PREPARATION OF OPHTHALMIC SOLUTIONS: MODERN CONCEPTS.

I.—Atropine Sulphate *

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FOR a long time there were no general rules regarding isotonia and isohydria of ophthalmic solutions and lotions, or rather no attention was given to this subject when prescribing or preparing drugs.

The pH of human tears varies between 7 and 8, the average being 7.3. This was also found by Swan. Other findings were :

Charlton	7.2	Pelláthy	7.6-8.0
Györfly, Verzella	7.2-7.5	Kusunocki	7.4-8.4
Dekking	7.6	Blok	7.8-8.0
Obrig	7.3-7.7	Roulet, Gifford	8.0
Gardilicé	7.5-7.9	Oguchi, Pedersen B.	8.5

It may be that higher values are due to lacrimation produced by the irritating effect of glass-electrodes. According to these authors pure tears are more alkaline than the conjunctival fluid (a mixture). The triple structure of the precorneal film was emphasized recently (Wolff).

Even in the latest pharmaceutical text-books there are but few directions regarding the preparation of ophthalmic solutions, and only in respect to isotonia. Thus for instance the V. Swiss Pharmacopoeia (1933) prescribes quite generally the use of distilled water and recommends addition of boric acid if the drops are turbid. According to the XI. Swedish Pharmacopoeia (1946) and the IX. Danish Pharmacopoeia all lotions are prepared with distilled water and made isotonic by adding sodium chloride (except silver nitrate). Another prescription demands that the solution be isotonic with tears (freezing point—0.8); schedules usually give the necessary directions.

Recently numerous investigators have taken up the question of isotonia. The older assumption that a 1.4 per cent. Na Cl solution is isotonic with lacrimal fluid is not substantiated (Krogh, 1945); the freezing point of tears is 0.56. (Dale and Husa, 1948; Pedersen-Bjergaard, 1948.) The osmotic pressure of tears is equal to that of physiological saline. Accurate adjustment is not imperative, as the eye tolerates a wide range of osmotic values (0.5-2.0 per cent. Na Cl; Hind and Goyan, 1947).

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Results obtained by various authors regarding the pH of eye drops were by no means uniform. While some recommended the value 6.75 as optimal for eye solutions, others ascertained long ago that it is neither necessary nor possible to prepare these drugs with exactly the same pH. Some consideration was given to the side effects, due to presence of active bases, irritating to eye tissues.

An up-to-date description of preparing isohydric lotions is to be found in the Commentary of the Swiss Pharmacopoeia. The pioneer work of Gifford (1935) influenced one of us in recommending alkaloids to be dispensed in the alkaline region; this must be reconsidered. Lower values were given by Skolaut (1948) 6.8-6.0; Blok (1945) reports that a half per cent. atropine solution underwent a 44 per cent. decomposition at pH 8.3, while homatropine showed a decomposition of 89 per cent. A very comprehensive study by Hind and Goyan (1947) emphasized the importance of the chemical stability of ophthalmic solutions. We refer to their excellent paper, where five groups are given in dispensing stabilized drugs. They found the concentration of free atropine base at pH 8.2-2 per cent. and at pH 9 18.3 per cent. The role of free base liberated by hydrolysis is stressed in evaluating activity and side effect. The reactions encountered in a solution of an alkaloid are as follows:—

The concentration of free base at various pH levels may be calculated from the constant of the base (Table I of Hind and Goyan, 1947), expressed in mol per cent. of free base. As to the question of preventing mould growth, we recommended previously nipa-bodies (esters of p-hydroxybenzoate). Zephyran† seems to be very adequate; the value of sodium propionate, a new fungicide should be investigated. The problem of eserine will be discussed elsewhere.

Experimental Findings

As the views of the different authors regarding decomposition of alkaloids are often contradictory (*e.g.*, Hind as against Blok) it proved necessary to examine this question. The behaviour of atropine sulphate solution (1 per cent.) was studied. By using modified Palitsch (1915) boric acid-sodium borate buffers we prepared a series ranging from pH 5 to 9 as shown in Table I; zephyran was added in a concentration of 1 : 50,000.*

† Zephyran (benzalkonium chloride) is a cationic detergent of high self-sterilizing quality, used at the Wilmer Institute in a concentration of 1:5000.

TABLE I

pH	0.2 mol Boric Acid cc.	0.05 mol Borax cc.	Grams NaCl per 100 cc.
5	0.356 M boric acid sol.	—	0.20
6	100	—	0.20
7.09	94	6	0.22
8.08	70	30	0.25
8.98	20	80	0.29

The physiological activity was studied by measuring the pupillary diameter with Wessely's keratometer before instillation and then, after 20, 40, and 60 minutes, at constant illumination. It is a well known fact that mydriasis sets in 10 minutes after instillation, attains its maximum degree 30 minutes later, and then decreases gradually. Blinking was avoided if possible, and colour of iris noted.

TABLE II

Mydriatic effect of 2 drops 1 per cent. atropine sulph. (mean values)

pH	No. of cases	Increase of pupillary diameter (mm.)	
		after 40 mins.	after 60 mins.
5	6	2.4	2.4
6	3	2.5	2.5
7	4	3.0	3.1
8	3	3.0	3.1
9 (fresh)	7	2.4	3.7
9 (2 months old)	3	2.6	3.0

The degree of mydriasis rises gradually in proportion with the increase of pH. Then we compared the behaviour of a fresh solution pH9 with one which was two months old.

The difference between the two drugs with regard to pharmacological activity is negligible.

