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# Supersensitivity of salivation in response to pilocarpine after withdrawal of chronically administered hyoscine in the mouse

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**Summary**

1. The duration of the effect of a single large dose of hyoscine, in reducing the salivary response of mice to pilocarpine, was established as less than 66 hours.

2. Supersensitivity was observed, after daily oral dosing with hyoscine, in the increased salivation of mice in response to pilocarpine injected at least 66 h after withdrawing hyoscine.

3. The minimum duration of pretreatment with hyoscine that resulted in supersensitivity was 5 days. The daily dose was more effective if divided.

4. The period after withdrawal for which supersensitivity could be detected was 6 days.

5. The maximum salivary response to pilocarpine was increased by chronic hyoscine pretreatment.

6. The antagonism of a single dose of hyoscine to pilocarpine salivation, as expressed by the dose-ratio of pilocarpine, was not altered by chronic hyoscine pre-treatment.

## Introduction

Supersensitivity of the salivary glands to sympathomimetic amines has been reported after chronic pre-treatment with cholinoceptor blocking agents in cats (Emmelin & Muren, 1950; Emmelin, 1961), in rats (Ohlin & Perec, 1966) and in rabbits (Nordenfelt & Ohlin, 1957). Emmelin & Muren (1951) pretreated cats chronically with atropine and failed to demonstrate supersensitivity of the salivary glands to acetylcholine or pilocarpine 24 h later, although supersensitivity to sympathomimetic amines was shown to be present at that time. It was assumed that persistence of the antisialogogic activity of atropine prevented detection of cholinoceptor supersensitivity. An increase in parotid secretion induced by acetylcholine has, however, been demonstrated by Mózsik, Jávor, Dobi, Petrássy & Szabó (1967) after 2–4 weeks treatment of patients with atropine.

In the following investigation of the effect of chronic pretreatment of mice with hyoscine upon the salivary response to pilocarpine, an interval adequate for recovery from the antisialogogic effects of hyoscine was allowed after withdrawal.

## Methods

The measurement of salivation in mice was based on that described by Richter (1966) and Lavy & Mulder (1969). All mice were sedated with diazepam, 50 mg/kg

intraperitoneally, 30 min before testing. Immediately after the injection of pilocarpine hydrochloride into a tail vein, they were laid in plastic trays with their heads projecting and their jaws in contact with a sheet of Whatman No. 54 filter paper, on a perspex sheet inclined at 5° to the horizontal. The mice were thus resting slightly head downwards; in this way only saliva was absorbed by the filter paper. The trays were moved backwards a short distance when the stained area reached about 2·5 cm diameter; this was done repeatedly through the period of collection.

The stained areas were quickly outlined in ink and, when dry, were cut out and weighed. The total weight of paper that had been stained during the collection period provided an estimate of spot size equivalent to that given by planimetry, used by both Richter (1966) and Lavy & Mulder (1969) and shown to be related to the quantity of saliva secreted.

Drugs used were: diazepam (Roche); hyoscine hydrobromide (Macfarlane Smith); pempidine tartrate (May & Baker): pilocarpine hydrochloride (B.D.H.). All were given dissolved in distilled water or 0·9% NaCl w/v (physiological saline), except for diazepam, which was injected as the ampoule solution. All doses refer to the forms given above.

## Results

### *Sialogogic activity of pilocarpine*

The mean salivary response, represented by the total weight of filter paper absorbing the secretion, of groups of 10 mice injected intravenously with 0·5, 1 and 2 mg/kg pilocarpine hydrochloride, is plotted against the duration of collection in Figure 1. This shows that the salivary response to these doses may last for up to 40 min with clear regression upon dose. A collection period of 10 min

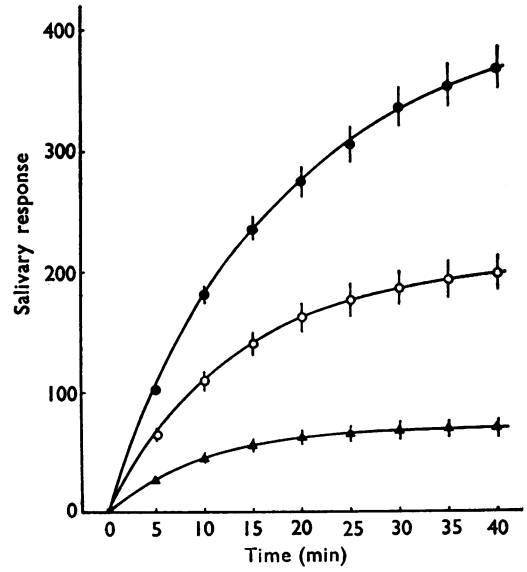


FIG. 1. The time-course of salivation, as measured by the dry weight of collecting filter paper (see **Methods**), plotted as means ± S.E. for groups of 10 mice injected intravenously with pilocarpine hydrochloride, 0·5 mg/kg—▲, 1 mg/kg—○ and 2 mg/kg—●.

after injection of pilocarpine was chosen for subsequent experiments as being the shortest period ensuring adequate regression, low variance and also adequate sensitivity to the antagonist effect of hyoscine.

