

# Provocative outflow test

## Combining water drinking and homatropine

D. A. LEIGHTON, C. I. PHILLIPS, AND A. C. C. GIBBS

*Departments of Ophthalmology and Social and Preventive Medicine, University of Manchester, and Manchester Royal Eye Hospital*

We have combined instillation of guttae homatropine 2 per cent. with water drinking as a new and more powerful provocative test in a series of 24 patients suspected of open-angle glaucoma in order to compare their effect on ocular tension and facility of outflow with the effect of guttae homatropine alone and water drinking alone. The amount of water given to each patient was varied according to body weight, 20 ml./kg. being given, with the object of decreasing the variance of the observations. Spaeth (1967) used 14 ml./kg. in his water drinking tonometry test.

Becker and Christensen (1956) demonstrated a greater fall in C after one litre of water in 188 cases of open-angle glaucoma than in 175 normal eyes; a  $Po/C > 100$  was found in 97 per cent. of glaucomatous eyes and only 1 per cent. of normal eyes. Galin, Aizawa, and McLean (1961), however, could not find a significant fall in the outflow facility of 41 cases of glaucoma after 1 litre of water.

Christensen and Pearce (1963) reported a rise in tension after guttae homatropine 5 per cent. in open-angle glaucoma. This occurred only occasionally in normal eyes in which there was usually a fall. It was accompanied by a fall in the outflow facility in 28 per cent. of glaucomatous and 15 per cent. of normal eyes. Bárány and Christensen (1967) demonstrated an increase in the outflow resistance in normal and glaucomatous human eyes after cycloplegia with guttae homatropine 5 per cent.; the effect was greater in patients with open-angle glaucoma than in normal subjects and there was a small overlap between these two groups.

Kirsch (1965) and then Kristensen (1967) combined a dark-room test with water drinking and mydriasis as a provocative test in suspected closed-angle glaucoma.

### Methods

The test subjects were 24 patients suspected of open-angle glaucoma who had borderline or raised ocular tensions and/or discs which were considered suspicious of glaucoma. All had open angles and any patient with angles judged to be in danger of becoming closed by mydriasis was excluded.

Each attended as an out-patient on three separate mornings, the same eye being tested on each occasion. When water was to be taken, the patient was told to have nothing to eat or drink from the previous evening. In all, thirteen right and eleven left eyes were chosen at random for investigation by the toss of a coin except for one in which corneal opacities were present in one eye.

The procedure was as follows:

- (1) Applanation tension.
- (2) Schiötz tension.
- (3) 4-minute tonography.
- (4) One of the following at each visit:
  - (a) Water by mouth (20 ml./kg. body weight)
  - or
  - (b) Guttae homatropine 2 per cent. to the eye for testing
  - or
  - (c) Both (a) and (b).

then, after 45 minutes,

- (5) Applanation tension.
- (6) Schiötz tension.
- (7) 4-minute tonography.

These tests were carried out by a technician using a Schwarzer electronic tonometer. The order of these three tests *a*, *b*, and *c* under (4) above was randomized. There are six possible different sequences, so that four subjects were allotted to each sequence (see Table I).

**Table I** Order in which tests were carried out

Sequence		I	II	III	IV	V	VI	
Order of tests	1st visit	<i>a</i>	<i>b</i>	<i>c</i>	<i>c</i>	<i>b</i>	<i>a</i>	} see key below
	2nd visit	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>c</i>	
	3rd visit	<i>c</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>c</i>	<i>b</i>	
No. of subjects allotted to each		4	4	4	4	4	4	Total no. of subjects 24

*a* = Water drinking tonography test

*b* = Homatropine tonography test

*c* = Water drinking homatropine tonography test

## Results and discussion

Table II compares pre- and post-provocation values of C, Po, etc. for each test on individual eyes, *i.e.* "within test" differences, and Table III compares the effects of one test with another. Statistical evaluation of the results was by the Wilcoxon Matched Pairs Signed Ranks Test (Siegel, 1956). This is non-parametric and suitable to the data because a normal distribution does not have to be assumed. A two-tailed version of this test was used. The Wilcoxon T score is given for each of the tests. The lower the T score, the less likely this is to arise by chance. The borderline of significance has been taken as  $P = 0.05$ , and for clarity the Wilcoxon T score for this probability is added in brackets for each result in the tables.

