The Interactions of Neuromuscular Blocking Agents in Man: The Role of Hexafluorenium

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Hexafluorenium bromide, a bisquaternary ammonium compound, has marked anticholinesterase and weak non-depolarizing neuromuscular blocking effects in man. The effects of hexafluorenium alone and in combination with d-tubocurarine, gallamine, decamethonium and succinylcholine were investigated in 118 patients under light thiopental, N₂O-O₂, meperidine anesthesia by means of electromyography and recording of the force of contraction of the indirectly stimulated adductor pollicis muscle. Intravenous injection of 1.0 mg/kg hexafluorenium caused slight to moderate non-depolarizing block in 10 of 24 subjects. The hexafluorenium induced neuromuscular block was potentiated by ether anesthesia. Hexafluorenium and d-tubocurarine always increased and neuromuscular effects of each other. The interactions of hexafluorenium and gallamine were not consistent. The neuromuscular effects of hexafluorenium and decamethonium were mutually antagonistic. The previous administration of hexafluorenium markedly potentiated and prolonged the neuromuscular effects of succinylcholine. Hexafluorenium dibromide [hexamethylene-1,6-bis-(fluorenyl)-dimethyl-ammonium dibromide; Mylaxen; HFL] is a potent cholinesterase inhibitor and also has some neuromuscular blocking effect. In man, after intravenous administration, its inhibitory effect on plasma cholinesterase (PChE) is predominant (I₅₀ = 1.5 × 10⁻⁷ M). In unanesthetized subjects intravenous administration may be followed by nausea and mild abdominal cramps, but causes no bradycardia, and in anesthetized subjects usually induces a transient elevation of the pulse rate. There is a marked species variation in its neuromuscular activity. In the dog, on a mg/kg basis, it is about as potent as d-tubocurarine chloride (d-te), but in the mouse its neuromuscular blocking action is about twenty times less than that of d-te. In man its neuromuscular blocking action is clinically manifested only in relatively deep planes of ether anesthesia. Because of its inhibitory effect on PChE, which interferes with the metabolic transformation of succinylcholine dichloride (Anectine; SCh), HFL potentiates and prolongs the neuromuscular effect of SCh.

The neuromuscular effect of HFL alone, and in combination with SCh, was recently investigated by Nastuk and Karis in the isolated frog nerve muscle preparation. They concluded that the potentiating effect of HFL on the myoneural action of SCh in this preparation is not due to its anticholinesterase activity and they entertained the possibility that the sites of action (receptors) of HFL and SCh may not be identical. Katz et al. studied the effects of HFL and edrophonium (EDR) on the neuromuscular blocking action of SCh, decamethonium bromide (Syncurine; C10), hexamethylene-1,6-bis-carbaminoylcho-
line bromide (Imbretil) and d-tc. Their studies failed to demonstrate any neuromuscular blocking action of HFL in human subjects anesthetized with various anesthetic agents.

The purpose of the present study has been to ascertain whether, in the absence of deep ether anesthesia, HFL has any neuromuscular effect of its own in man, and to obtain information on the mechanism of its myoneural effects from its interaction with several other neuromuscular blocking agents.

Material and Methods

This investigation was carried out on 118 patients undergoing elective surgical procedures on the lower extremities not requiring muscular relaxation. In 116, general anesthesia was maintained with thiopental sodium, nitrous oxide (N₂O)-oxygen (O₂)-meperidine hydrochloride (Demerol); or droperidol (Inapsine)-fentanyl citrate (Sublimaze)-N₂O-O₂. In two others, thiopental induction followed by ether-O₂ anesthesia, was used.

The electromyogram (EMG), obtained from the abductor digitii quinti muscle after electrical stimulation of the ulnar nerve, was used to estimate the degree of neuromuscular block in all the experiments. In 62 subjects, the force of contraction of the adductor pollicis was also recorded through a Grass FT 03 force-displacement transducer. The subject's hand and arm were immobilized on a padded arm board. Two subcutaneous needle electrodes were placed near the ulnar nerve above the level of the medial epicondyle. Square wave stimuli of 0.2 milliseconds duration were supplied by a Grass SC 4 stimulator, isolated from ground by a CR 578 B transformer. Through two similar electrodes, inserted in the hypothenar eminence, the EMG was recorded, using a Grass 5P5 pre-amplifier, a type 5D amplifier and a pen writer. The ulnar nerve was then blocked at the medial epicondyle, with 2 per cent lidocaine hydrochloride (Xylocaine) containing 1:200,000 epinephrine, to eliminate reflexes evoked by the stimulation. When the absence of EMG response to stimulation of the ulnar nerve confirmed the effectiveness of the block, the stimulating electrodes were reinserted at the wrist in close proximity to the ulnar nerve.