Figure 2 shows the relation between injected dose of pilocarpine hydrochloride and the salivary response over the first 10 min collection period after injection. The relation is linear between 0·25 and 2 mg/kg, confirming the findings of Richter (1966).

### *Duration of the antisialogogic effect of hyoscine*

The effect of increasing the interval between giving an oral dose of hyoscine hydrobromide and the intravenous injection of pilocarpine hydrochloride 1 mg/kg upon the reduction of the salivary response measured during the first 10 min after the pilocarpine is shown in Table 1. It will be seen that a dose of 10 mg/kg

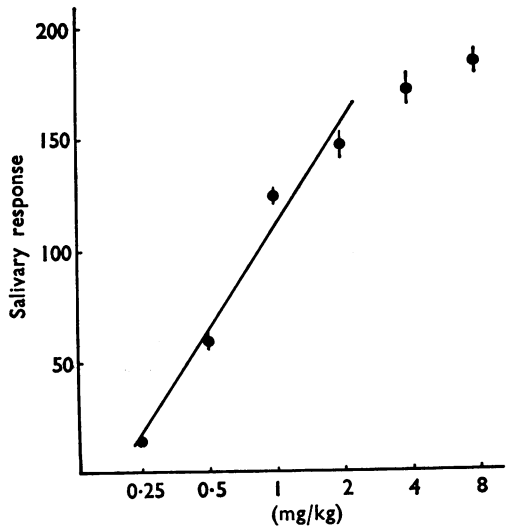


FIG. 2. Relation between dose of pilocarpine hydrochloride injected intravenously in groups of 40 mice and the salivation measured over the first 10 min after injection (b=153·5±13·6; $n$=160).

TABLE 1. *Duration of the effect of an oral dose of hyoscine hydrobromide in reducing the salivation occurring in groups of 5 mice during 10 min after the injection of pilocarpine hydrochloride (1 mg/kg, i.v.)*

| Dose of hyoscine HBr (mg/kg) | Pre-treatment interval (h) | Mean salivary response ± S.E. | |
|---|---|---|---|
| | | After hyoscine | Control |
| 10 | 24 | 14·0± 8·8* | 72·6± 9·6 |
| | | 16·8±12·7* | 76·6± 5·5 |
| | 42 | 82·6±10·0 | 88·0±10·3 |
| | | 68·8± 9·6 | 46·4± 3·1 |
| 20 | 42 | 43·3±13·2 | 64·8±13·1 |
| | | 28·2± 9·7* | 80·8± 8·4 |
| | 48 | 55·6±11·6 | 46·3± 4·7 |
| | | 66·5±24·3 | 83·2±14·9 |
| 40 | 66 | 48·6± 5·0 | 31·4± 5·2 |
| | | 45·5± 7·5 | 61·7± 8·8 |

* Significant difference from control ($P$<0·05).

hyoscine hydrobromide was still effective 24 h later, though not at 42 h, while 20 mg/kg was ineffective after 48 h and 40 mg/kg after 66 hours.

This being so, in subsequent experiments an interval of between 66 and 72 h was allowed after a dose of hyoscine when assessing changes in sensitivity to pilocarpine.

*Effect of withdrawal of hyoscine after repeated dosing*

Mice were dosed orally with hyoscine hydrobromide, 20 mg/kg, twice daily, for various periods and their salivary response to pilocarpine hydrochloride (1 mg/kg i.v.) was tested on the third day of withdrawal (the first day being the one following the last dose of hyoscine). Comparisons were made between pretreated mice and a similar number of controls dosed only with distilled water; they were compared in groups of 5, in several runs spaced through the day. The significance of differences due to the treatment was established by analysis of variance; in almost every series, significant difference between runs was also observed.

