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ON THE RENAL THRESHOLD FOR CHLORIDE IN MAN.

By R. S. AITKEN.

(*From the Physiological Laboratory, Oxford, and the Medical Unit, the London Hospital.*)

INTRODUCTION.

THE conception of a renal threshold for chloride seems to rest more upon analogy and inference than upon precise observation. It is still true, as Haldane and Priestley(6) said in 1915, that "the stimulus to the excretion or the retention of this or that substance by the kidneys is presumably a slight increase or diminution in its concentration in the blood plasma; but quantitative data on this subject are as yet hardly existent." The idea of a "threshold" originated with Claude Bernard(3), who described it in the excretion of glucose. Magnus(7) extended it to chloride: he injected sodium chloride and sodium sulphate solutions into the veins of rabbits and dogs and observed the resulting diuresis and the concentration of the salts in blood and urine; after sodium sulphate injections he obtained urine which was almost chloride-free, secreted by the kidneys from blood which contained 0·6 p.c. of NaCl; so he came to the conclusion (which he unfortunately extended to the sulphate as well as the chloride) that the kidney acts as an "overflow-valve," excreting chloride when the amount in the blood exceeds "a certain threshold," and to all intents and purposes excreting none when it sinks below that threshold.

The idea was taken up by Ambard(1) in his attempt to represent the behaviour of the kidney by algebraical equations. He dealt first with urea, and developed his well-known formula and his "ureo-secretory constant." Turning to chloride, he accepted the idea of a threshold as used by Magnus(7), and gave some data(2) in support of it. These consist of determinations of serum chloride in two human subjects, with corresponding determinations of the rate of excretion of chloride over short periods; the figures show that, with falling serum chloride, the chloride excretion falls at a rate, which, if continued, would bring it to zero when the serum chloride is about 0·565 p.c. Ambard(1) also observed that in a normal subject living on a low chloride diet, the serum NaCl did not fall below 0·581 p.c. He then made two assumptions: (*a*) that his urea formula is applicable to the excretion of chloride, provided that the total serum concentration in the case of urea be replaced by the excess of serum chloride concentration over a threshold, (*b*) that a "chloro-secretory constant" exists, identical numerically with the "ureo-secretory constant" in a given individual. Making those assumptions, he proceeded to calculate the chloride threshold in various subjects, from determinations of the serum chloride concentration, the urine chloride amount and concentration, and the ureo-secretory constant. He found that the threshold was not fixed, but varied: it rose after a meal, and it also rose slightly when the subject lived on a low chloride diet. Finally he concluded that "the mobility of the threshold for Cl is the essential factor in the regulation of the concentration of Cl in the blood." Ambard's chloride threshold, then, is arbitrarily defined in terms of an equation whose validity and whose constant are assumed, and he finds the threshold to be movable, not fixed.

Cushny(4) uses the idea of threshold to classify urinary constituents into "threshold bodies" and "no-threshold bodies." Chloride is a "high-threshold body," resembling sodium and glucose in "possessing a definite threshold above which it is excreted by the kidney." Cushny crystallizes the idea into a simple formula:

$$\frac{C_B - T}{C_U} = K,$$

where C_B and C_U are the concentrations of chloride in blood and urine, T is the threshold, and K is described vaguely as a "constant for each sample of urine"; but he gives no evidence to suggest that this formula can be applied quantitatively, and in fact he admits that "the relation between the chloride of the plasma and of the urine cannot be followed very exactly."

Observations on chloride excretion in pneumonia are often quoted in illustration of the threshold phenomenon, in spite of the fact that the kidney of a pneumonic patient cannot be regarded as normal. They show that in pneumonia the plasma chloride concentration is unusually low, and the urinary chloride excretion may be very small, but not zero; for instance, the lowest 24-hour excretion of chloride found by Peabody(9) in pneumonic patients was 0·13 grm., while Haden(5) recorded 0·05 p.c. NaCl in the urine of one case, and a "faint trace" in two others.

There is in addition one striking observation recorded by Moss(8) who tested the urine passed by a collier (subject to miners' cramp) after working a shift in a hot damp mine: "it gave not even the slightest cloudiness with silver nitrate, although only 5 c.c. were secreted during $4\frac{1}{2}$ hours."

Speaking generally, then, the facts on which the idea of a chloride threshold is based seem to be:

1. That the plasma chloride concentration is found to vary only within narrow limits, while the urine chloride varies widely in amount and concentration.

