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ON THE CHLORINE CONTENT OF HUMAN MUSCLE AND SKELETAL TISSUE, WITH SPECIAL REFERENCE TO THE DEGENERATION OF CARTILAGE[1]

BY

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Chapter VI

DISCUSSION OF RESULTS

Muscle.

The average values for the chlorine content are:

Cardiac muscle: embryos and infants 0.26 ± 0.03%, adults 0.27 ± 0.02%.

Skeletal muscle: embryos and infants 0.33 ± 0.01%, adults 0.26 ± 0.04%.

Thus no essential difference of the chlorine content either between cardiac and skeletal muscle or between infants and adults was found. On the other hand, the individual values show wide variations: 0.09-0.49% for cardiac muscle and 0.10-0.48% for skeletal muscle. In the present investigation the correlation between this variation and, e.g. the patho-anatomical condition of the muscle was not studied.

A comparison with values reported in the literature (Table 2), shows that the latter vary considerably (0.03-0.16%) and are much lower than those obtained in this investigation (0.26-0.33%). Undoubtedly this is explained by the fact that the methods used for determination of chlorine were all based on the principle of destruction of the organic substance in an open vessel (v. Slyke's method or its modifications), which

[1] Continued from p. 368, fasc. 3.

does not exclude the possibility of loss of chlorine (Chap. III, p. 355).

The implication of the higher values obtained in the present investigation, is that we must revise the view that muscle merely contains chloride extra-cellularly. This view is accepted by a great many authors (Kerpel-Fronius (53), Eichelberger (64), Cameron & Wilton (29), Darrow et al (10), Darrow & Yannet (54), Everett (55), Eichelberger & Hastings (48, 56, 57), Lavietes et al. (58, 59), Muntwyler et al. (60), Peters (61, 62, 63)), who base their opinion on the analytical methods already mentioned. If we accept the new value for the chlorine content of the musculature—0.26%—we are forced to assume that chlorine is also present intra-cellularly. The extra-cellular volume of muscle being 14% (Eichelberger (64)), and the chloride concentration of the extra-cellular fluid 0.41% (Gamble (65)), it can be calculated that only 22% of the chlorine present in the tissue is extra-cellular and that the remainder is intra-cellular. Conceivably this intra-cellular portion may occur in different forms. Theoretically it might be in solution as chloride— in a concentration of 0.24%. However, it is thought that the cell's intracellular osmotic pressure is maintained by, in addition to cations, protein, phosphate and bicarbonate, whereas chloride ions are not present (Darrow et al. (10), Peters (61)). If this were so, we should be compelled to assume that the intra-cellular chlorine occurs in a non-dissociated linkage with muscle protein and plays no part in the maintenance of the cell's osmotic pressure. A linkage with glycogen is perhaps conceivable, but glycogen occurs in such variable amounts and is converted so rapidly that any such linkage seems very improbable. A direct study of the way in which chlorine combines in cardiac muscle gave the following values, which suggest a linkage with muscle protein, since there can hardly have been any glycogen left in this specimen, which was taken from a necropsy case: 0.22% chlorine in fresh tissue and 0.99% in dried. Water content 78.0%. The dried specimen was extracted for three days with 0.1 N nitric acid. The final portion of nitric acid used for the

TABLE 2

Author	Year	Animal	Tissue	No. of determinations	% of chlorine	Method of chlorine determination
Katz 45	1896	Pig, etc.[1]	skeletal muscle	10	0.03—0.08	Meillérs
Wahlgren (46)	1906	Dog	„ „	6	0.07	Bunge
Magnus-Levy (47)	1910	Man	„ „	1	0.06	„
„ „	„	„	heart muscle	1	0.12	„
Cameron & Wilton (29)	1928	Rat	skeletal muscle	6	0.06	V. Slyke
„ „	„	Dog	„ „	5	0.07	„
„ „	„	Man	„ „	1	0.16	„
Close (8)	1933	„	heart muscle	79	0.13	„
„	„	„	skeletal muscle	37	0.08	„
Winter (28)	1934	Horse etc.[2]	„ „	10	0.05—0.12	„
„	„	„	heart muscle	10	0.08—0.12	„
Eichelberger & Hastings (48)	1937	Dog	skeletal muscle	20	0.08	V. Slyke-Wilson
Amberson et al. (49)	1938	Cat	„ „	5	0.05	Sunderman-Williams
Hastings & Manery (50)	1939	Rat	„ „	—	0.08	Fiske-Sokhey
Eichelberger & Bibler (9)	1940	Dog	„ „	20	0.08	V. Slyke
Muntwyler et al. (51)	1940	„	„ „	24	0.07	Sunderman-Williams
Clark et al. (52)	1942	Rat	„ „	24	0.06	Schales

