CXXV. CHLORIDE AND WATER IN THE CONSTITUTION OF TISSUES.

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It has already been shown [Osman and Close, 1930] that the bicarbonate and chloride of the blood-plasma as well as the water of subcutaneous tissues show variations in the human subject with age and sex and in health and debility. A similar investigation has now been carried out on the chloride and water of other tissues, and it has been found that the differences due to age, sex, *etc.* were not so marked as in the observations made on the plasma.

The chloride and water values in the tissues were also considered from two other points of view.

(a) Their relationship to each other and to the other constituents of tissues.

(b) Their correlation with clinical manifestations and pathological states.

Subsequently the former of these (a) became the chief line of investigation when it was noticed that there is law and order underlying the apparently haphazard arrangement of the principal constituents of tissues, though unfortunately it is not possible at present to do more than indicate the broad outlines of the chemical pattern as revealed in the relationship between chloride and water and between these two substances and the hydrogen ion concentration, the protein, sodium and potassium contents. No relationship could be established between the chloride and water and the electrical conductivity [Crile *et al.*, 1922].

Material.

The tissues examined were taken from human subjects at routine *post mortem* examinations, and, as it was found impossible to select a normal type of case, the average figures shown in Table IV (water content) and in Tables V and VI (chloride content) were obtained from duplicate estimations on a large series of consecutive cases—90 in the case of the organs; 80 in the case of the brains. Many types are, therefore, included and no particular type predominated.

Amongst the several disadvantages that render material obtained in this way far from ideal is the inability to gauge the amount of blood and fibrous tissue present in the mass of tissue taken, so that the figures obtained may not represent the amount of water or chloride in the actual cell of that tissue. On the other hand, it is equally difficult to obtain tissues in normal subjects free from these two substances.

The estimations were at least made under comparable conditions, as all bodies were put in a refrigerator two hours after death and were examined within the next following 24 hours. Within this period the effect of autolysis on the chloride and water distribution in tissues taken in bulk is minimum. This was shown in the following experiments.

¹ Based on a thesis approved for the degree of Doctor of Medicine in the University of London.

A. An amputated human limb was left on a laboratory bench for 48 hours after removal. Apart from a covering of cloth to prevent loss of surface water, no other precautions were taken. Pieces of muscle, cartilage, fibrous tissue and bone were removed at intervals of 2 hours, 24 hours and 48 hours after operation and their chloride and water contents were estimated. (Methods described later.) Tables I and II show the results of the chloride and water estimations.

Table I. *Cl estimations.*

(As % of wet weight.)

Tissue	At 2 hours	At 24 hours	At 48 hours
Tendo Achillis	0.24	0.25	0.24
Articular cartilage	0.21	0.21	0.22
Skin	0.21	0.22	0.22
Voluntary muscle	0.15	0.15	0.15
Bone (fibula)	0.09	0.08	0.09

Table II. *Water estimations.*

(As %.)

Tissue	At 2 hours	At 48 hours
Voluntary muscle	72.86	73.13
Articular cartilage	65.24	65.41
Tendon	63.57	63.44

B. The chloride contents of the liver and kidneys of three healthy guinea-pigs were estimated immediately after death, and 24 hours after death.

The results are shown in Table III.

Table III.

	Immediately after death Cl %	24 hours after death Cl %
Guinea-pig A. Liver	0.12	0.13
Kidney	0.23	0.24
Guinea-pig B. Liver	0.12	0.12
Kidney	0.27	0.26
Guinea-pig C. Liver	0.11	0.11
Kidney	0.18	0.17

The results shown in Tables I, II and III, make it clear that there is little or no disturbance of the chloride and water contents or tissues taken from the body within 24 hours of death.

The water content of tissues.

In Table IV are shown the average water percentages and the standard deviation of a single observation in the organs and tissues investigated—the grey matter (Rolandic area), white matter (lateral portion of the corpus callosum), kidney, liver, thyroid, heart muscle and voluntary muscle (psoas). The average percentages of water in several other tissues, collected from the literature, are also shown.

The water content was measured by heating about 20–30 g. of tissue (cut into small pieces) in an oven at a temperature of 105–110° until the weight from day to day became constant.

