalent weight for weight to the therapeutic dose in man (3 $\mu$g./ml. blood), has not been shown to cross the placental barrier in sufficient quantity to cause respiratory arrest in the foetus.

5. D-Tubocurarine chloride crosses the placental barrier from the foetus to the mother when present in the foetal blood stream in an approximate concentration of 140 $\mu$g./ml.

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REFERENCES

Bourne, A. (1947). *Lancet* (1), 849.
Coghill, G. E. (1934). *Proc. Soc. exp. Biol., N.Y.*, **31**, 62.
Euler, V. S. von & Wahlund, H. (1941). *Acta physiol. scand.* **2**, 327.
Gonzalez, A. W. Angulo y (1935). *Proc. Soc. exp. Biol., N.Y.*, **32**, 621.
Gray, T. C. (1947). *Brit. med. J.* (1), 444.
Griffiths, H. R. & Johnson, G. E. (1942). *Anesthesiology*, **3**, 418.
Harroun, P., Beckert, F. E. & Fisher, C. W. (1947). *Surg. Gynec. Obstet.* **84**, 491.
Holmes, P. E. B., Jenden, D. J. & Taylor, D. B. (1947). Demonstrated to the Physiol. Soc.
Kuo, Z. Y. (1939). *J. exp. Zool.* **82**, 371.
Preyer, W. (1885). *Specielle Physiologie des Embryo*, pp. 218 and 448. Leipzig: Grieben.
Whitacre, R. J. & Fisher, A. J. (1945). *Anesthesiology*, **6**, 124.
Wien, R. (1948). *J. Physiol.* **107**, 44.