[1] Pig, cow, calf, deer, rabbit, dog, cat, fowl, frog, eel

[2] Horse, cattle, pig, sheep, dog, cat, rabbit, mouse, guinea-pig, rat

TABLE 3

Author	Year	Animal	Tissue	No. of determinations	% of chlorine	Method of chlorine determination
Cameron & Wilton (29)	1928	Dog	Tendon	3	0.26	V. Slyke
Close (8)	1933	Man	„	33	0.22	„
Amberson et al. (49)	1938	Cat	„	5	0.29	Sunderman-Williams
Danielson et all. (66)	1938	Rat	„	14	0.26	V. Slyke
„ „	„	Cat	„	14	0.31	„
„ „	„	Rabbit	„	14	0.43	„
Hastings & Manery (50)	1939	„	„	2	0.44	Fiske-Sokhey
Muntwyler et all. (51)	1940	Dog	„	5	0.42	Sunderman-Williams
Brown & Eichelberger (11)	1945	„	„	14	0.28	V. Slyke

TABLE 4

Wahlgren (46)	1906	Dog	Bone	6	0.18	Pringsheim
Cameron & Wilton (29)	1928	Rat	„	4	0.13	V. Slyke
„ „	„	Dog	„	3	0.10	„
Close (8)	1933	Man	„	6	0.11	„
Winter (28)	1934	Dog, etc.[1]	„	8	0.08-0.11	„
Amberson et al. ((49)	1938	Cat	„	5	0.10	Sunderman-Williams

TABLE 5

Close (8)	1933	Man	Articular cartilage	1	0.20	V. Slyke
„	„	„	Costal cartilage	6	0.13	„
Manery & Hastings (50)	1939	Rabbit	Ear cartilage	2	0.42	Fiske-Sokhey

[1] Dog, guinea-pig, rat, cat, fowl

extraction was chlorine-free, indicating that the extraction was complete. Subsequent determination of chlorine in the specimen gave the following values: corresponding to fresh tissue 0.17% of chlorine, and to dried tissue 0.78% (A 472, A 473). In this case, then, 23% of the chlorine was diffusible by extraction, and 77% in "bound" form in combination with the muscle protein—which certainly agrees well with the foregoing assumption.

Summary: in the material under discussion the chlorine content of cardiac muscle was 0.27%, and of skeletal muscle, 0.26%.

The writer concludes that chlorine is also present within the cells of the muscles, where it is bound to the muscle protein and osmotically inactive.

Tendons.

The average values obtained for the content of chlorine in tendons was 0.60 ± 0.04%. The variations between the individual values are considerable: 0.22-0.83%. The few analyses made of material from infants afford no indication of any difference between the chlorine contents of infants and adults.

If we compare these values with those given in the literature (Table 3, p. 480), we find that the latter are much lower, ranging between 0.22 and 0.44%. The observations already made on the methods used for determining chlorine also apply here (Chap. III, p. 355).

Tendons have come to play a comparatively important part in the discussion on extra- and intra-cellular spaces. Some authors (Hastings & Manery (50), Muntwyler et al. (51)) have considered that connective tissue is to be regarded as belonging to the extra-cellular space in a given tissue, and as tendons have been considered to represent a "massive" form of connective tissue they have been examined with special reference to chlorine, which is considered to be definitely an extra-cellular ion. The result has been that more chlorine is found in tendons than had been computed. Amberson et al. (49), for example,

found that in cats the chloride space (the volume in which the chlorine found upon analysis may be expected to be distributed with the concentration of chloride existing in extracellular fluid) was 75%, while the water content was only 70%. From this they conclude that chlorine must be present intra-cellularly and is probably organically bound. As the average content found in the present investigation was 0.60%, there is all the more reason to assume that osmotically inactive chlorine bound to protein actually occurs. If we take as our premise that the entire water content of the tissue contained chloride in a concentration corresponding to the content of the extra-cellular fluid (according to Gamble (65), 0.41%), we have thereby fixed the maximum amount of soluble chloride that can exist. If any chlorine that may possibly remain is calculated on the basis of the dry substance, we thus obtain a figure for "bound" chlorine. For tendons, this value will be 0.95%. (The calculation is made from the average value of the group: see also Table 1, p. 365).

Summary: the chlorine content in the tendons examined (Achilles tendons) was on an average 0.60%, with a variation between 0.22 and 0.83%. The writer concludes from this that, in addition to chloride, there exists bound, osmotically inactive chlorine in a concentration of 0.95% calculated on the basis of the dry substance of the tissue.