Table IV. *Water content of tissues.*

Tissue	Number of observations	Mean water %	Standard deviation $\pm$ (of a single observation)	Group
C. S. fluid	—	99 (a)	—	Body fluids
Lymph (thoracic duct)	—	95 (b)	—	
Synovial fluid	—	94 (c)	—	
Serum	—	92 (b)	—	
Grey matter (Rolandic area)	21	85	1.23	Nuclear tissues
Lung	—	84 (d)	—	
Kidney	68	82.5	1.76	
Heart muscle	37	80.3	1.00	
Plain muscle	—	80 (e)	—	
Suprarenal	—	80 (f)	—	
Spleen	—	79 (g)	—	
Pancreas	—	79 (g)	—	
Nerve	—	78 (f)	—	
Liver	88	76.9	2.10	
Vol. muscle	37	76.8	2.21	
Thyroid gland	43	76.3	2.38	
Artic. cartilage	—	73 (f)	—	Anuclear tissues
White matter	21	71.3	1.94	
Costal cartilage	—	68 (f)	—	
Tendo Achillis	—	66.7	2.86	
Red blood cell	—	66 (h)	—	
Connective tissue	—	62 (b)	—	
Bone	—	30 (i)	—	
Adipose tissue	—	30 (f)	—	

(a) Levinson [1929]; (b) Cameron [1929]; (c) Cajori *et al.* [1926]; (d) Wells [1925]; (e) Evans [1926]; (f) Charles [1884]; (g) Hill [1906]; (h) Peters and Van Slyke [1931]; (i) Morse [1927].

The division of the body into three groups may be made on the average values of the water contents.

In Group A, the body fluids, the water content ranges from 90 to 99 %.

In Group B, the nuclear (or cellular) tissues, the water content ranges from 75 to 85 %.

In Group C, the anuclear tissues, the water content ranges from 10 to 73 %.

As the nucleus plays the most important part in the oxidative processes of the cell [Warburg, 1924] the nuclear tissues (group B) are the most active metabolically. The anuclear tissues (group C) on the other hand, consist largely of matrix with relatively few nuclear cells and will have a lower metabolism. In fact, Warburg [quoted by Rous, 1925] was doubtful if respiration or glycolysis occurred at all in this latter group (C).

The water content is thus greatest where metabolism is nil—in the body fluids, but in the two groups of tissues, the higher the rate of metabolism, the greater is the water content. This latter principle applies to living tissues in general, as is seen for example in the parallel decline of water content and active metabolism with the progress of age.

The two groups B and C differ in many other characteristics as well. The anuclear tissues for example, are mainly supporting in function. The ability to “buffer” of the anuclear tissues has been shown to be almost non-existent [Rous and Beattie, 1926], while in the nuclear group where metabolism is most active, “buffering” is a prominent feature. There is also a marked difference in the type of proteins in each group. In the nuclear tissues nucleoproteins,

albumin and globulin are typical, but in the anuclear tissues, the protein consists mainly of collagen or elastin, both of which are closely related to gelatin. Other differences will become apparent later.

As regards the individual tissues investigated, least variability in the water content appears to occur in the heart muscle and grey matter. It was in these same two tissues that Voit [*cf.* Starling, 1930], in a study of the effects of starvation, also found the least disturbance of weight.

The chloride in the tissues.

The method of Van Slyke [1923] was used in estimating the chloride of the several organs and tissues of 108 *post mortem* subjects.

In Table V are shown:

- (a) The number of cases examined.
- (b) The average chloride % *i.e.* g. chloride per 100 g. of tissue (not dried).
- (c) The standard deviation of a single observation.

Table V. *Chloride % in various parts of the body (brain excepted).*

Tissue		Number of observations	Mean Cl %	Standard deviation $\pm$ (of a single observation)
Group A.	Cerebrospinal fluid	—	0.44	—
	Lymph	—	0.41	—
	Serum	—	0.36	—
	Synovial fluid	—	0.34	—
Group B.	Lung	55	0.22	0.031
	Kidney	97	0.19	0.033
	Thyroid gland	80	0.18	0.032
	Suprarenal	15	0.17	0.046
	Spleen	55	0.17	0.026
	Pancreas	41	0.17	0.035
	Nerve	—	0.17	—
	Plain muscle	—	0.16	—
	Liver	97	0.15	0.032
	Heart muscle	79	0.13	0.022
	Voluntary muscle	37	0.08	0.016
Group C.	Tendo Achillis	33	0.22	0.026
	Connective tissue	—	0.20	—
	Articular cartilage	—	0.20	—
	Red blood cell	—	0.19	—
	Bone	6	0.11	0.019
	Costal cartilage	6	0.13	0.016

In the body fluids, the average chloride % is much greater than in any of the tissues.

