# Positive correlation between initial pupil diameter and amplitude of miotic response to pilocarpine in rabbits and to carbachol in man

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A linear correlation between amplitude of miotic response and initial pupil diameter has been demonstrated in rabbits using various concentrations of pilocarpine HCl. The same correlation was found when published results in humans using carbachol were examined and it is likely that the correlation also applies to pilocarpine in humans. No such correlation was observed with physostigmine.

It has been generally assumed that the decrease in pupil diameter following miotics in rabbits and in man is independent of the initial diameter.

In some recent investigations using the rabbit pupil to study new formulations of pilocarpine, we found an apparent increase in potency when we used a group of relatively untrained rabbits. On further examination we found that this could be explained if there was a positive linear correlation between the starting diameter and the change in diameter after pilocarpine. Surprisingly this correlation does not seem to have been reported previously.

This paper describes our results using pilocarpine in rabbits and also the calculated linear coefficients for pilocarpine and carbachol using human data reported in the literature (Ogle, Whisnant & Hazelrig, 1966).

**Methods.**—The animals used were male New Zealand white rabbits. All animals were allowed food (Dixon's RGP pellets) and water *ad libitum*.

Pilocarpine HCl was made up in 0·9% saline in the following concentrations: 0·25%, 1·0%, 5·0%, 10·0%.

*Experimental procedure.* Rabbits were housed in individual cages in a room which was temperature controlled between 60 and 70° F.

Before testing, animals were transferred from their normal housing conditions to a small laboratory with controlled lighting conditions. The laboratory had walls of uniform colour and was illuminated by fluorescent lighting which could be controlled in intensity over a wide range. Light intensity was selected for the experiments with two points in mind. First, the pupil should be sufficiently dilated for a good miotic effect to be produced and, second, the light intensity should be sufficient to permit the pupil to be measured optically by the operator.

During the experiments the rabbits were packed securely into standard metal stocks. Training was found to be essential as the rabbits otherwise exhibited excitement. This training was carried out for 1 h periods at least three times in the week preceding the experiment.

*Measurement of pupil diameter.* Pupil diameters were measured using an instrument which incorporates a long focal length microscope held in line with a plane mirror angled at 45° to the axis of the microscope. This arrangement permits the operator to view the rabbits' eyes from a position in front of the animal, resulting in much less operator movement and therefore less disturbance to the animals. An eyepiece graticule is incorporated in the microscope and diameters were always read in the horizontal plane since it was found that the pupils were seldom exactly circular in shape. This is also found in human pupils under conditions of low light intensity (Kristek, 1965a).

*Drug administration.* Animals were transferred from their home cages into the test laboratory and allowed 20 min for dark adaptation, preliminary experiments having shown that this time is sufficient. After this time, base-line readings were taken from each rabbit. Thirty minutes after entering the room each rabbit had drug solution instilled into its eyes. These solutions were instilled via a Hamilton micro-syringe, dispensing volumes of 0·05 ml into each eye. After a further 20 min the pupil diameters were measured in the manner described.

*Statistics.* The correlation between the two variables, initial pupil diameter and amplitude of miotic response, was investigated using the Bravais-Pearson coefficient of linear correlation.

**Results.**—A highly significant correlation between initial pupil diameter and amplitude of miotic response following various concentrations of pilocarpine HCl was obtained in rabbits (Table 1).

TABLE 1. *Correlation coefficients for miotic response versus initial pupil diameter in rabbits and in man following various concentrations of miotic drugs*

| Species | Drug/concentration | | Correlation coefficient | *N* | *P* value | Slope | Intercept |
|---|---|---|---|---|---|---|---|
| Rabbit | Pilocarpine HCl | 0·25% | 0·7650 | 30 | 0·001 | 0·7352 | −3·3009 |
| | | 1·0% | 0·5883 | 37 | 0·001 | 0·6677 | −2·3509 |
| | | 5·0% | 0·8821 | 18 | 0·001 | 1·1543 | −4·9009 |
| | | 10·0% | 0·6057 | 12 | 0·05 | 0·7765 | −2·3950 |
| Man (calculated values from results in literature (Ogle *et al.*, (1966)) | Pilocarpine HCl | 1·0% | 0·5058 | 12 | 0·1 | 0·6891 | −2·2780 |
| | Carbachol | 0·75% | 0·5992 | 12 | 0·05 | 0·7692 | −0·4506 |
| | Physostigmine salicylate | 0·25% | −0·0172 | 6 | Not sig. | — | — |

**Discussion.**—Analysis of published results (Table 1) showed that the relationship which we have demonstrated in rabbits also holds for carbachol in humans but not for physostigmine. In the case of pilocarpine, the correlation coefficient is of the same order as that for carbachol and although $P=0{\cdot}1$, in view of the above evidence it seems likely that a further experiment using a greater number of subjects would confirm the relationship.

This correlation is of obvious importance where serious attempts are being made to reduce variation in assays of miotic drugs. A relationship between the amplitude of a response to a drug and the initial value of the parameter exists in other areas of pharmacology, thus blood pressure responses to hypotensive and hypertensive agents are related to the initial blood pressure in both rats (Nicholas & Hughes, 1968) and dogs (Korol & Brown, 1967).

A similar relationship has been shown to govern photoreactions in the normal human eye (Kristek, 1965b; Peleska, 1965). In this instance Peleska has suggested expressing intensity of contraction of the pupil by contraction index (the ratio of amplitude and the initial width of the pupil), rather than by the amplitude of the contractions alone.

We have attempted to use a modification of the contraction index to adjust the results, but this did not reduce scatter and so far we are unable to suggest a satisfactory remedy.

It is of interest that the correlation is also found in human eyes, with carbachol and probably pilocarpine, but not with physostigmine. The latter result may not be entirely surprising as its mode of action —that is, inhibition of acetylcholinesterase, is different from that of the cholinergic agonists, pilocarpine and carbachol.

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