2. That as the plasma chloride concentration falls (within those limits), the urine chloride diminishes rapidly.

3. That in pneumonia, where the plasma chloride is very low, the excretion of chloride is slight.

These facts indicate that there is a region of plasma chloride concentration above which chloride excretion by the kidney is relatively great and below which it is relatively small, but they do not justify the statement that a certain fixed level or concentration of plasma chloride exists above which chloride excretion goes on and below which it ceases. The latter is, however, the commonly accepted interpretation of the threshold idea, an interpretation whose precision is not warranted by the available experimental results.

In view of this an attempt was made, in the experiments to be described below, to observe the relationship between plasma chloride concentration and urinary chloride excretion in man, especially at low rates of chloride excretion. Parallel determinations of plasma and urine chlorides were made on two subjects for about a week, during which time they lived under practically uniform physiological conditions, taking a standard chloride-free diet, and accelerating the output of chloride from their bodies by undergoing periods of water drinking and sweating. The latter process is necessary if minimal rates of renal excretion are to be attained within a reasonable time, for when the intake of chloride is reduced to zero the kidneys are tolerably efficient in conserving the chloride already in the body; the skin is much less efficient, and allows the loss of 2 or 3 grm. of sodium chloride in every litre of sweat.

First experiment.

Methods.

The subject, J. G. P., lived for six days on a uniform and completely chloride-free diet consisting of cane sugar, "casein jelly" and water, measured amounts of which were taken at frequent intervals throughout the day. One day's ration of "casein jelly" was made as follows. Forty grm. of "ashless casein" (Glaxo Company), said to contain less than 1 p.c. of calcium, were shaken with a litre of water, and sufficient chloride-free sodium hydroxide was added to make the granules of casein go into opalescent solution (about 7·5 c.c. of 20 p.c. NaOH). The reaction was adjusted by adding 1 p.c. acetic acid till a few drops of added phenolphthalein just gave a faint pink colour. Twenty-five grm. of Coignet's photographic gelatine were added to the solution, and dissolved by warming. Finally 50 grm. of cane sugar and a little vanillin dissolved in alcohol were added, and the mixture poured into 200 c.c. beakers, in which it cooled and set. The resulting jelly was quite palatable. Samples of casein and gelatine had previously been ashed and tested for chloride; none was found. The energy value of this jelly and additional sugar consumed (which varied a little from day to day) was between 1060 and 1580 calories per day.

The experiment occupied eight days in all. The subject's general activities were kept as far as possible uniform; he motored to the laboratory every morning, sat in a chair or moved quietly about the building all day (except when sweating), and motored home in the evening. On the first day he ate a normal breakfast with a generous allowance of salt, and nothing else all day. On the second day he began the diet just described, and returned to a normal one on the evening of the seventh. On each of the first five days he spent two hours (10.30 a.m.–12.30 p.m.) in a closed respiration chamber, in which the atmosphere was laden with moisture, and the wet-bulb temperature raised by an electric heater to between 27° and 30° C. While in the chamber he drank 1500 c.c. of water, and pedalled for four periods of 10 minutes each on a bicycle ergometer, doing as strenuous work as was comfortable in the circumstances. The result was copious sweating on every occasion.

Three times every day the urine secreted in a period of one hour was collected, and a sample of blood was taken from an arm vein exactly in the middle of that period. Urine passed in the intervening periods was also collected. Urines were passed into clean dry measuring cylinders, and measured when cool. Their chlorides were estimated by the usual

Volhard method, duplicate determinations being made on the one-hour specimens. In highly coloured urines containing very little chloride, some difficulty was experienced with the end-point in the titration with thiocyanate. When the nitric acid was added, a brownish colour developed which tended to mask the brown of the iron alum indicator. In these circumstances the titration was done in a wide glass boiling tube held against the light, instead of in the usual white porcelain dish, and a similar tube with its contents incompletely titrated was set up for comparison; in this way satisfactory end-points were obtained. Blood samples were taken from veins at the elbow through a small needle, using only sufficient compression of the arm to make the vein prominent at the moment of puncture. The blood was led directly under paraffin, through a rubber connection and a glass tube which had a weighed amount of heparin (4 mg. per 10 c.c. blood) moistened with saline, on its inner surface. It was centrifuged for 20 minutes and the plasma pipetted off. No trace of hæmolysis occurred, except in one specimen, which was discarded. Plasma chlorides were estimated by the method described by van Slyke(10), in duplicate. In spite of special precautions, including that of stirring during the titration mentioned by Whitehorn(12), the duplicates in a number of cases failed to agree. This was attributed to incomplete digestion of the proteins in the water-baths, which were not self-filling, and had to be re-filled at intervals with cold water. Where duplicates differed by more than 2 p.c. they were discarded; two-thirds of those retained differed by less than 1 p.c. Mean values have been used.