(1) *Fall in outflow facility "C"* (uncorrected for ocular rigidity K)

The fall in C for the homatropine tonography test and also the water drinking homatropine tonography test was significant ( $P < 0.01$ ) in each case. Surprisingly there was no significant fall for the water drinking tonography test. The magnitude of the fall was significantly greater for the water drinking homatropine tonography test than for the water

drinking tonography test ( $T = 67\frac{1}{2}$ ;  $0.02 < P < 0.05$ ;  $T$  corresponding to  $P = 0.05$  is 73). In neither of the other two between-test comparisons was there a significant difference (Table III).

**Table II** "Within test" differences. Comparison of immediately pre-test values of  $C$ ,  $P_0$ ,  $R$ ,  $P_0/C$ , and Flow, with these values after 45 minutes. 24 subjects

Test	Water drinking tonography		Homatropine tonography		Water drinking homatropine tonography	
	Wilcoxon T score	P	Wilcoxon T score	P	Wilcoxon T score	P
Fall in C	103 $\frac{1}{2}$ (81)	> 0.05 (NS)	37 (81)	< 0.01 (S)	30 $\frac{1}{2}$ (81)	< 0.01 (S)
Rise in $P_0$	7 (66)	< 0.01 (S)	41 (40)	> 0.05 (NS)	0 (81)	< 0.01 (S)
Rise in R	83 $\frac{1}{2}$ (81)	> 0.05 (NS)	41 $\frac{1}{2}$ (81)	< 0.01 (S)	34 $\frac{1}{2}$ (81)	< 0.01 (S)
Rise in $P_0/C$	57 (73)	< 0.01 (S)	42 $\frac{1}{2}$ (81)	< 0.01 (S)	8 (81)	< 0.01 (S)
Rise/Fall in Flow	111 Rise (81)	> 0.05 (NS)	49 $\frac{1}{2}$ Fall (81)	< 0.01 (S)	146 Fall (81)	> 0.05 (NS)

S = significant;  $P < 0.05$ . NS = not significant;  $P > 0.05$ . Figure in brackets represents T score for  $P = 0.05$ , i.e. borderline of significance for that particular test.

When T is less than 81, a tie or ties have occurred, so that the number of ranks for that particular test is reduced.

**Table III** "Between test" differences in the same 24 subjects as in Table II

Comparison of tests	Water drinking tonography versus Homatropine tonography		Homatropine tonography versus Water drinking homatropine tonography		Water drinking tonography versus Water drinking homatropine tonography	
	Wilcoxon T score	P	Wilcoxon T score	P	Wilcoxon T score	P
Fall in C	90 $\frac{1}{2}$ (73)	> 0.05 (NS)	140 $\frac{1}{2}$ (73)	> 0.05 (NS)	67 $\frac{1}{2}$ (73)	$0.02 < P < 0.05$ (S)
Rise in $P_0$	7 $\frac{1}{2}$ (40)	< 0.01* (S)	0 (81)	< 0.01* (S)	28 (52)	< 0.01* (S)
Rise in R	103 (81)	> 0.05 (NS)	133 (81)	> 0.05 (NS)	88 (81)	> 0.05 (NS)
Rise in $P_0/C$	132 (81)	> 0.05 (NS)	88 (81)	> 0.05 (NS)	74 (81)	$0.02 < P < 0.05$ (S)
Fall in flow	48 (81)	< 0.01 (S)	102 (81)	> 0.05* (NS)	115 (81)	> 0.05 (NS)

+ = result favours test mentioned second at head of column.

\* = result favours test mentioned first at head of column.

S = significant;  $P < 0.05$ . NS = not significant;  $P > 0.05$ .  $0.02 < P < 0.05$  means P between 0.02 and 0.05.

Figure in brackets represents T score for  $P = 0.05$ , borderline of significance for that particular test comparison.

When T is less than 81, a tie has occurred, so that the number of ranks for that particular test comparison is reduced.