To reduce the effect of transmitter release by nerve traffic during withdrawal from hyoscine, pempidine tartrate, 20 mg/kg, was given orally, twice daily on the first and second day following the withdrawal of hyoscine after daily dosing for 8 days in the first 3 experiments shown in Table 2. It will be seen that hyoscine pretreatment increased the salivary response of mice to pilocarpine by about 80% of that of controls. In two further experiments shown in Table 2, where pempidine was withheld, it will be seen that a significant increase in response again followed

TABLE 2. *Effect upon salivary response to pilocarpine hydrochloride (1 mg/kg, i.v.) of the pretreatment of groups of mice with hyoscine hydrobromide (20 mg/kg, orally) twice on each of 8 successive days, the last dose being given 66–72 h before test*

| Additional pretreatment | *n* | Mean salivary response ± S.E. | | % Increase in response |
|---|---|---|---|---|
| | | Hyoscine treated | Control | Pretreated/control* |
| Pempidine tartrate | | | | |
| 20 mg/kg orally twice on | 20 | 174·1± 8·1 | 101·8±5·7 | 71·1 |
| each of the 2 days after end | 15 | 164·5± 8·1 | 82·9±8·6 | 98·4 |
| of hyoscine treatment | 15 | 197·8±13·8 | 109·6±7·5 | 80·5 |
| Saline only during 2 days | 15 | 147·5±11·0 | 89·7±6·9 | 64·4 |
| after hyoscine | 15 | 157·2±11·4 | 106·7±8·7 | 47·3 |

* All differences were significant to 95% or better by analysis of variance.

TABLE 3. *Effect of duration of pre-treatment with hyoscine hydrobromide (40 mg/kg, orally) daily, for different periods in one or two daily doses, upon the salivary response to pilocarpine hydrochloride (1 mg/kg, i.v.) given 66–72 h after withdrawing hyoscine*

| Duration of pretreatment (days) | % Increase in response ± S.E. (mean of 6 comparisons) | *P* for difference pretreated-control (analysis of variance) |
|---|---|---|
| Two divided doses | | |
| 3 | 8·9± 2·9 | >0·2 |
| 4 | 42·8±16·7 | <0·001 |
| | 13·5± 4·2 | 0·1><0·2 |
| 5 | 50·9±17·3 | <0·001 |
| | 32·5± 6·5 | <0·001 |
| Single daily doses | | |
| 5 | 43·5±15·4 | <0·001 |
| | 17·3± 5·0 | >0·2 |

hyoscine withdrawal, though to a lesser extent than when pempidine was used; the difference was significant by analysis of variance.

Table 3 gives the results of further experiments in which the period of repeated dosing with hyoscine hydrobromide was varied. A significant increase in response over controls was produced consistently after only 5 days dosing, though the extent of the increase was less than that found after 8 days (Table 2). It may also be seen in Table 3 that dosing once daily with 40 mg/kg for 5 days was less reliably effective in increasing sensitivity than two daily doses of 20 mg/kg.

### *Duration of supersensitivity*

Table 4 gives the results of experiments in which the salivary response to a fixed dose of pilocarpine was tested at various intervals after the last of repeated doses of hyoscine. It will be seen that supersensitivity was apparent by the 3rd day and persisted until the 6th day of withdrawal, the level declining so that significance could no longer be established at the 7th day.

TABLE 4. *Duration of enhanced salivary response to pilocarpine (1 mg/kg, i.v.) in mice pretreated with hyoscine hydrobromide (20 mg/kg, orally) twice daily for 5 days*

| Day of withdrawal from hyoscine | % Increase in response ± S.E. (mean of 6 comparisons) | *P* for difference pretreated-control (analysis of variance) |
|---|---|---|
| 3 | 56·6±15·0 | <0·001 |
| 4 | 48·5±10·0 | <0·001 |
| 5 | 37·5± 9·5 | <0·01 |
| 6 | 40·0±11·5 | <0·001 |
| 7 | 19·6± 6·4 | >0·2 |