Results.

The effect of the régime on the renal excretion of chloride is shown in Table I, which gives the 24-hour outputs of water and chloride.

TABLE I.

First Experiment.

Urinary output in 24 hours from 9 a.m. Of day	Water c.c.	NaCl grm.	Remarks
1	1995	8·240	Normal breakfast only
2	2326	1·393	Régime begun: chloride-free diet with water-drinking-plus-sweating every morning
3	1398	0·277	
4	2093	0·189	
5	1907	0·122	
6	1411	0·116	(No drinking-plus-sweating)
7	949	0·134	Normal diet resumed at 5 p.m.
8	1199	1·088	

On the first day the chloride excretion was high, because the subject had previously been eating salt freely with his food. From the second day it fell rapidly, and by the fourth day of the régime (fifth day of the experiment) it was as low as 122 mg. in 24 hours. On the sixth day of the experiment it was 116 mg. in 24 hours. On the seventh day, when normal diet was resumed in the evening, fluids were taken freely (800 c.c. in excess of the usual allowance); both salt and water, however, were retained in the body, for the 24-hour urine up to 9 a.m. the following day contained only 0·134 grm. NaCl and less than a litre of water. By the eighth day the NaCl excretion had risen to something over 1 grm.

The chloride concentrations of the samples of plasma, with the corresponding amounts of chloride in the urine, are given in Table II, and plotted one against the other in Fig. 1, where they seem to justify

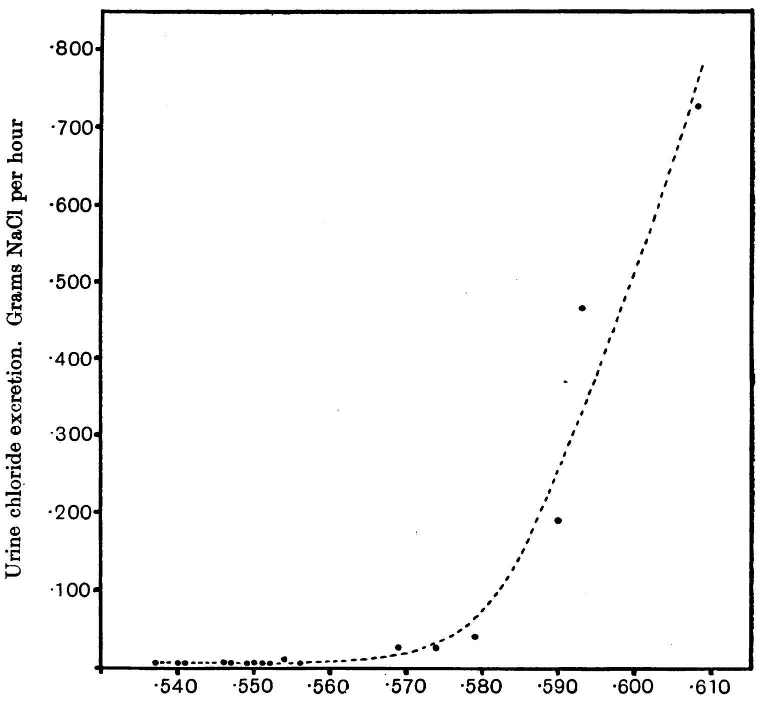

Fig. 1. Plasma chloride concentration and urinary chloride excretion in the first experiment (Table II).

a curve of the shape shown. This curve represents the relation of plasma chloride concentration to urinary chloride excretion in this subject, under strictly uniform physiological conditions. Its upper part descends steeply; it bends fairly sharply in the region of plasma chloride concentration between 0·555 p.c. and 0·585 p.c., but the number of points obtained there does not fix it very satisfactorily; and below that level it runs horizontally down to 0·537 p.c. This lowest part, which is the most difficult to achieve experimentally, is shown on a larger scale in Fig. 2. The points appear to scatter somewhat, but if those representing