Fig. 1 (overleaf) shows the changes in C for the three tests in the form of bar charts. Means and standard deviations are indicated. The results were not normally distributed, as Prijot (1961) has already found.

In Fig. 2 (overleaf), the changes in C are shown in chronological order of performance, i.e. irrespective of which test was done on a particular visit. The initial C values became progressively smaller from Visit 1 to Visit 3 but the maximum difference, i.e. between Visit 1 and Visit 3 was not significant ( $T = 97\frac{1}{2}$ . A T of 81 is required for  $P = 0.05$ ). This probably explains the fact that for Visit 3 the provocation-induced fall in C is least,

although it is not significantly less than for Visit 1 ( $T = 120$ ;  $T(0.05) = 81$ ). This interesting trend is presumably due in some way to habituation of the patient (rather than to the weather, experience of the tonographer, etc.) and it underlines the importance of our rigid randomization (!) of the order of our series of tests—note the remarkably standard average of initial C in Fig. 1.

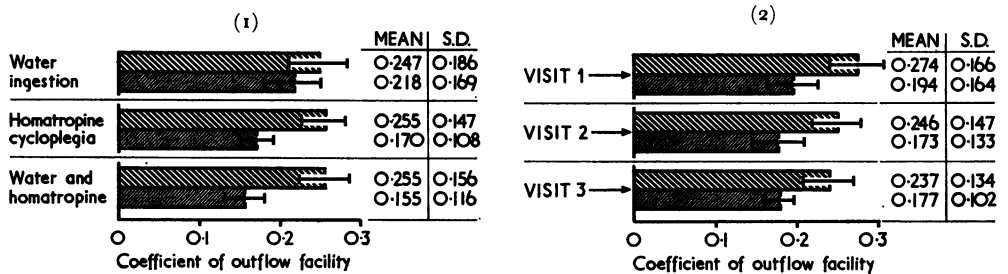


FIG. 1 Bar Chart showing outflow facility in 24 subjects, before and after provocation for :

- Water drinking tonography test;
- Homatropine tonography test;
- Water drinking combined with homatropine tonography test.

The standard error of the mean is indicated on each bar.

To the Wilcoxon Matched Pairs Signed Ranks Test there was a significant fall in the outflow facility for (b) and (c) at  $P < 0.01$ , but no significant fall for (a) ( $P > 0.05$ ). The fall was significantly greater for (c) than for (a) ( $0.02 < P < 0.05$ ).

FIG. 2 Bar Chart showing outflow facility in 24 subjects, before and after provocation for :

- Visit 1
- Visit 2
- Visit 3, *i.e.* irrespective of which test was done on a particular visit.

The standard error of the mean is indicated on each bar.

Note that initial tonography appears to show a steady decrease from Visit 1 to Visit 3, which is the main factor in reducing the test response; however, the change in outflow facility at Visit 3 was not significantly less than at Visit 1 (Wilcoxon Matched Pairs Signed Ranks Test).

In the determination of outflow facility, the 5.5 g. weight was used throughout the tests, except for the post-provocation values in four water drinking homatropine tonography tests, when the 7.5 g. weight was used. However the total fall in C in the water drinking tonography test for these four individuals when the 5.5 g. weight was used consistently was greater than in the water drinking homatropine tonography test; accordingly, the use of the 7.5 g. weight is unlikely to have biased the results in favour of the latter test.

## (2) Rise in $P_o$ applanation tension

Obviously the changes in ocular tension during the water drinking tonography test are not comparable with those in a conventional water drinking tonometry test because of the effect of the initial tonography, but examination of these data was of interest. For both the water drinking tonography test and the water drinking homatropine tonography test, the rise in  $P_o$  was significant (see "Within test" differences, Table II), that for the latter being significantly greater ( $P < 0.01$ ) (see "Between test" differences, Table III).