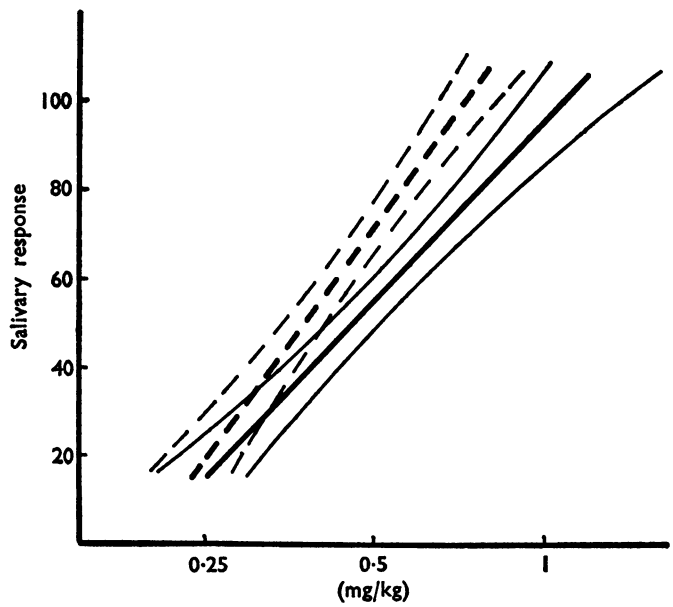


FIG. 3. Regression lines computed for the relation between salivary response and intravenous dose of pilocarpine hydrochloride in mice dosed orally twice daily for 5 successive days with either water (unbroken lines) or hyoscine hydrobromide 20 mg/kg (broken lines), the last dose being given 66–72 h before commencing test. The envelopes enclose the 95% confidence limits.

*Dose-response relations of pilocarpine after hyoscine withdrawal*

Figure 3 shows the dose-response relations for pilocarpine hydrochloride, injected intravenously, in mice on the 3rd day after withdrawal of hyoscine and in control mice. Each relation was computed from 60 values and that for the hyoscine-treated mice may be seen to be displaced to the left of that for the controls. Tested by the method used by Conolly, Davies, Dollery & George (1971), these relations did not diverge significantly from parallelism but were significantly non-coincident ($P<0.01$). An estimate from analysis of variance of the relative sialogogic potency of pilocarpine in these two groups gave 1·23 (95% confidence limits—1·07 to 1·41), i.e. an increase in sensitivity by 23% as the result of the pretreatment.

As shown in Table 5, the response of hyoscine-pretreated mice to doses of pilocarpine found to give maximal response in control mice (see also Fig. 2) was increased by 23% of that in control mice.

*The antisialogogic activity of hyoscine after hyoscine withdrawal*

Figure 4 shows the effect of an acute dose of 4 $\mu$g/kg hyoscine hydrobromide, i.p., on the relation between salivary response and dose of pilocarpine hydrochloride, injected intravenously 30 min later, in control mice and in mice 3 days after stopping chronic treatment with hyoscine. Each relation was computed from 90 values. It will be seen that the regression line for pilocarpine is shifted to the left in withdrawn mice (b) compared to that in control mice (a), as already seen in Fig. 3: the relations were once again shown to be significantly non-coincident. The shift of the relations to the right in the presence of hyoscine is seen to be the same for both groups. From analysis of variance, the dose-ratio for pilocarpine representing the antagonist activity of 4 $\mu$g/kg hyoscine hydrobromide was:

In control mice—2·1 (1·84–2·39)

In hyoscine-withdrawn mice—2·04 (1·79–2·34),

the parentheses enclosing the 95% confidence limits. These figures indicate that the antagonist activity of hyoscine is not affected by supersensitivity.

## Discussion

The development of supersensitivity to cholinoceptor stimulants as the consequence of prolonged treatment with cholinoceptor blocking agents has been reported by Friedman, Jaffe & Sharpless (1969) for increased sensitivity to the hypothermic action of pilocarpine in mice after the withdrawal of hyoscine. The dose of hyoscine used by these authors (reaching 130 to 200 mg/kg daily) was much greater than that found effective in the present study, though they were able to demonstrate increased sensitivity after only 24 withdrawals. In the work reported here, a minimum interval of 66 h was found to be necessary to ensure that the

TABLE 5. *Effect of 5 days pretreatment with hyoscine hydrobromide (20 mg/kg, orally) twice daily, upon the salivary response of groups of 20 mice to large doses of pilocarpine hydrochloride injected i.v. 66–72 h after withdrawing hyoscine*

| Dose of pilocarpine (mg/kg) | Mean salivary response ± S.E. | | % Increase in response | *P* for difference pretreated-control (analysis of variance) |
|---|---|---|---|---|
| | Pretreated | Control | | |
| 2 | 177·4± 8·1 | 144·7±8·3 | 22·6 | <0·01 |
| 4 | 201·8± 7·5 | 160·9±8·2 | 25·4 | <0·001 |
| 8 | 194·4±11·6 | 159·7±8·2 | 21·7 | <0·05 |

effects of the last dose of hyoscine did not prevent demonstration of increased sensitivity to the sialogogic action of pilocarpine.