The voltage required for maximal response, as indicated by the amplitude of the EMG tracing, was established. Supramaximal stimuli, of twice this voltage, were used throughout the experiments. Usually the stimulation rate was 0.1/second (one every 10 seconds). In addition, in some experiments the effects of tetanus were investigated with stimulation rates of 100/sec. applied for 20 seconds. This frequency and duration of stimuli was found to be optimal for evoking post-tetanic facilitation (PTF). * Stimulation rates of 1.0/sec. were used, to demonstrate the fatigability encountered during partial non-depolarization block.¹⁰,¹¹

For the recording of the force of muscular contraction (ergogram) the strain gauge was attached to the abducted thumb by a loop of pre-stretched, braided silk. A stable base line indicated that the resting muscle tension remained constant throughout the experiments. The recording of the ergogram through the strain gauge was accomplished with a Grass 5P1 pre-amplifier and an amplifier pen writer, similar to that used for the EMG recording.

All experiments were carried out during operation. Observations of pulse rate, blood pressure and respiratory rate were made at intervals of five minutes or less.

Respiration was assisted or controlled as indicated. All drugs were administered through the sleeve of a freely flowing intravenous infusion. In 6 experiments SC was infused by means of a Harvard infusion pump.

* Personal communication, J. C. Crul, 1964.

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**Table 1. The Neuromuscular Blocking Effects of Hexafluorium, d-Tubocurarine Gallamine, Decamethonium and Succinylcholine**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg./kg.)</th>
<th>Number of Subjects</th>
<th>Intensity of the Block*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Hexafluorium</td>
<td>1.00</td>
<td>10†</td>
<td>16.2 ± 3.8</td>
</tr>
<tr>
<td>d-Tubocurarine</td>
<td>0.15</td>
<td>12</td>
<td>38.1 ± 5.3</td>
</tr>
<tr>
<td>Gallamine</td>
<td>1.00</td>
<td>24</td>
<td>47.2 ± 6.0</td>
</tr>
<tr>
<td>Decamethonium</td>
<td>0.027</td>
<td>20</td>
<td>43.3 ± 6.3</td>
</tr>
<tr>
<td>Succinylcholine</td>
<td>0.10†</td>
<td>6</td>
<td>—</td>
</tr>
</tbody>
</table>

* Expressed as per cent decrease of the amplitude of the control EMG.
† Represents the 10 of 24 subjects in whom discernible block developed.
‡ Followed by the continuous intravenous infusion of SC at 0.03 to 0.04 mg./kg./minute.
No muscle relaxants were administered until stable tracings were obtained for at least five minutes. This period served as control for the changes induced by the subsequently administered drugs.

The neuromuscular blocking agents (and their intravenous mg./kg. dose) investigated alone, or in combination with one another, were: HFL (0.4 or 1.0 mg./kg.); d-tc (0.1 or 0.15 mg./kg.); gallamine triethiodide (Flaxedil, gal.) (1.0 mg./kg.); Sch (0.1 mg./kg.) and C10 (0.027 mg./kg.).

The effects of the relaxants were expressed as percentage changes in the amplitude of the control EMG or ergogram. In experiments where two relaxants were administered in sequence, sufficient time was allowed for the development of the maximum effect of the first drug before the second drug was injected.

Unless otherwise stated, the results reported were obtained from EMG studies. Neuromuscular block was considered slight, if the decrease in the amplitude of the EMG tracing was less than 20 per cent of control; moderate, if it was greater than 20 per cent but less than 50 per cent, and marked if it exceeded 50 per cent. In the illustrations, the ergograms are shown where they demonstrate relevant points more clearly than the EMG. It must be noted that the frequency response of the pen writer is such that EMG response to tetanic stimuli cannot be recorded with any degree of accuracy.