Bone.

The average value obtained for the chlorine content of bone is 0.50 ± 0.04%. The variations between the individual values are considerable: 0.17-1.49%. If the chlorine content of costal bones of infants and adults (0.42 and 0.50% respectively) is compared we find no statistically significant difference. Nor can any distinct difference be established between patellar, costal or other bones that have been examined.

If the values published in the literature (Table 4) are compared with these, we find that they are far lower, varying between 0.08 and 0.18%, and the remarks already made on

the methods of determining chlorine also apply here (Chap. III, p. 355). The comparatively high chlorine content found in the present material (on an average 0.50%) must be attributable to the fact that most of the chlorine exists in a "bound" form, particularly as the water content of bones is relatively low. Calculation of this value on the same principles as for tendons gave a value of 0.55%, computed on the basis of the dry substance.

Summary: The chlorine content in the bone examined was on an average, 0.50%, and most of this occurred in a "bound" form.

Normal cartilage tissue.

The average values obtained for the chlorine content of normal cartilage are:

costal cartilage, infants 0.46 ± 0.04%, adults 0.63 ± 0.09%
("bound" chlorine 0.62 and 0.96% respectively).

patellar cartilage, infants 0.32 ± 0.03%, adults 0.42 ± 0.03%
("bound" chlorine 0 and 0.43% respectively).

nucleus pulposus, infants 0.22 ± 0.03%, adults 0.24 ± 0.02%
("bound" chlorine 0 and 0% respectively).

annulus fibrosus, infants 0.45 ± 0.05%, adults 0.53 ± 0.03%
("bound" chlorine 0.68 and 0.81% respectively).

All cartilages, then, show an increase in chlorine content from infants to adults, although the increase is not statistically significant for any group. With regard to the cartilage of the intervertebral disc, it is worth mentioning that in the inner portion of the disc, i.e. in the nucleus pulposus, there is about half as much chlorine as in the external portion, i.e. the annulus fibrosus. This greater chlorine content in the annulus fibrosus has been a rule quite without exception in the present series of investigations, the chlorine content of the annulus being greater than that of the nucleus of the same disc in every case. As it is known that in adults the intervertebral discs are practically non-vascular (67, 68), and that the exchange of metabolites thus proceeds by diffusion, one might

expect to find a higher chlorine content as the result of some influence from without which spreads secondarily to the centre. In fact, examination of material taken in three different places from the periphery towards the centre of an intervertebral disc, showed that the chlorine content did gradually decrease towards the centre (A 273-A 275).

Comparatively little research has been published on cartilage tissues from this aspect (Table 5, p. 480). The values vary between 0.13 and 0.20% for man, while the values for cartilage in the rabbit's ear is 0.42%. The methods of determining chlorine were the same as for the preceding groups of tissues.

Summary: The chlorine content in normal cartilage tissue varied, on an average, between 0.24 and 0.63%, the value depending on the origin of the cartilage. In the costal cartilage, annulus fibrosus and patellar cartilage of adults, part of the chlorine is present in a "bound" form.

Comparison between normal and pathological patellar cartilages.

The chlorine content of normal patellar cartilage (infants and adults aggregate) is 0.35%, of malacic 0.53%, of paramalacic 0.58% and of osteo-arthritic cartilage 0.52%. Thus, there is a very considerable increase in the chlorine content when the cartilage undergoes a pathological change. If, instead, we convert the values into the rates of "bound" chlorine (Table 1, col. 5, p. 365. The computation uniform with that for tendons), we find that the increase is still more pronounced: normal cartilage 0.08 %, malacic 1.03 %, paramalacic 1.10% and osteo-arthritic 0.89%. Comparison with the normal cartilage group shows the increase in chlorine content in the different pathological cartilages to be statistically significant. The chlorine content of malacic cartilage is somewhat lower than that of paramalacic cartilage, though this may be accounted for by the higher water content, the oedema, of the malacic cartilage (Öwre (69), Lindahl (70)). If, instead, we consider the chlorine content calculated from the dry substance, the