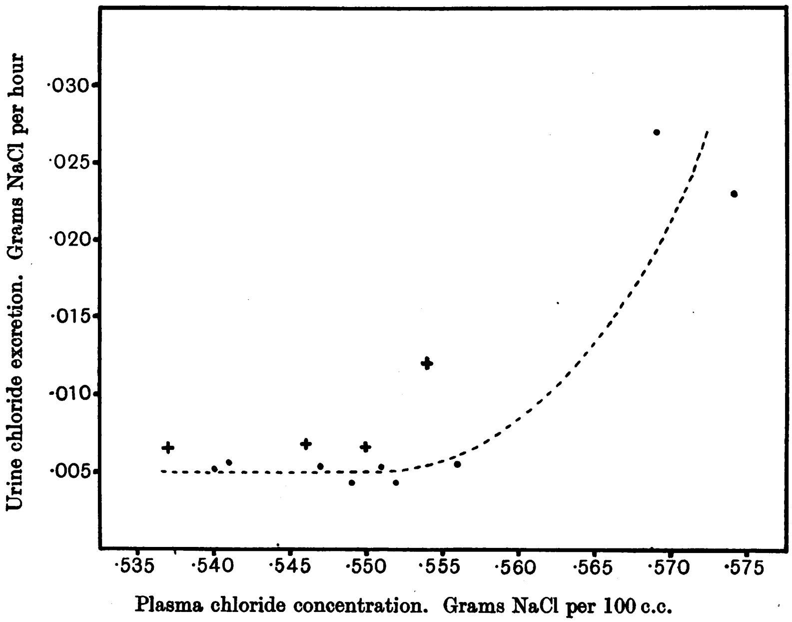

Fig. 2. Plasma chloride concentration and urinary chloride excretion in the first experiment (Table II).

+ Points 9, 10, 12, 13: water excretion over 90 c.c. per hour.

● Points 7, 8, 11, 14–19: water excretion under 60 c.c. per hour.

periods when the water excretion of the kidneys exceeded 90 c.c. per hour be differentiated from the rest (in which it was less than 60 c.c. per hour—*vide* Table II) the former are seen to lie above the curve established by the latter. In other words, at these very low levels of chloride excretion, a diuresis of 100 or 200 c.c. an hour is accompanied by a relatively large increase in chloride excretion.

TABLE II.

Parallel determinations of plasma chloride concentration and urinary chloride excretion at intervals throughout the first experiment.

Day	Urine (1 hour samples) Volume c.c.	Urine (1 hour samples) NaCl grm.	Plasma NaCl p.c.	No.
1	48	0·727	0·608	1
	29	0·466	0·593	2
	560	0·190	0·590	3
2	63	0·155	0·578	4
	94	0·050	0·511*	5
	150	0·039	0·579	6
3	29	0·023	0·574	7
4	13	0·0054	0·566	8
	259	0·012	0·554	9
	92	0·0064	0·537	10
5	25	0·0052	0·551	11
	121	0·0065	0·550	12
	150	0·0068	0·546	13
6	33	0·0056	0·541	14
	56	0·0051	0·540	15
7	49	0·0053	0·547	16
	32	0·0042	0·552	17
	21	0·0042	0·549	18
8	36	0·027	0·569	19

* This value is evidently in error, and has been omitted from the figures.

SECOND EXPERIMENT.

Methods.

An essentially similar set of observations was made on a second subject, R. S. A., with a special attempt to obtain more points on the transitional part of the curve between 0·555 p.c. and 0·585 p.c. plasma chloride. The sweating periods were a little less severe than in the first experiment. Specimens of blood and urine were obtained twice daily; during the hour while the urine was being secreted and during the preceding half-hour, in each case, the subject remained seated so that circulatory disturbances should affect the kidneys as little as possible. The interval from the last ingestion of food to the collection of specimens was always the same. The diet of casein jelly, sugar and water (1860 cals. per day) was absolutely constant. In other respects the general procedure and technique resembled those in the first experiment, except that plasma chlorides were estimated in triplicate, using improved water-baths, and results agreeing within 1 p.c. were obtained.

The effect of the régime on the renal excretion of chloride and water is shown in Table III. The reduction in chloride output is rapid, but not so great as in the first experiment.

