CONCENTRATION OF GLUCOSE AND TOTAL CHLORIDE IN TEARS*

BY

A. GIARDINI AND J. R. E. ROBERTS

Ophthalmological Research Unit, Medical Research Council, Institute of Ophthalmology, London.

ALTHOUGH numerous studies of the chemical composition of tears have been made, some points still remain obscure or contradictory. In this paper we confine ourselves to a consideration of the concentration of glucose and chloride at progressive stages of lacrimation, in subjects with a normal glucose metabolism.

In Table I values of the concentration of glucose found by the more recent workers are shown. It is apparent that a great diversity of opinion still exists, since the values range from a maximum of 65 mg. per 100 ml. to a complete absence of glucose.

TABLE I

Sugar Content of Tears

Investigator	Date	Method	Average Sugar (mg. per 100 ml.)
Wada	1922	Unknown	Present in some subjects
Ridley and Brown	1930	Maclean	65
Borsellino	1935	Bang II	27
Michail, Vancea, and Zolog	1938	Hagedorn-Jensen	Absent
Giardini	1949	Hagedorn-Jensen	10.2

It should also be noticed that reducing value does not necessarily indicate sugar content; thus one of us (Giardini, 1948) found a reducing value of 10·2 mg. per 100 ml. but failed to observe crystals of phenylglucosazone which, in biological fluids, will identify glucose in a concentration of approximately 5 mg. per 100 ml. (Rossi, 1933). In the present work reducing values have been estimated before and after fermentation with yeast.

The values of the chloride concentration found by other workers

* Received for publication October 25, 1950.

are shown in Table II, where the figures are given as mg. NaCl per 100 ml. In addition, the equivalent values in millimoles per kg. H_2O are shown.

TABLE II

Chloride Content of Tears

Investigator	Date	NaCl (mg. per 100 ml.)	NaCl as Chloride mM. per kg. H_2O)
Frerichs	1846	500	87.5
Magaard	1882	400	70.0
Arlt	1885	1,300	218.0
Ridley and Brown	1930	658	115.4
Michail	1938	823	142.4
Smolens and others	1949	490	85.05

Since it is possible that chloride concentration may vary with intensity of lacrimation, the chloride content has been determined by us in tears collected at definite stages of secretion.

Methods

Collection Procedure.—Lacrimation was induced by tear gas, tears being collected from each eye with a 12 in. Pyrex tube of 4 mm. internal diameter drawn to a 4 in. capillary of 1 mm. internal diameter at the tip. The annealed tip was placed in the conjunctival sac near the outer canthus, and the collected tears transferred to a corked Wassermann tube graduated at 0·2 ml.*

For the glucose determinations blood samples were necessary as the subjects were not under basal conditions. Our determinations of the sugar content were made by the Hagedorn-Jensen method combined with a fermentation process whereby the glucose is estimated by difference. For the preparation of the 10 per cent. yeast solution 10 gm. fresh baker's yeast were shaken with a large excess of distilled water, centrifuged at 3,000 r.p.m., and the supernate completely removed; this was repeated five times. The final yeast precipitate was made up to 1 l. with distilled water at 0°C. and a 10 ml. aliquot run into a test-tube placed in ice-water until ready for use. Vigorous yeast activity of the stock solution is maintained for about a week if the suspension is refrigerated, but before further use the stock solution must again be washed repeatedly. Blood and tear samples for fermentation were introduced into 10 ml. ground glass stoppered test-tubes; to each blood tube was added one drop (0·04 ml.) heparin solution containing 40 International Units per ml. aqua dist. Of the tears for fermentation, 0·2 or 0·1 ml., depending on the lacrimation stage under investigation, were placed in a stoppered test-tube, and the protein of the corresponding unfermented tears precipitated. To all fermentation tubes was added 0·2 of the freshly washed and chilled 10 per cent. yeast solution. The fermentation tubes were placed in a water bath and incubated for half an hour at 36 to 38°C. with occasional gentle shaking. On cooling, the protein was precipitated by the Somogyi (1930) technique, and the solution boiled for 4 min. and allowed to stand for 8 hours. All the solutions were centrifuged for 10 min. at 3,000 r.p.m., and the supernate run into prepared tubes containing the Hagedorn-Jensen reagent. The precipitate was washed twice with 3 ml. aqua dist., and again centrifuged to ensure that no yeast passed into the reaction tubes. Reagent

* We are indebted to Mr. F. T. Ridley, F.R.C.S., for his advice regarding the procedure for the collection of tears.

blanks, and yeast-water blanks, were run alongside a standard solution containing 20 mg. per cent. glucose. A fermented standard glucose was included to check the activity of the yeast.