Results

The observations made after the intravenous administration of the various neuromuscular blocking agents alone will be discussed first. Subsequently the interaction of HFL with the other compounds will be considered.

Hexafluorurium. The intravenous administration of 0.4 mg./kg. HFL failed to produce any EMG or ergographic evidence of neuromuscular block in 9 subjects tested. HFL, 1.0 mg./kg., was administered to 24 other subjects. In 14 of these no neuromuscular effect could be observed. In the remaining 10, the mean intensity and standard error of the block was 16.2 ± 3.8 per cent (table 1). In one subject, however, there was a 50 and 90 per cent decrease in the height of the EMG and ergographic tracings respectively (fig. 1).

The characteristics of the HFL-induced neuromuscular block were studied in 5 of these 10 subjects. With stimulation rates of 100/sec. tetanus was poorly maintained and there was post-tetanic facilitation. Furthermore, increasing the stimulation rate from 0.1 to 1/sec markedly decreased the amplitude of the EMG and the ergogram. These findings indicated that HFL produces a non-depolarization or phase II 12,13 block. The results of the ergographic studies were similar to those of the EMG studies. The intravenous administration of 0.2 mg./kg. edrophonium hydrochloride (Tension), however, had only a slight antagonistic effect on the HFL block (fig. 1) in the 3 subjects tested.

In 2 subjects, anesthetized with ether, 1.0 mg./kg. HFL caused a 35 to 40 per cent and a 65 to 75 per cent decrease of the EMG and the ergogram, respectively. Severe fall of blood pressure and tachycardia developed after the administration of HFL in both subjects anesthetized with ether. In the first patient the blood pressure decreased from 140/90 to 50/0 and the pulse rate increased from 72 to 160. In the second patient the blood pressure fell from 135/85 to 95/65 and the pulse rate rose from 80 to 140. Pulse rate
Fig. 2. The neuromuscular blocking action of d-tubocurarine (DTC) and gallamine (GAL). Upper tracings: DTC (EMG and ergogram). Lower tracings: GAL (EMG and ergogram). Symbols: 1. Tetanic stimulation; 2. Rest; 3. Stimulation rate of 1/second. Note poorly sustained tetanus, post-tetanic facilitation, fatigability at stimulation rate of 1/second and the antagonistic effect of edrophonium (EDROPH).

Fig. 3. The neuromuscular blocking actions of decamethonium (C10) and succinylcholine (SCH). Upper tracings: C10 (EMG and ergogram). Lower tracings: SCH (ergogram and EMG). Symbols: 1. Tetanic stimulation; 2. Rest; 3. Stimulation rate of 1/second. Note well sustained tetanus and absence of post-tetanic facilitation and fatigability at stimulation rate of 1/second.

and blood pressure returned to pre-injection values in 30 minutes in the first, and 15 minutes in the second patient.

d-Tubocurarine and Gallamine. Eight subjects received 0.1 and 12 subjects 0.15 mg./kg. d-tc. The 0.1 mg./kg. dose had no demonstrable effect on neuromuscular transmission in 2 and caused a slight block in 6 subjects. The 0.15 mg./kg. dose caused a 38.1 ± 5.3 per cent (range 8 to 67 per cent) block (table 1). Tetanus was poorly maintained and was followed by post-tetanic facilitation. Increasing the rate of stimulation to 1/second caused fatigue. The block was reversed by edrophonium. The results of the ergographic studies were similar to those of the EMG experiments (fig. 2).

The administration of 1.0 mg./kg. gal. to
The intravenous administration of 0.1 mg./kg. SCh to 2 subjects had a slight and transient effect (fig. 3). In 6 other subjects the initial 0.1 mg./kg. dose of SCh was followed by the intravenous infusion of SCh at the rate of 0.03 to 0.04 mg./kg./minute. After the development of a steady state the amplitude of the EMG and the ergograms decreased by 40 to 96 and by 42 to 96 per cent, respectively. The total dose of SCh used in these studies did not exceed 3 mg./kg.