In the nuclear tissues there is more chloride in the glands than in the muscles. The difference in the chloride contents of the three muscles is of interest as it corresponds roughly with the difference in rate of conducting the impulse. The highest chloride value (plain muscle) is associated with the slowest rate of conduction, the lowest chloride (voluntary muscle) with the highest rate, while heart muscle is intermediate in both respects.

Although the least variable in their water contents, grey matter and heart muscle are not exceptional in regard to the variability of their chloride contents.

In the individual estimations it was found that all the tissues of one case might have at the same time a higher or lower chloride % than the average, but that the relative order of the chloride % was not always the same. In the

brain on the other hand, there were fluctuations between one brain and another, but the relative order of the chloride % of the several regions of any one particular brain almost invariably remained unchanged. The average chloride % of the various parts of the brain is shown in Table VI.

Table VI. *Chloride % in the brain.*

Region	Number of observations	Mean Cl %	Standard deviation $\pm$ (of a single observation)
Grey matter. Frontal	52	0.21	0.036
Rolandic	64	0.20	0.036
Temporal	51	0.20	0.032
Occipital	51	0.18	0.031
Cerebellar	67	0.16	0.035
White matter. Central	50	0.18	0.038
Lateral	57	0.14	0.024
Mixed. Caudate N.	61	0.19	0.032
Medulla	82	0.15	0.030
Thalamus	51	0.15	0.030
Pons	78	0.14	0.026

It may be shown that (with the exception of the difference between the mean of the frontal and of the temporal or Rolandic regions) the differences in the means in each of the three groups in the above table, are significant ones, *i.e.* the chloride % is greater in the frontal area than in the occipital; is greater in the central part of the corpus callosum than in the lateral; is greater in the medulla than in the pons, *etc.*

A. *The relationship of chloride and water to the cH of tissues.*

Rous [1925], has made interesting qualitative observations by injecting certain dyes into the peritoneal cavity and then dissecting the tissues under oil after the animal had been killed. He was able to note the reaction of each tissue by the colour. In this way, though no measurements were possible, he was able to arrange the tissues in the relative order of their degree of alkalinity, and this arrangement is shown in the first column of Table VII, beginning with

Table VII.

Tissues arranged by Rous [1925] in order of their p_H (alk. to acid)	Tissues arranged in the order of their Cl contents, as given in Table V (high to low)
—	C. S. fluid
Plasma	Serum
Connective tissue	Connective tissue
Tendon	Tendon
Bone	—
Articular cartilage	Articular cartilage
—	Red blood corpuscle
—	Grey matter (Rolandic)
—	Thyroid gland
Epidermis	—
Kidney	Kidney
—	Pancreas
Spleen	Spleen
—	Plain muscle
Liver	Liver
Gall bladder	White matter
Heart muscle	Heart muscle
Voluntary muscle	Voluntary muscle
Pancreas	(See No. 10 this column)

the most alkaline. For comparison, the tissues, in descending order of their chloride values, have been placed in the second column.

It is apparent, therefore, that the body fluids which have already been shown to contain most chloride are also the most alkaline, and the nuclear tissues with the least chloride are the most acid of the three groups. Rous's findings, however, become more interesting when the individual tissues are considered. Here the resemblance between the two columns of Table VII is either a remarkable coincidence only, or suggests that the chloride varies inversely with the cH of the tissues. (The one exception is the pancreas, an organ notoriously subject to *post mortem* changes.)

In the four fluids or tissues where various independent observations of the p_H values are in good agreement, this inverse ratio probably exists in actual fact—as will be seen in Table VIII where the p_H , cH and the cCl values of these

Table VIII.

	p_H	cH	cCl	$cH \times cCl$
C. S. fluid	7.45	0.35×10^{-7}	4.4	1.54×10^{-7}
Serum	7.4	0.4×10^{-7}	3.9	1.56×10^{-7}
Red blood cell	7.25	0.56×10^{-7}	2.9	1.62×10^{-7}
Voluntary muscle	6.9	0.13×10^{-6}	1.2	1.56×10^{-7}

four tissues or fluids are given. In the last column it will also be seen that the product of the cH and cCl of each is approximately a constant with a value of about 1.6×10^{-7} , or

$$\frac{cH_{\text{serum}}}{cH_{\text{r.b.c.}}} = \frac{cCl_{\text{r.b.c.}}}{cCl_{\text{serum}}} = K$$

(cCl = concentration of chloride in g. per 1000 g. of cell water).