value in the case of paramalacic cartilage is 2.39% and in that of malacic 2.71%—that is to say, actually a somewhat higher chlorine content in malacic cartilage. It may also be mentioned that the chlorine content of paramalacic, macroscopically normal cartilage is quite close to that of pathological, malacic cartilage. The writer has regarded this fact as an indication that, from the chemical point of view, the macroscopically normal cartilage adjacent to a malacic focus is already pathological and is predisposed to malacia, i.e. is *premalacic*—a term which the writer would like to propose instead of the term "paramalacic" hitherto used. Accordingly, in my opinion, the difference between premalacic and malacic cartilage consists in the greater exposure of the cartilage in the malacic area to mechanical strain, in response to which a malacic lesion has developed in the already chemically changed cartilage. Wiberg (71) has shown that malacia arises in just those parts which have been particularly subjected to mechanical strain. If we compare the figures representing the chlorine contents of normal cartilage in infants and in adults, and of malacic cartilage, we find a progressive increase: 0.32; 0.42; 0.53%. This upward trend is still more pronounced in the case of "bound" chlorine (Table 1, col. 5, p. 365): 0; 0.43; 1.03%, though the differences arising from such a grouping are, owing to the groups being so small, merely significant between the first and last groups. In the present material, then, we have with increasing age, an increase in the chlorine content of patellar cartilage which progresses in premalacic cartilage, to reach its maximum in malacia (provided we allow for the oedema of the malacia in our calculation) and declines somewhat as osteo-arthritic changes set in. In 1944, Hirsch (72) demonstrated histologically and chemically that the patellar cartilage, when malacic, showed conspicuous changes in its content of chondroitin sulphuric acid. In this process the chondroitin sulphuric acid content decreased already at a very early stage in the development of malacia (before any actual macroscopical changes took place), and was still lower in the actually malacic lesion. These observations, it seems

to me, may prove to be in accord with my own findings. From the chemical standpoint it seems fairly obvious that in the combination of protein with chondroitin sulphuric acid, i.e. chondromucoid, the chondroitin sulphuric acid is gradually replaced by the stronger acid (HCl), if this is present. The writer has not been able to find in the literature any account of comparative studies of chlorine in normal and pathological patellar cartilages.

Summary: The chlorine content of malacic, paramalacic and osteo-arthritic cartilages is considerably higher than that of normal cartilage, the increase being chiefly due to the presence of "bound" chlorine.

Comparison between normal and pathological intervertebral discs.

The chlorine content of the normal nucleus pulposus is 0.23%; of the degenerated nucleus pulposus (post-mortem specimens) 0.46%; and of disc prolapses and disc hernias (operation specimens) 0.50%. The difference between the figures for normal and pathological nucleus pulposus in adults is statistically significant. If we divide up the material into the following groups: normal nucleus in infants; in adults; disc prolapses; degenerated nucleus; and disc hernias; we obtain the following chlorine values, 0.22; 0.24; 0.45; 0.46; and 0.52%. Here the differences between the normal groups, each severally, is statistically significant as against each one of the other groups, whereas the difference between the various groups of pathological nuclei is not statistically established. It may thus be observed that in the present material the chlorine content of pathologically changed discs is approximately twice that of normal discs. If we convert the values into "bound" chlorine, we find for normal nucleus pulposus, both in infants and in adults, 0%, and for disc prolapses, degenerated nucleus, and disc hernias: 0.50; 0.62; and 0.92% respectively, that is to say, on this computation a still more pronounced difference.

Hence, the increase which was previously observed in the chlorine content of patellar cartilage (chiefly "bound" chlorine) with degeneration, also occurs with degeneration of intervertebral discs. No information on this point could be found in the literature.

Summary: The chlorine content of pathological nuclei pulposi (disc hernias, disc prolapses and degenerated nuclei pulposi from necropsies) was greatly increased in comparison with that of normal nuclei pulposi, the increase being mainly due to the presence of "bound" chlorine.

Age changes in chlorine content.

It is extremely difficult to gauge the behaviour of the chlorine content with increasing age from the present material. The material has been compiled chiefly with a view to ascertaining any possible difference between the contents of normal and degenerated cartilages. Consequently the groups in which we may compare the chlorine content in infants and adults (both normal) are comparatively small. Whenever the material has been divided into these groups the chlorine content has been higher in adults, but the analyses are so few, that the differences have not been significant. If, on the other hand, we include in the patellar cartilage group all the cartilage specimens irrespective of the patho-anatomical condition of the cartilage, there is a significant difference between infants and adults. Examination of the correlation between chlorine content and age in patellar cartilage taken from adults indicates an increase in the chlorine content with age, but this increase is not significant (correlation coefficient 0.11 ± 0.12). If, however, the age group 4-20 years had been represented in the material, a significant correlation would probably have emerged. In this series all normal, paramalacic and osteoarthritic cartilages have been included, while the malacic cartilages have been excluded, because in practically every case the latter were taken from a patella from which paramalacic cartilage had also been secured. (The chlorine content

in paramalacic and malacic cartilage does not differ significantly, but owing to its greater surface compared with that affected by malacia the paramalacic cartilage may be regarded as being more representative of the patella concerned). An increase in chlorine content with age might also be expected from another point of view: it is a well-known fact that degenerative changes in patellar cartilage increase with age. Seeing that the chlorine content is significantly higher in degeneration, we even indirectly reach the conclusion that the chlorine content must increase with age.