Determination of Total Chloride.—In order to detect any dilution effects, we made our determinations at widely separated lacrimating stages; thus a relationship might be established between the varied results of previous workers.

The total chloride was determined by the Sendroy technique (1936). Our subjects were divided into four groups according to the lacrimating stages involved. In the first three lacrimation was induced, and tears collected, as described above.

Group I. 0·22 ml. taken from the first lacrimation of each eye from three subjects, followed by a further 0·22 ml. Right and left samples at each lacrimating stage bulked. Six analyses made.

Group II. 0·22 ml. taken from the first lacrimation of three subjects and bulked for analysis.

Group III. 0·11 ml. taken from each eye of six subjects. Right and left samples bulked. Six independent analyses made.

Group IV. Two analyses made on the bulked tears of two groups of ten subjects, each contributing approximately 0·01 ml. from each eye. Collection was made by pipette, the tip of which when moved slightly in the conjunctival sac, induced the minute quantity of tears required for analysis. In order to reduce the loss by evaporation which must occur to some extent in Groups I, II, and III, the small traces of tears in Group IV were run directly into the weighed quantity of 0·085 M. phosphoric acid required by the Sendroy method, and their weights obtained by difference.

Determination of Total Solids.—The total solids were determined by evaporating to dryness at 105°C. tears weighing 0·6028 gr. The solid residue was cooled in a dessicator and weighed immediately after removal; this weighed 0·0110 gr. The total solid was therefore 1·84 per cent.

Results

Glucose.—The results from twelve subjects are shown in Table III (overleaf). From the first seven subjects 0·22 ml. tears was taken from both right and left eyes, and when possible a further 0·22 ml. In the last five subjects only 0·1 ml. was collected from each eye, both being combined for analysis.

In 25 determinations the Hagedorn-Jensen analysis for total reducing substances gave a range of values equivalent to 2·8 to 7·7 mg. per 100 ml. of glucose, with an average of 6·06 mg. per 100 ml. Agreement between the two eyes in the same subject was always within 0·8 mg. per 100 ml. with no significant predominance of either eye. At different lacrimating stages, a decrease of concentration, varying from 2·6 to 0·2 mg. per 100 ml., was found in successive samples in four cases, and an increase ranging from 0·4 to 1·28 mg. per 100 ml. in three cases. With such variability and minute changes in concentration between the first and second samples, no generalization may be made concerning the possibility of the dilution of glucose in tears during a continuous lacrimation.

The true glucose determined by the fermentation method varied between 1·0 and 5·0 mg. per 100 ml., with an average of 2·6 mg. per 100 ml.; in one case and in one eye only, there appeared to

TABLE III

Sugar Content of Blood and Tears

TRS—Concentration of total reducing substances.
NGS—Non-glucidic substances.
TGS—True glucose.

Subject	Blood			Eye	Tears		
	TRS	NGS	TG		TRS	NGS	TG
1	117	22	95	R1	2.8	1.2	1.6
2	171	28	142	R1	3.8	3.8	0
				L1	3.6	2.4	1.2
3	137	30	107	R1	7.4	2.4	5
				R2	4.8	3.4	1.4
				L1	7.4	2.4	5
4	153	18	125	R1	6.15	2.72	3.43
				R2	5.70	2.55	3.15
				L1	5.37	1.62	4.75
				L2	5.04	2.87	2.17
5	106	28	78	R1	6.40	2.8	3.6
				R2	7.36	4.8	2.56
				L1	7.16	3.51	3.65
6	102	18	84	R1	6.23	2.72	3.51
				R2	6.63	3.77	2.86
				L1	5.52	3.77	1.75
				L2	6.80	4.4	2.4
7	187	12	175	R1	6.46	4.07	2.39
				R2	5.83	3.6	2.23
				L1	6.31	3.85	2.46
8	112.5	24.7	77.8	R + L	5.4	4.8	1.6
9	120	22.5	97.5	R + L	7.7	5.6	2.1
10	—	—	—	R + L	7.3	5.06	2.3
11	111.6	26.5	84.1	R + L	6.02	4.6	1.42
12	124	20	104	R + L	6.9	4.9	2

R1 ... Concentration in 1st lot of tears from right eye.
R2 ... Concentration in 2nd lot of tears from right eye.
L1 ... Concentration in 1st lot of tears from left eye.
L2 ... Concentration in 2nd lot of tears from left eye.
} concentration in mg. equivalent glucose per 100ml.

be a complete absence of glucose. The true glucose of tears represented an average of 41·0 per cent. of the total reducing substances, while the true glucose of the same subject's blood was 81 per cent.