**Interaction of Hexafluorenum with Other Neuromuscular Blocking Agents**

*Hexafluorenum and D-Tubocurarine.* Whatever the sequence of administration of these two agents the subsequent administration of one increased the neuromuscular blocking activity of the other. In 4 experiments 1.0 mg./kg. HFL was followed by 0.1 or 0.15

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Fig. 4. The interaction of hexafluorenum (HFL) and d-tubocurarine (DTC). Upper tracing: HFL followed by DTC, (EMG). Lower tracing: DTC followed by HFL, (EMG). Symbols: 1. Tetanic stimulation with pen writer switched off. Note additive effect of HFL and DTC which is independent of sequence of administration. Post-tetanic facilitation shown in lower tracing.

24 subjects caused a 47.3 ± 6.0 per cent (range 10 to 95 per cent) decrease of the amplitude of the EMG tracing. In 15 of these, in whom the force of contraction was also recorded, the amplitude of the ergographic tracing decreased by 15 to 100 per cent. The characteristics of the gal-induced neuromuscular block, were the same as those produced by d-tc (fig. 2).

*Decamethonium and Succinylycholine.* In 20 subjects a single dose of 0.027 mg./kg. C10 decreased the amplitude of the EMG by 43.3 ± 6.3 per cent (range 6 to 90 per cent). The decrease of the height of the ergographic tracing in 7 subjects varied from 10 to 85 per cent. A short period of facilitation preceded the development of the C10-induced neuromuscular block in 9 subjects. Facilitation of neuromuscular transmission was also present in 5 of these 9 subjects after recovery from the C10 block. In 2 subjects in whom the first 0.027 mg./kg. C10 caused a slight to moderate neuromuscular block a second, identical dose of C10 administered during peak effect of the first dose, caused a 64 and 100 per cent decrease of the height of the EMG, respectively. Tetanus was well maintained, there was no post-tetanic facilitation, and increasing the rate of stimulation to 1/second caused no fatigue, indicating that C10 produced a typical depolarization block (fig. 3).
mg./kg. d-tc. In the 3 out of 4 cases when HFL produced a detectable block this was increased by the subsequent administration of d-tc (fig. 4). In 3 experiments the 8, 18, and 60 per cent block produced by 0.15 mg./kg. d-tc was increased by the administration of 1 mg./kg. HFL to 43, 60 and 80 per cent, respectively (fig. 4).

Hexafluoronium and Gallamine. The interaction of HFL and gal. at the neuromuscular junction is more complex than that of HFL and d-tc. In 6 experiments the administration of 1.0 mg./kg. HFL was followed by 1.0 mg./kg. gal. In the 2 cases in which this dose of HFL had a detectable effect, the intensity of the block was increased by the subsequent administration of gal. (fig. 5). In the 4 cases where 1.0 mg./kg. HFL had no EMG effect, 1.0 mg./kg. gal. produced a 15 to 70 per cent decrease of the amplitude of the EMG. The results of the 17 experiments in which the administration of 1.0 mg./kg. gal. was followed by 1.0 mg./kg. HFL were not uniform. In 5 experiments the subsequent administration of HFL increased the intensity of the block by 4 to 34 per cent; in 2 other experiments HFL had no detectable effect on the gal.-induced neuromuscular block; and in the remaining 10 experiments HFL antagonized the neuromuscular effects of gal. (fig. 5).

Hexafluoronium and Decamethonium. HFL and C10, whatever the sequence of their administration, consistently antagonized the neuromuscular effects of one another (fig. 6). In 8 subjects the administration of 0.4 mg./kg. HFL after 0.027 mg./kg. C10 resulted in the prompt reversal of the C10-induced neuromuscular block. In 6 experiments the administration of 0.027 mg./kg. C10 preceded by 0.4 mg./kg. HFL had no detectable neuromuscular blocking action. A second, identical dose of C10 administered 3 to 5 minutes after the first dose to 4 subjects caused a variable (3 to 50 per cent) decrease of the height of the EMG (fig. 6). In 2 subjects the neuromuscular block produced by 1.0 mg./kg. HFL was antagonized by the administration of 0.027 mg./kg. C10 (fig. 6) and the height of the EMG returned to above control level.