The above equation of course, expresses the relationship which would be expected to hold if equilibrium was reached between a living cell and its surrounding fluid, but it is pointed out later that, except in the case of the red blood cell and serum, there are reasons why it is improbable that such equilibrium does exist.

Table IX.

	cCl	Protein %	Na %	K %	Ratio Na/K
Group A.					
Cerebrospinal fluid	4.4	0.02	0.35	0.02	18/1
Lymph	4.3	5.0	—	—	—
Serum	3.9	7	0.33	0.02	16/1
Group B.					
Grey matter	2.5	8	—	—	4/1
Kidney	2.2	13.5	0.20	0.26	0.8/1
Pancreas	2.0	—	0.07	0.27	0.26/1
Plain muscle	2.0	16	—	—	—
Liver	1.8	12	0.09	0.29	0.3/1
Heart muscle	1.6	—	0.10	0.28	0.36/1
Voluntary muscle	1.2	19	0.06	0.30	0.2/1
Group C.					
Connective tissue	3.4	34	—	—	—
Red blood cell	2.9	32	? Nil	0.4	—
White matter	2.0	8.6	—	—	—

Na % and K % as given by Gerard [1912].
Protein % from references to Table IV.

B. *Relationship of water content to cH of a tissue.*

From a comparison of Tables IV and VII, it will be seen that in the constitution of the nuclear tissues and body fluids, decrease of water content is associated with increase of acidity, while in the constitution of the anuclear tissues, decrease of water content with increasing alkalinity appears to be the rule.

Relationship of chloride to the protein content.

Since most of the "solids" of a tissue is protein, the more protein there is in a tissue, the less water it can have. The chloride : protein relationship will thus become the reverse of the chloride : water relationship.

In the body fluids and nuclear tissue group, the chloride should vary indirectly with the protein. When the concentration of protein is calculated from the protein % and allowance is made for the source of these published figures, this statement is found to be more or less correct. ($c\text{Protein}$ = concentration of protein; i.e. g. of protein per 1000 g. of cell water.)

	$c\text{Cl} \times c\text{Protein}$ (Approx.)
Serum	$3.9 \times 78 = 300$
Kidney	$2.2 \times 163 = 360$
Plain muscle	$2.0 \times 200 = 400$
Voluntary muscle	$1.2 \times 250 = 300$

In the anuclear tissue group, however, the greater the amount of protein in a tissue, the greater will be the chloride content—directly proportional in the case of at least the red blood corpuscle and fibrous tissue.

The comparatively high chloride of the red blood cell may serve a useful purpose, as Green [1932] has shown that the more chloride there is present, the greater will be the solubility of haemoglobin.

Relationship of Na and K contents to the chloride content.
(See Table IX.)

It is now well established that Na is the principal base of the body fluids and also of the anuclear tissues, the red blood cell excepted, while in the cells of the nuclear tissues K predominates. Na and chloride thus show a preference for the same group of tissues, the ratio Na/K being high in the more alkaline groups with high chloride values, and low in the more acid cellular tissues of low chloride content and high metabolism.

Selectivity of disease in relation to the above chemical findings.

Roberts [1905] showed many years ago that the deposition of urates in gout took place at those sites where Na was present in greatest amounts. In view of what was said in the preceding paragraphs, it may perhaps be the high degree of alkalinity of these tissues which is really the determining factor.

That the nature of the reaction may be an important factor in determining the liability of a tissue to be attacked is suggested also in the arterial disease called by Gull and Sutton, "arterio-capillary fibrosis"; and later by Evans [1920] "diffuse hyperplastic sclerosis" (of the arterioles).

According to both Evans [1920] and Fishberg [1925] the incidence of arterio-capillary fibrosis occurs in the various organs in the following order of decreasing frequency—kidney, spleen, pancreas, suprarenal and brain. The intestines and liver are affected only occasionally and Jores [1913] states that he has never

found changes of this nature in the vessels of the lungs, heart and skeletal muscle. Arranged in the form of a table, these organs are:

Most affected—	Kidney
	Spleen
	Pancreas
	Suprarenal
Least affected—	Intestines
	Liver
Never affected—	Lungs
	Heart muscle
	Voluntary muscle.