Considering the irritating effect of more acid lotions we were surprised to see that such symptoms were absent. Not once did we observe hyperaemia, burning sensation, etc., after a single application of the drug. However, frequent instillation during the long course of an illness may possibly lead to intolerance. (It should be mentioned that lacrimation diminishes the lysozyme-content of tears: atropine—by reducing secretion—raises it again). It may be stated that the mydriatic effect is more or less the same, showing no irritative side-effects: the activity of a solution with higher pH is only very slightly intensified. This clinical statement is contrary to the experimental data of Cori, etc., who used cats and the isolated iris.

Concurrently we determined the change of atropine sulphate into base: we worked out the following method, led by the general characteristic of alkaloids, that alkaloidal salts dissolve in water, but not in chloroform and *vice versa*, bases dissolve in chloroform and not in water. So if chloroform is shaken with the test substance and the chloroform-solution is dehydrated, only the alkaloid in base form will dissolve. Our supposition proved to be right. We also studied the alkaloid-content according to the method of Schulek-Szeghő, with the difference that we did not add ammonia to the aqueous solution.

1 cc. of the solution was shaken together with 70 cc. of chloroform for several minutes: then anhydric sodium sulphate was added to the mixture, to give it a powder like aggregation non-adhesive character. We then poured the chloroform solution through filter paper containing a few grams of anhydric sod. sulphate and evaporated the chloroform. 10 cc. 0.02 N. sulphuric acid was added to the residue and retitrated with 0.02 N. NaOH, using methyl red indicator.

The solution was kept in transparent bottles in a dark place so that changes could be observed. As 3 to 4 weeks after dispensing the drug moulds appeared, we raised the concentration of zephyran to 1:20,000.

We estimated the concentration of free base directly after dispensing, then after 3, 14, and 25 days. We determined further the whole atropine content (salt); it was thought that atropine having an esteric character, would decompose in an alkaline milieu. For the sake of comparison the changes were observed in an unbuffered solution as well.

TABLE III

pH	Time of determination after preparation							
	Immediately		3 days		14 days		25 days	
	a	b	a	b	a	b	a	b
5	95.4	16.2	94.7	23.7	91.3	25.0	93.4	31.8
6	94.7	14.9	94.0	19.6	91.3	21.7	90.7	30.4
7	95.4	6.8	94.0	10.2	92.7	14.9	92.0	29.8
8	95.4	94.0	91.3	60.2	88.6	73.1	86.6	32.5
9	94.7	93.4	90.0	64.2	78.5	76.4	79.1	29.1
unbuffered	96.4	4.1	93.4	4.8	80.5	13.5	82.5	27.7

a = total atropine-content, expressed as atropine sulphate (percentage).

b = free base, expressed as percentage of a.

For the sake of comparison b is given as atropine sulphate also. The difference between a and b represents atropine in ionic state.

As the data prove, after preparation the atropine sulphate content of all the solutions was approximately the same (94.7-96.4 per cent.). The exactitude of determination can be said to amount to ± 1 per cent. The concentration of the free base after preparation was lowest in pH 7 solution, somewhat higher in the pH 5 and 6 types. The atropine was almost entirely in base form in solution of pH 8 and 9. During storage the base content slowly increased in pH 5-7 drugs, while in pH 8 and 9 it gradually diminished.

FIG. 1.—Total atropine content, expressed as percentage (ordinate), related to the time element in days (abscissa). The lines indicate solutions of varying pH. v = unbuffered solution.

Finally after 25 days the base concentration of atropine showed values of about 30 per cent. irrespective of hydrogen ion concentration.

While being stored the total amount of salt diminishes: in type 5-7 this decrease is negligible, in pH 9 the dissociation is appreciable after 2 weeks. The change in simple aqueous solutions without buffers is also interesting: in spite of the fact that in the beginning the atropine-base value was low, the reduction was almost the same in 2 weeks as with pH 9 solution. During the last ten days the total amount of atropine sulphate scarcely underwent any change in the different types (Figs 1 and 2).

The published data should not be considered as absolute values; possibly atropine, developing during decomposition, dissolves.

The rate of decomposition, however, is borne out by complete uniformity of experimental circumstances.

Our results show clearly why the data of different authors vary so widely regarding decomposition of atropine, the *concentration of the free base being a function of time*. According to our findings the base concentration changes considerably, but the

FIG. 2. Change of the free base concentration (percentage) as a function of time in days.

The lines indicate solutions of varying pH. v = unbuffered solution.

investigators quoted never mentioned how long after dispensing the solutions they carried out their tests. The decomposition of the atropine molecule in simple aqueous solution, as well as in pH 8 and 9 types is very remarkable. According to biological tests, with isotonic solutions the effect is in no close relation to the change of pH 5-9, or rather the increase of activity in relation to higher pH levels is not significant. Type pH 7 is recommended in order to obtain a stable solution, without sacrificing pharmacological activity.

Summary

Isotonic atropine sulphate solution — regardless of its pH — does not irritate the eye: simple aqueous solution (unbuffered) causes a burning sensation.

There is no distinct difference between the activity of atropine base and ion, not even when the solutions contained atropine entirely as bases or ion. The proportion of base and ion concentration varies after a certain time.

In aqueous solutions (unbuffered) and in types above the neutral point (e.g., pH 8-9) atropine splits hydrolytically.

From the standpoint of chemical stability the optimal is a pH 7 solution, prepared with boric acid-sodium borate buffer, which approached physiological circumstances. As a preservative we recommend zephyran (1:20,000). It is advisable to prepare ophthalmic drugs with distilled sterile water.

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