Supersensitivity to cholinoceptor effects by treatment with blocking agents has been attributed to the absence of an action on the cell of transmitter release by nerve impulses or spontaneous leakages from the endings of the postganglionic neurones (Emmelin & Strömblad, 1957; Emmelin, 1960; 1961; Assarson & Emmelin, 1964). In order to reduce the effect of transmitter release by nerve traffic during withdrawal from hyoscine in the present study, mice were treated with pempidine during this period. It was shown, by comparison with others not so treated, that this had some effect on the extent of supersensitivity that developed, since a lesser, though still significant, degree of supersensitivity developed without it; moreover, the pempidine treatment that was used was itself without effect on

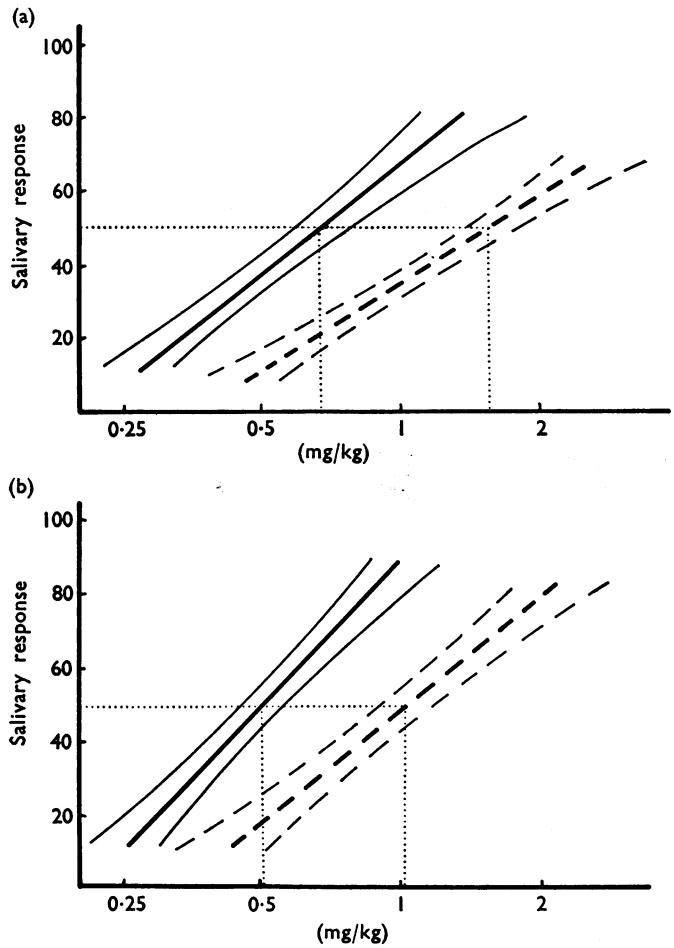


FIG. 4. Regression lines computed for the relation between salivary response and intravenous dose of pilocarpine hydrochloride in mice dosed orally twice daily for 5 successive days with either water (a) or hyoscine hydrobromide, 20 mg/kg (b), terminating 66–72 h before test, and also injected intraperitoneally, 30 min before test with either saline (unbroken lines) or hyoscine hydrobromide, 4 $\mu$g/kg (broken lines). The envelopes enclose the 95% confidence limits; the dotted lines indicate the dose of pilocarpine hydrochloride to produce a response of 50.

sensitivity to pilocarpine (Table 2). These findings are consistent with the hypothesis.

The duration of the supersensitivity observed was determined as 6 days. This is of a similar order to that reported for supersensitivity of cat salivary glands to adrenaline on withdrawal of atropine (Emmelin & Muren, 1952) but longer than the 3 days found for the supersensitivity of mice to pilocarpine hypothermia after hyoscine (Friedman *et al.*, 1969).

It was notable that not only was pilocarpine more effective in producing a salivary response after hyoscine withdrawal but the maximum response obtainable was also increased. Other workers have not found this to be the case; Mószik *et al.* (1967) found that the maximum response of salivation in man to citric acid was not increased after atropine; Emmelin & Muren (1951) also found no increase in the salivary response of cats to large doses of pilocarpine after nerve section. Emmelin, Malm & Strömblad (1960) found a decreased maximum response after nerve section but attributed this to consequent atrophy of the gland. Friedman *et al.* (1969) showed an increase in the maximum hypothermic response of mice to pilocarpine after hyoscine withdrawal, though they did not comment upon it.