TABLE IV

Chloride Concentration in Tears

Group	Chloride (NaCl in mg. per 100 ml.)	Chloride (mM. per Kg. H_2O)	Volume Tears taken (ml.)	Number of Subjects
I	A. 628	110.2	0.22	3
	B. 603	105.8	0.22	—
	A. 635	111.4	0.22	—
	B. 600	105.0	0.22	—
	A. 732.5	128.2	0.22	—
	B. 688	120.5	0.22	—
II	665	116.5	0.22	3 (bulked)
III	698	122.4	0.11	6
	715	125 0	—	—
	680	119 3	—	—
	689	120.8	—	—
	726	127.0	—	—
	689	120.8	—	—
IV	770	135.0	0.01	10 (bulked)
	776	136.0	0.01	10 (bulked)

Group I lacrimation profuse: A, first withdrawal. B, second withdrawal.
Group II lacrimation also profuse
Group III lacrimation less profuse
Group IV only 0.01 ml. could be taken, no tear gas used

Average chloride Gp. I. B ... ... 630 (110.4 mM.)
Average chloride Gp. I A and Gp. II ... ... 665 (116.8 mM.)
Average chloride Gp. III ... ... 697 (122.4 mM.)
Average chloride Gp. IV ... ... 773 (135.5 mM.)
} see Figure (overleaf).

Chloride.—As shown in Table IV, the second 0·22-ml. samples, after profuse lacrimation, had an average chloride concentration of 110·4 mM. per kg. H_2O (Group I.B), while the average values taken after progressively earlier lacrimating periods showed a continuous increase, *i.e.*, 116·8, 122·4 and 135·5 mM. chloride after 0·2 ml., 0·1 ml., and 0·01 ml. respectively. The dilution between the first (approx. 0·01 ml.) and the last (0·4 ml.) lacrimal secretion was 18·5 per cent. The Figure illustrates this progressive dilution.

Discussion

As demonstrated, the total reducing substance of tears is extremely low compared with that of blood. In tears true glucose is represented to the extent of only 40 per cent., while in the blood, as is well known, the true glucose represents 80 per cent. The deficiency of this important metabolite may be one reason why tears are a poor culture medium for pathogenic organisms.

The concentration of chloride may have some bearing on problems still under discussion, especially those regarding corneal physiology. From this point of view, the significant concentration in tears is that obtaining at the earliest stage of lacrimation, a value that presumably approximates to that of the normal film in contact with the corneal epithelium. The average value found

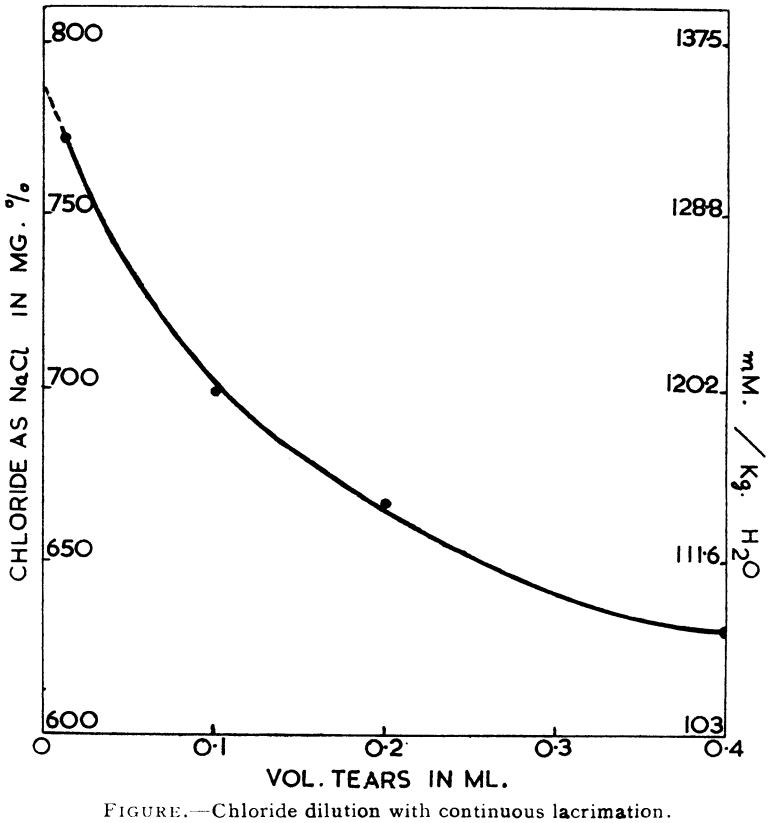

FIGURE.—Chloride dilution with continuous lacrimation.