TABLE III.

Second Experiment.

Urinary output in 24 hours from 8 a.m. Of day	Water c.c.	NaCl grm.	Remarks
1	—	—	Normal breakfast Chloride-free diet begun in the afternoon 7·5 grm. NaCl taken in addition
2	2691	6·393	Chloride-free diet continued, with periods of drinking-plus-sweating
3	1957	2·635	,, ,,
4	2057	1·093	,, ,,
5	827	0·499	12 grm. NaCl taken in addition in the evening
6	—	—	13 grm. NaCl taken in addition about 9 a.m. Specimens No. 8, Table IV taken at noon. Normal diet resumed after that

The plasma chloride concentrations and the corresponding rates of urinary excretion are given in Table IV, and plotted in Fig. 3, where

TABLE IV.

Parallel determinations of plasma chloride concentration and urinary chloride excretion at intervals throughout the second experiment.

Day	Urine (1 hour samples) Volume c.c.	NaCl grm.	Plasma NaCl p.c.	No.
2	142	0·723	0·600	1
	74	0·247	0·586	2
3	55	0·216	0·579	3
4	122	0·112	0·577	4
	65	0·062	0·563	5
5	24	0·034	0·553	6
	163	0·023	0·556	7
6	35	0·183	0·604	8

the scale is the same as in Fig. 1. With the exception of No. 8, the points lie close to the curve shown, which is quite similar to that in Fig. 1, although lying a little further to the left; they establish the transitional bend of the curve where the points in Fig. 1 were too few.

The ingestion of large amounts of NaCl in a short time at the end of the experiment (Table III) led to two interesting results. In the first place the 24-hour specimen of urine completed at 8 a.m. on the sixth day contained only 0·499 grm. NaCl, although 12 grm. had been swallowed the previous evening. This emphasises the degree to which the body, if depleted of salt, retains what salt may be subsequently offered to it. Along with the salt it retains water, corresponding to that lost during the period of depletion (Veil(11)); hence the low volume, 827 c.c., of the 24-hour urine of the fifth day. Secondly, specimens of blood and urine were obtained about midday on the sixth day, after 25 grm. in all of salt had been taken, and while the restoration of the normal salt and water equilibria of the body was presumably still in process. Their chloride values gave the point 8, which is completely off the curve in Fig. 3, indicating that although the plasma chloride value

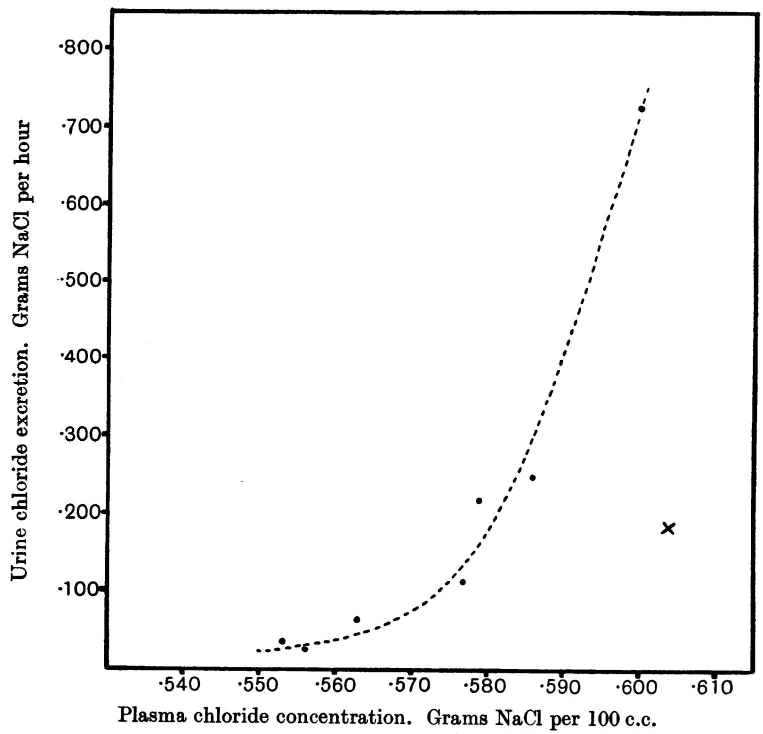

Fig. 3. Plasma chloride concentration and urinary chloride excretion in the second experiment (Table IV).