In the homatropine tonography test, the rise in  $P_o$  was not quite significant, although the fall in  $C$ , rise in  $R$  (outflow resistance), and rise in  $P_o/C$  had all been significant. It is worthy of note that the water drinking homatropine tonography test produced a greater rise in  $P_o$  than the homatropine tonography test in all 24 cases. Therefore water loading in addition to cycloplegia was required to increase the tension within the test. This was well illustrated by estimation of aqueous flow with the formula (Gloster, 1966):  $\text{Flow} = C(P_o - P_v)$ , where  $P_v$  is the episcleral venous pressure. This was assumed to be 11 mm. Hg throughout the tests; there is general agreement in the literature that it lies in the region of 10 to 12 mm. (Gloster, 1966). The assumptions here are probably large, but the concept is useful in this context. On this basis (see Tables II and III), a significant fall in flow occurred between the first and second tonographies in the homatropine tonography test. In the water drinking tonography test there was a net rise, which was not significant, and in the water drinking homatropine tonography test the change in flow was negligible, a slight fall being found. The difference in change of flow between the water drinking tonography and homatropine tonography tests was significant, the latter giving the greater fall. This absence of rise in tension and apparent fall in aqueous flow in homatropine tonography raises interesting possible explanations like the opening up of unconventional aqueous outflow channels (Bill, 1966) and the reduction of aqueous formation by homatropine cycloplegia, but they may quite simply be due to the hypotensive effect of the initial tonography. To clarify this point would require a comparison of the homatropine tonography test with a further test situation involving two tonographies with an interval of 45 min. between them, but no medication, etc.; in the latter case the  $P_o$  of the second tonography might well not have had time to return to the level at the start of the first tonography.

### (3) Rise in outflow resistance $R$ .

$R$  is the reciprocal of  $C$  (Gloster, 1966). The rise in  $R$  for the water drinking tonography test was not significant, but this time the water drinking homatropine tonography test was not significantly more effective than the water drinking tonography test in increasing  $R$ , whereas it had been more effective in reducing  $C$ . The different result when the reciprocal is used was probably due to some tests in which the rise in resistance was large compared with the corresponding fall in  $C$  or *vice versa*.

Bárány and Christensen (1967), in their examination of similar data for a homatropine tonography test, quoting Prijot (1961), plotted logarithms of the outflow resistance so as to obtain a nearer approximation to a normal distribution. Fig. 3 shows a graph of

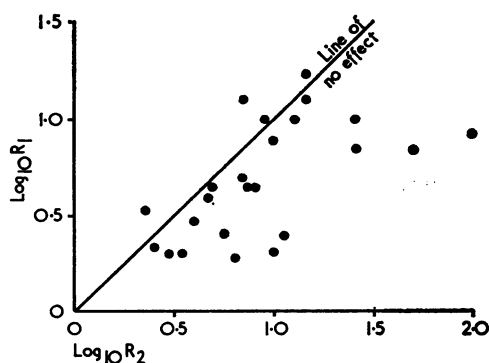


FIG. 3 Scattergram of  $\log_{10}$  outflow resistance in 24 subjects.

$R_1$  before and  $R_2$  after provocation with water drinking combined with instillation of guttae homatropine

$\log_{10} R$  from our data before and after water *and* homatropine. The distribution of points is very similar to that recorded by Bárány and Christensen (1967) for homatropine tonography. A normal distribution for these results being assumed, a Student's *t* test was done for  $\log_{10} R$  in the water drinking homatropine tonography and the water drinking tonography test. This indicated a significantly greater rise in  $R$  for the former than for the latter ( $t = 2.35$ ;  $0.02 < P < 0.05$ ). With data from eleven additional subjects who had had the homatropine tonography test and water drinking homatropine tonography test only, *i.e.* 35 subjects in all, a Student's *t* test for rise in  $\log_{10} R$  was done. This gave a significantly greater rise in outflow resistance for the water drinking homatropine tonography test than for the homatropine tonography test. ( $t = 2.158$ ;  $P = 0.02 < P < 0.05$ ). Unfortunately, these results are open to criticism because the tests were not randomized, 21 patients having had the homatropine tonography test first and fourteen the water drinking homatropine tonography test first. However, this does constitute some additional evidence that water combined with homatropine provided the most effective provocation of all three tried in this series.