An increase in the maximum response obtainable suggests that the supersensitivity results from changes induced by treatment at the level of the receptors or beyond. Alterations in accessibility of the drug to the receptors could be expected to affect the range of effective doses but not the maximum response.

The activity of hyoscine in antagonizing the stimulant action of pilocarpine was shown to be unaltered by supersensitivity, in terms of the dose-ratio of pilocarpine for a given effect level. Thus no tolerance to the action of hyoscine was found, as claimed for various anticholinoceptives by other authors, including Friedman *et al.*, (1969) in mice and Mószik & Jávor (1969) in man.

The unaltered anticholinoceptor activity of hyoscine suggests that the affinity of the receptor for the drugs remains unaltered. The findings are consistent with the hypothesis attributing supersensitivity to an increase in cholinoceptor area, as adduced for skeletal muscle after denervation (Axelson & Thesleff, 1959) or botulinum toxin (Thesleff, 1960). The evidence presented does not, however, allow distinction between such an effect and a change in the relationship between receptor occupation and response.

## REFERENCES


Assarson, N. & Emmelin, N. (1964). Leakage of transmitters in salivary glands. *Br. J. Pharmac. Chemother.*, **22**, 119–125.

Axelson, J. & Thesleff, S. (1959). A study of supersensitivity in denervated mammalian skeletal muscle. *J. Physiol., Lond.*, **147**, 178–193.

Conolly, M. E., Davies, D. S., Dollery, C. T. & George, C. F. (1971). Resistance to β-adrenoceptor stimulants (a possible explanation for the rise in asthma deaths). *Br. J. Pharmac.*, **43**, 389–402.

Emmelin, N. (1960). Is there leakage of acetylcholine in postganglionic parasympathetic nerve endings? *Nature, Lond.*, **185**, 297–8.

Emmelin, N. (1961). Supersensitivity following "pharmacological denervation". *Pharmac. Rev.*, **13**, 17–27.

Emmelin, N., Malm, L. & Strömblad, B. C. R. (1960). Effect of denervation on the maximal secretory capacity of salivary glands. *Q. Jl. exp. Physiol.*, **45**, 349–351.

Emmelin, N. & Muren, A. (1950). Supersensitivity of denervated organs to chemical stimuli. *Nature, Lond.*, **166**, 610.

Emmelin, N. & Muren, A. (1951). Sensitization of the submaxillary gland to chemical stimuli. *Acta physiol. scand.*, **24**, 103–127.

EMMELIN, N. & MUREN, A. (1952). The sensitivity of submaxillary glands to chemical agents in cats under various conditions over long periods. *Acta physiol. scand.*, **26**, 221–231.

EMMELIN, N. & STRÖMBLAD, B. C. R. (1957). Sensitisation of the submaxillary gland above the level reached after section of the chorda tympani. *Acta physiol., scand.*, **38**, 319–330.

FRIEDMAN, M. J., JAFFE, J. H. & SHARPLESS, S. K. (1969). Central nervous system supersensitivity to pilocarpine after withdrawal of chronically administered scopolamine. *J. Pharmac.*, **167**, 45–55.

LAVY, U. I. & MULDER, D. (1969). Salivary inhibition in mice and rabbits by a number of anticholinergics. A methodological investigation. *Arch. int. pharmacodyn.*, **178**, 437–445.

MÓSZIK, G. & JÁVOR, T. (1969). Development of drug cross-tolerance in patients treated chronically with atropine. *Eur. J. Pharmac.*, **6**, 169–174.

MÓSZIK, G., JÁVOR, T., DOBI, S., PETRASSY, K. & SZABO, A. (1967). Development of "pharmacological denervation phenomenon" in patients treated with atropine. *Eur. J. Pharmac.*, **1**, 391–395.

NORDENFELT, I. & OHLIN, P. (1957). Supersensitivity of salivary glands of rabbits. *Acta physiol. scand.*, **41**, 12–17.

OHLIN, P. & PEREC, C. (1966). Effects of atropine treatment on the submaxillary gland of rats. *Q. Jl. exp. Physiol.*, **51**, 196–201.

RICHTER, W. (1966). Estimation of anticholinergic drug effects in mice by antagonism against pilocarpine-induced salivation. *Acta pharmac. Tox.*, **24**, 243–254.

THESLEFF, S. (1960). Supersensitivity of skeletal muscle produced by botulinum toxin. *J. Physiol., Lond.*, **151**, 598–607.

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