● Points 1 to 7. X Point 8.

has risen to its initial level the chloride excretion rate is still quite low: the kidney's response to the presence of chloride in that concentration in the plasma has altered.

Discussion.

The conclusion to be drawn from these two experiments is that, in the human subject, if the physiological conditions of diet, water intake, muscular activity etc., are rendered sufficiently uniform, and if the body is gradually depleted of chloride by a chloride-free régime and forced sweating, then the response of the kidneys to variation in the concentration of chloride in the plasma can be defined as a reasonably smooth curve, relating plasma chloride concentration to rate of renal chloride excretion. In the two subjects used the curve is found to be steep above a plasma NaCl concentration of about 0·585 p.c., indicating a large increase in excretion for a small increase in plasma concentration. Below about 0·555 p.c. of plasma NaCl it is horizontal, indicating a steady rate of excretion at about 5 mg. per hour, down at least to 0·537 p.c. of plasma NaCl. The intermediate transitional part of the curve occupies the range 0·555 p.c. to 0·585 p.c. plasma NaCl, and this part of the curve is the most precise definition that can be given of the term "renal threshold for chloride." The threshold is not a definite point, but simply a neighbourhood in which the relation of renal excretion to plasma concentration changes rapidly; below this neighbourhood, the excretion is small and apparently steady, but it is not zero.

There is no evidence that even this roughly defined neighbourhood is constant in physiological (or pathological) conditions other than those of these experiments. On the contrary, the discrepancy of point 8 in Fig. 3 suggests strongly that during such a disturbance as the transition from a low-salt to a high-salt régime, the whole curve will be considerably altered; and the scatter of the points in Fig. 2, already referred to, points to a relationship between rates of excretion of chloride and of water, especially in the lower part of the curve. In other words, the chloride-excreting activity of the kidney is not determined solely by the chloride concentration of the plasma, but is influenced by other factors; therefore it is extremely unlikely that even the transitional neighbourhood, approximately defined in these experiments, is constant in varying circumstances.

These considerations throw doubt on the usefulness of applying the threshold idea to chloride at all. It is obvious that a fixed threshold value on which accurate calculations might be based does not exist. The study of the behaviour of the human kidney would be advanced if the conception of a chloride threshold were discarded, and, under controlled and suitably varied physiological conditions, more extended observations were made of the kidney's response to the stimulus of varying plasma chloride concentration.

Summary.

1. The relation between the rate of excretion of chloride by the kidney and the concentration of chloride in the plasma has been observed in two human subjects, over a wide range of plasma chloride concentrations, under strictly uniform physiological conditions.

2. The nearest approximation to a renal threshold for chloride appears to be in the neighbourhood of 0·555 p.c. to 0·585 p.c. plasma NaCl, where the above relationship is rapidly altering. There is no evidence that this neighbourhood is constant under varying physiological conditions. It is suggested that the idea of a renal threshold for chloride be abandoned.

I wish to thank Dr J. G. Priestley for his advice and help throughout these experiments, and for his kindness in suffering himself to be the subject of the first one.

References.

1. Ambard, L. Physiologie normale et pathologique des reins, p. 145 et seq. Paris. 2nd ed. 1920.
2. Ambard, L. and Weill, A. Journ. Phys. et Path. Gén. 14. p. 753. 1912.
3. Claude Bernard. Leçons sur le Diabète et la Glycogenèse animale, p. 132. Paris. 1877.
4. Cushny, A. R. The Secretion of the Urine, ch. II, London, 2nd ed. 1926.
5. Haden, R. L. Journ. Lab. and Clin. Med. 10. p. 337. 1924–5.
6. Haldane, J. S. and Priestley, J. G. This Journ. 50. p. 296. 1916.
7. Magnus, R. Arch. f. exp. Path. und Pharm. 44. p. 396. 1900.
8. Moss, K. N. Proc. Roy. Soc. B, 95. p. 181. 1923.
9. Peabody, F. W. Journ. of Exp. Med. 17. p. 71. 1913.
10. van Slyke, D. D. Journ. Biol. Chem. 58. p. 523. 1923–4.
11. Veil, W. H. Bioch. Zeitschr. 91. p. 267. 1918.
12. Whitehorn, J. C. Journ. Biol. Chem. 74. p. 299. 1927.