Hexafluoronium and Succinylcholine. The administration of 0.4 mg./kg. HFL was followed by the injection of 0.1 mg./kg. Sch in 3 subjects, and that of 1.0 mg./kg. HFL followed by 0.1 or 0.2 mg/kg. Sch in 6 others. While the effect of 0.1 mg./kg. Sch injected alone was either not detectable or minimal and short lasting (fig. 3) this dose of Sch injected after HFL produced a prolonged 20 to 100 per cent neuromuscular block (fig. 7). In 3 experiments facilitation of neuromuscular transmission preceded the Sch-induced neuromuscular block. In 2 subjects in whom a stabilized block of neuromuscular transmission was produced by the continuous intravenous infusion of Sch, the administration of 0.4 mg./kg. HFL caused a significant increase in the intensity of the block (fig. 7). In 5 experiments tetanus was not maintained and there was post-tetanic facilitation after the administration of 1 mg./kg. HFL (fig. 8). After the administration of Sch to these subjects tetanus was not maintained, but there was no post-tetanic facilitation. In 2 of these subjects increasing the rate of stimulation to 1/second caused a decrease in the height of the ergogram (fatigue).

The interaction of d-Tubocurarine with Decamethonium and Succinylcholine. The
preliminary administration of small, by themselves ineffective, doses of d-tec consistently inhibited the neuromuscular actions of otherwise effective doses of C10 and Sch. Similarly d-tec antagonized the neuromuscular effects of C10 and Sch (fig. 9). When 0.027 mg./kg. C10 was administered after 0.15 mg./kg. d-tec to 5 subjects, the intensity of the block increased in 1, remained unchanged in 2, and was partly reversed in the remaining 2 (fig. 10).

**Discussion**

Contrary to earlier reports stating that 1.0 mg./kg. HFL, during thiopental, nitrous oxide-oxygen, meperidine anesthesia did not have any clinically detectable or experimentally demonstrable neuromuscular effect, the findings of the present study indicate that this dose of HFL produced neuromuscular block of variable intensity in about 40 per cent of similarly anesthetized human subjects. Considerable individual variation was also encountered in the neuromuscular effects of the 4 other relaxants (d-tec, gal., C10 and Sch) tested by us and others. The HFL-induced neuromuscular block had all the characteristics (poorly sustained tetanus, post-tetanic facilitation, fatigability) of a typical non-depolarization block, except that it was antagonized by edrophonium to a lesser degree than a d-tec or gal. block (figs. 1 and 2). This discrepancy is probably due to the fact that HFL is, in its own right, a relatively potent inhibitor of the acetylcholinesterase of human muscle ($IC_{50} = 2.2 \times 10^{-8} M$). Its effect at the neuromuscular junction represents the balance between its non-depolarizing neuromuscular blocking action and its inhibitory effect on the cholinesterase of the endplate. This enzyme similarly to the cholinergic receptors is located on the post-junctional membrane and is accessible to quaternary ammonium compounds like HFL. Consequently it is understandable that the administration of another anticholinesterase, edrophonium, will result in only moderate antagonism of the neuromuscular block.

It is not surprising, that whatever the sequence of their administration, HFL and d-tec, two non-depolarizing neuromuscular blocking agents, increased the effects of each other on neuromuscular transmission. The variable result of the interaction of HFL and another non-depolarizing relaxant, gal., at the neuromuscular junction is more difficult to explain. The subsequent administration of gal. always increased the neuromuscular effect of HFL. In contradistinction, when gal. was administered first, HFL in different subjects increased, antagonized, or had no effect on the neuromuscular effect of the former (fig. 6). A possible explanation of this puzzling finding may be that the affinity of gal. for the cholinergic receptors is less than that of d-tec and therefore it is more easily displaced from these agents.
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Fig. 8. The interaction of hexafluorourium (HFL) and succinylcholine (SCH). Ergogram. Symbols: 1. Tetanic stimulation; 2. Stimulation rate of 1/second. Note HFL block, with poorly sustained tetanus and post-tetanic facilitation. Transient increase in force of contraction follows administration of SCH, preceding development of more intense block. Tetanus is still poorly sustained, fatigability is present at stimulation rate of 1/second but post-tetanic facilitation is absent. The effect of edrophonium (EDROPH) is slight.

structures than d-tc. Because of this decreased affinity, in subjects in whom the anticholinesterase effect of HFL is predominant over its curare-like effect, it will antagonize the neuromuscular effects of gal.; where the reverse is true, the myoneural effect of the two compounds will be additive; and when the balance is even, there will be no change in the intensity of the gal. block.