#### (4) *Rise in $P_0/C$*

In all three tests a significant rise was found, and that for the water drinking homatropine tonography test was significantly greater than in the water drinking tonography test ( $0.02 < P < 0.05$ ).

#### (5) *Changes in ocular rigidity $K$*

During water drinking tests, Becker and Gay (1959), in patients suspected of open-angle glaucoma, reported a significant fall in  $K$ , and Drance (1963) a more frequent fall in  $K$  in glaucomatous than in normal eyes. Drance used his own formula (Drance, 1960) for estimating  $K$ .

In these tests,  $K$  was estimated by Friedenwald's Nomogram (1955 calibration) and paired applanation and Schiötz readings.

Our tests differ from a conventional water drinking tonometry test in that a 4-minute tonography has been carried out after the initial tension readings and before the ingestion of water and/or the instillation of guttae homatropine. The direction of changes in  $K$  did not show a significant trend towards either an increase or a decrease (Sign test; see Siegel, 1956).

### **Further discussion**

In the practical clinical care of patients with the overt manifestations of glaucoma, we need to place little or no reliance on provocative tests, and to use tonography to a very limited extent. However, our responsibility for assessing at least the first-degree relatives of our patients with glaucoma as well as borderline cases has re-awakened our interest in provocative tests; hence the present study of a new combination of two known methods, *viz.* water drinking and homatropine.

In so far as the greatest change in "ocular fluid dynamics" was produced by the water drinking homatropine tonography test, it seems likely that this would be the best of the three tests examined here in differentiating a normal from a glaucoma population. A separate study of a series of normal subjects and patients with glaucoma would, of course, be required to define criteria of normality and judge the discriminating value of the tests.



(To assess the test's value in predicting ultimate glaucoma, a cohort study of a large population would be required.)

The mechanism by which water drinking reduces the C value is presumably related to decreased osmolality of the blood, which causes either increased production of aqueous or decreased outflow, presumably because of the swelling of the trabecular meshwork, or both, although the evidence for this is circumstantial. It is interesting that Spaeth (1967) showed that, in 20 per cent. of his 234 water drinking tests, the rise in ocular tension actually preceded the decrease in serum osmolality. A more indirect mechanism may therefore be involved.

Graphs were drawn to plot test responses against body weight (as a check on whether our linearly weight-related dose of water was effective) and against age (to check whether poorer absorption might occur as age increased), but inspection suggested no correlation.

In practice, a test involving two tonographies with only 45 minutes between them is rather unpleasant for the patient and time-consuming for the examiners, and also has the intrinsic disadvantage that the state of the eye after the first tonography must be somewhat altered even when the second tonography is done 45 minutes later. For all these reasons, a water drinking homatropine *applanation* test may be more practical, even if not clearly better than the water drinking homatropine *tonography* test assessed above, and these two tests are at present being compared in another series. The possibility of using an injection of hypotonic saline and/or pitressin is being considered. We have also found some evidence that the rise in ocular tension produced by water is less in a large eyeball than in a small one, as would be expected, and more data are being accumulated for this study.

### Summary

In each of 24 "open-angle glaucoma suspects", an initial tonography on one eye was immediately followed by provocation,

- viz.*: (a) water by mouth (20 ml./kg. body weight),  
 (b) guttae homatropine 2 per cent.,  
 or  
 (c) both (a) and (b),

in strictly randomized order, and a second tonography was done on the same eye 45 minutes later.

The most effective test in producing a fall in the C value was the water drinking homatropine tonography test (see Fig. 1 and Table III) and the difference was statistically significant for the comparison of (c) with (a) but not with (b). The inclusion of eleven additional cases, not strictly randomized however, showed that (c) was significantly more effective than (b) in increasing resistance to outflow. The effect of the tests on Po (see Table III) was significant in all the comparisons,

- viz.*: (c) greater rise than (b)  
 (c) greater rise than (a)  
 (a) greater rise than (b).

Because of the disadvantages in a test requiring two tonographies with an interval of only 45 minutes, a separate study is being done to assess whether a water drinking homatropine *applanation* test may be as good as or better than a water drinking homatropine *tonography* test.

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