The order of these organs very nearly corresponds with the order of the organs shown in Table VII; that is to say, the more alkaline organs with their higher chloride contents are more prone to changes in their blood vessels than the more acid organs with their corresponding low chloride values, or *vice versa*. In the serum of patients with high blood pressures, a similar tendency is found—high normal alkali reserve and chloride percentage [Muntwyler *et al.*, 1931].

Relationships of the body fluids to the tissues.

Tables IV and V reveal the marked difference between the levels of water and chloride inside and outside the cell, even though both substances can penetrate the cell membrane.

The Donnan equilibrium is believed to determine the distribution of diffusible ions between plasma and red blood corpuscle [Van Slyke, 1926] and between plasma and ascitic fluid [Loeb *et al.*, 1921–22], but not between the tissue cell and its body fluid. Hill [1932] has pointed out how impossible it would be, for example, to imagine equilibrium existing at the same time between the stomach cell (or kidney tubule cell) and the fluid on either side. He suggested that by liberation of energy, the cells are able to maintain themselves in what he calls “the steady state” of disequilibrium with their surrounding medium (or media) and that this ability to produce differences of concentration across their boundaries is a general property of living matter.

As pointed out above, it is not possible to say that the water and chloride figures obtained from a tissue considered in bulk will be the same as for the cell of that tissue, but it may be a significant fact in relation to the conception of the steady state of disequilibrium that the difference in concentration of chloride between tissue and body fluid is greatest in the nuclear tissues (group B), where the liberation of energy (as measured by the oxygen consumption) is greatest.

SUMMARY.

Post mortem material taken from the human body within 24 hours of death may be used for the estimation of at least the chloride and water in the tissues.

The relationships of chloride and water reveal a pattern in the chemical constitution of the body.

The nature of the relationships is not always the same in the three groups in the body—the body fluids, the nuclear tissues, and the anuclear tissues.

The chief characteristics of each group are summarised in Table X.

Table X.

Group	Number of cells	Meta- bolic rate	Re- action	Water	Cl	Na	K	Na/K	Protein
Body fluids	Nil	Nil	Alk.	High	High	High	Low	High	Low
Nuclear tissues	Many	High	Acid	Mod.	Low	Low	High	Low	Mod.
Anuclear tissues	Few	Low	Alk.	Low	High	High	Low	High	High

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REFERENCES.

- Cajori, Grouter and Pemberton (1926). *Arch. Int. Med.* **37**, 1.
 Cameron (1929). A Textbook of biochemistry. (Churchill, London.)
 Charles (1884). Physiological and pathological chemistry. (Smith, Elder and Co., London.)
 Crile, Hosmer and Rowland (1922). *Amer. J. Physiol.* **60**, 59.
 Evans (1920). *Quart. J. Med.* **14**, 215.
 — (1926). *Physiol. Rev.* **6**, 358.
 Fishberg (1925). *Arch. Int. Med.* **35**, 650.
 Gerard (1912). *Ann. Inst. Pasteur*, **26**, 986.
 Green (1932). *J. Biol. Chem.* **95**, 47.
 Hill (1906). Recent advances in physiology and biochemistry. (London.)
 — (1932). Adventures in biophysics. (London.)
 Jores (1913). Wesen der Entwicklung der Arteriosklerose.
 Levinson (1929). Cerebrospinal fluid. (London.)
 Loeb, Atchley and Palmer (1921–22). *J. Gen. Physiol.* **4**, 591.
 Morse (1927). Applied biochemistry. (Philadelphia.)
 Muntwyler, Way and Binns (1931). *J. Clin. Invest.* **10**, 489.
 Osman and Close (1930). *Quart. J. Med.* **23**, 393.
 Peters and Van Slyke (1931). Quantitative clinical chemistry. (Philadelphia.)
 Roberts (1905). Quoted from Allbutt's System of medicine.
 Rous (1925). *J. Exp. Med.* **41**, 451, 739.
 — and Beattie (1926). *J. Exp. Med.* **44**, 836.
 Starling (1930). Principles of physiology. (London.)
 Van Slyke (1923). *J. Biol. Chem.* **68**, 523.
 — (1926). Factors affecting the distribution of electrolytes, water and gases in the animal body. (Philadelphia.)
 Warburg (1924). Quoted from Bayliss, Principles of general physiology. (London.)
 Wells (1925). Chemical pathology. (Philadelphia.)