Independent of the sequence of their administration, HFL and C10 (fig. 6) always decreased and HFL and Sch (figs. 7 and 8) always increased the neuromuscular effects of one another. In view of the observations of Nastuk and Karis, these findings are of special interest. These investigators reported that in the sciatic nerve-sartorius muscle preparation of the frog, carbamylcholine, a depolarizing compound whose neuromuscular effect is similar to C10, increased the neuromuscular blocking effects of HFL. They found no basic difference between the interaction of carbamylcholine and SCH with HFL and suggested, that the site of action of HFL may be different from that of acetylcholine and similar compounds.

d-tc behaved differently from HFL and inhibited and antagonized the neuromuscular effects of both C10 and SCH (figs. 9 and 10). Since the neuromuscular effects of d-tc and HFL are qualitatively similar, but the two compounds differ from one another with regard to their inhibitory effect on plasma cholinesterase, it is highly probable that the potentiating effect of HFL on the SCH-induced neuromuscular block is the result of the marked inhibitory effect of HFL on this enzyme. This assumption is corroborated by the fact that the neuromuscular effects of SCH can also be potentiated by other anticholinesterases (e.g., neostigmine methylsulfate [Frostigmine] or tetrahydroammonacrine) which in the dose range used had no neuromuscular blocking effect.

Comparison of the findings of Nastuk and Karis with those of the present study indicates that the mechanism of the interaction of HFL with other neuromuscular blocking agents in man and frog are different. Because of the often demonstrated species variation in the pharmacological effects of drugs in general, and especially those of neuromuscular blocking agents, clarification of the mode of action of these compounds in man should be further pursued in suitably designed experiments carried out on human subjects.

Summary and Conclusions

The neuromuscular effects of hexafluorourium alone and in combination with d-tubocurarine, gallamine, decamethonium and succinylcholine were investigated in 118 lightly anesthetized subjects. The intravenous administration of 1.0 mg./kg. hexafluorourium caused typical non-
Fig. 9. The interaction of d-tubocurarine (DTC) with decamethonium (C10) and succinylcholine (SCH). Electromyographic tracings. Top tracing: DTC followed by C10. Second tracing: DTC followed by SCH. Third tracing: C10 followed by DTC. Fourth tracing: SCH infusion followed by DTC. Note the preventive and antagonistic effect of DTC on the neuromuscular action of C10 and SCH.

Fig. 10. The interaction of d-tubocurarine (DTC) and decamethonium (C10). Symbols: 1. Tetanic stimulation (100/sec); 2. Rest (no stimulus). Ergogram. Upper tracing: addition effect of DTC and C10. Lower tracing: antagonistic effect of DTC and C10.
depolarization block in 10 of 24 subjects. Hexafluorurium and d-tubocurarine always increased the neuromuscular effects of one another. The interaction of hexafluorurium and gallamine was not consistent. The hexafluorurium-induced neuromuscular block was increased by the subsequent administration of gallamine. When hexafluorurium was administered after gallamine the intensity of the block was increased in 5, unchanged in 2 and antagonized in 10 of the 17 subjects tested. There was a consistent mutual antagonism between the neuromuscular effects of hexafluorurium and decamethonium. The prior administration of hexafluorurium markedly potentiated and prolonged the neuromuscular blocking action of succinylcholine.

References
17. McCaul, K., and Robinson, G. D.: Suxamethonium "extension" by tetrahydroamino

Nervous System

NORADRENALENE RELEASE The view has been held for many years that the impulse which passes down the sympathetic postganglionic fiber releases noradrenaline directly. Current evidence indicates that the release is a more complicated process and that it closely resembles the mechanism of release of adrenaline from the adrenal medulla. The impulse passing down the fiber first releases acetylcholine, which, after leaving the fibers, makes the membrane of the fiber permeable to calcium ions. These ions enter the fiber and release noradrenaline from the granules in which they are held. (Burn, J. H.: Release of Noradrenaline from the Sympathetic Postganglionic Fibre, Brit. Med. J. 1: 197 (April) 1967.)