The average total chloride as NaCl of each stage is plotted against its respective volume. Ordinates, chloride in mg. per 100 ml., and mM. per kg. H_2O. Abcissae, total volume of tears withdrawn.

was 135·5 mM. per l. so that the cornea is apparently in contact with two hypertonic liquids (aqueous humour 124.3 mM. per l., Hodgson, 1937), compared with plasma (105·1 mM. per l., Hodgson, 1937). This finding could provide experimental evidence on the theories connected with the deturgescent state of the cornea (Kinsey and Cogan, 1942; Davson, 1949); however, the concentration of chloride taken alone is not a good index to the tonicity of the solution, since the bicarbonate ion represents a significant and possibly a highly variable fraction of the anions.

In a later paper we propose to discuss the concentration of sodium in tears, since this ion should be a better index to tonicity.

We may note in conclusion that the values found by previous workers (Magaard, 1882; Frerichs, 1846; Ridley and Brown, 1930; Smolens and others, 1949) are lower than ours, with the exception of that found by Michail (1938).

Summary

The true glucose was determined in tears of subjects with a normal glucose metabolism by the Hagedorn-Jensen method combined with a fermentation process. The average total reducing substance was equivalent to 6·06 mg. per 100 ml. glucose, while true glucose was present to the extent of 2·5 mg. per 100 ml. In contrast to the blood, the true glucose of tears represents only 41 per cent. of the total reducing substance. After continuous lacrimation the values were so small and variable that no generalization concerning the probability of a dilution of the sugar content was possible.

Analysis for the chloride was made by the Sendroy technique. After a profuse lacrimation 110·4 mM. per l. chloride were found, while the nearest to normal lacrimal secretion obtainable (first 0·01 ml. of tears to appear) gave a value of 135·5 mM. per l.

The dry weight of tears was 1·84 per cent.

We are indebted to Dr. Hugh Davson for his interest and advice in this work, to men and women of the London Metropolitan Police Force (E. Div.) for their co-operation in contributing tears and blood, and to members of the Staff of the Institute of Ophthalmology and the Ophthalmological Research Unit for their ready and cheerful assistance.

REFERENCES

ARLT (1855). *Graefes Arch. Ophthal.*, **1**, pt. 2, p. 135.
BORSELLINO, G. (1935). *Cultura med. mod.*, **14**, 299.
DAVSON, H. (1949). "Physiology of the Eye". Churchill, London.
FRERICHS, F. T. (1846). *In* Wagner, R., "Handwörterbuch der Physiologie", vol. 3, pt 1, p. 617.
GIARDINI, A. (1949). *Boll. Oculist.*, **28**, 649.
HAGEDORN, H. C., and JENSEN, B. N. (1923). *Biochem. Z.*, **135**, 46.
HODGSON, T. H. (1938). *J. Physiol., Lond.*, **94**, 118.
KINSEY, V. E., and COGAN, D. G. (1942). *Arch. Ophthal., Chicago*, **28**, 449.
MAGAARD, H. (1882). *Virchows Arch.*, **89**, 258.
MICHAIL, D. (1938). *Ann Oculist., Paris*, **175**, 565.
———, and ZOLOG, N. (1937). *C. R. Soc. Biol., Paris*, **126**, 1042.
———, VANCEA, P., and ZOLOG, N, (1937). *Ibid.*, **125**, 194, 1095.
——— ——— ——— (1938a). *Clujul. med.*, **19**, 129.
——— ——— ——— (1938b). *Bull. Acad. Méd. Roumanie*, **5**, 182.
RIDLEY, F. (1928). *Proc. roy. Soc. Med.*. **21**, 1495.
——— (1930). *Brit. J. exp. Path.*, **11**, 217.
ROSSI, A. (1933). *Arch. Sci. Biol., Bologna*, **19**, 320.
SENDROY, J., JR. (1937). *J. Biol. Chem.*, **120**, 335.
SMOLENS, J., LEOPOLD, I. H., and PARKER, J. (1941). *Amer. J. Ophthal.*, **32**, pt 2 (June), p. 153.
SOMOGYI, M. (1930). *J. Biol. Chem.*, **86**, 655.
WADA, H. (1922). Cited by Borsellino.