Summary: The chlorine content of patellar cartilage is higher in adults than in children. If only normal patellar cartilage is included in the calculation, this increase is not significant, but the writer nevertheless considers that it must be assumed that the increase found in this material would be confirmed if a larger series were investigated.

Water content of the specimens.

The various contents of dry substance in the specimens are shown in detail in Table 1, col. 2, p. 365, from which it is easy to calculate the water content in each case. It is worth pointing out in this connection that in all groups which were divided into infants and adults, the infants had a higher water content than the adults. The difference was significant for cardiac muscle, skeletal muscle, patellar cartilage, costal cartilage, nucleus pulposus and annulus fibrosus. If we compare adult normal catilage with degenerated cartilage, we note that the findings vary somewhat. There is no distinct difference between normal adult patellar cartilage and paramalacic cartilage or between normal adult patellar cartilage and osteo-arthritic cartilage. Malacic cartilage has a markedly higher water content than normal adult cartilage, paramalacic cartilage and osteo-arthritic cartilage. The latter has a higher water content than paramalacic cartilage. In these cases the differences are significant. If we compare normal adult nucleus pulposus with pathological nucleus (degenerated nuclei, disc

hernias, disc prolapses), all the pathological cartilages show a lower water content. The difference is significant for disc prolapses, and probably also for degenerated nuclei (post-mortem specimens) .

Summary: The water content of the tissues examined decreased from infants to adults and in degeneration of the nucleus pulposus, and increased in patellar cartilage when affected by malacia and by osteo-arthritis.

Chapter VII

MECHANISM OF INCREASE IN THE CHLORINE CONTENT OF CARTILAGE ACCOMPANYING DEGENERATION

At the present stage any attempt to explain the results presented here must be regarded as a mere working hypothesis. It will be the task of future investigators to test by experimental and clinical methods the conclusions that are drawn. However, since the results are, from the bio-chemical point of view, quite new, in that they demonstrate the presence of "bound" chlorine and thus to some extent open up fresh paths of approach, the writer feels justified in discussing the various possible ways of explaining them.

As early as 1905, Ambard and Beaujard (4) introduced the term *"Retention chlorurée sèche"*. Since then a large number of workers (73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90) have further developed, described and discussed the phenomenon. These authors consider that balance studies have shown chlorine to be retained in the body in acidosis, and a number (4, 73, 79, 80, 81) consider that the chlorides of the blood pass into the tissues and are there linked to the protein of the cells; or, to express it in typically French fashion, *hypochlorémie plasmatique avec chloropexie tissulaire* (53). However, no tissue analyses which could substantiate this assumption were made, though for one tissue, the blood, it has been found to agree with the facts. This tissue has, of course, been more easily and more frequent-

ly accessible to analysis than others. Various authors (91, 92, 93) have shown that the acid-base regulation in the blood proceeds partly as follows: in acidosis, chloride ions pass into the red blood corpuscles, where they are assumed to become bound as acid albuminates. Through this process the ratio between the chloride in the red blood corpuscles and the chloride in the serum (chloropectic index) is raised. Feldman & Ulanowskaja (91) suggested that a rise in the chloropectic index was a pronounced early symptom of acidosis, which actually appeared before the alkali reserve began to diminish. With regard to all body tissues other than blood, the view has been propounded up to recent times that, with the single exception of the red blood corpuscles, the cells were free from chlorine (Darrow et al. (10), Lavietes et al. (59), Peters (61), Gamble (94)). V. Slyke et al. (93), moreover, thought that the chloride ions which passed into the red blood corpuscles were not bound to the protein, but that as a stronger acid they simply displaced a certain amount of protein which functioned as anion. Of recent years, however, various authors (Amberson et al. (49), Darrow & Yannet (95), Eichelberger et al. (96), Hastings & Manery (50)) have also come to the conclusion, on the basis of chlorine analyses, that chlorine also exists intracellularly in certain tissues (connective tissue, gastric mucous membrane, testicle, lung). Nor, in theory, is there anything against the possibility that other cells of the body besides the red blood corpuscles may also be permeable to chloride ions. Dell'Acqua (97) showed experimentally by measuring the difference in the chloride contents of arterial and venous blood that, in diseases with a tendency towards acidosis, such as diabetes mellitus and nephropathy, a retention of chloride in the tissues took place. However, he did not inquire further into the question of how this retention might occur.

In addition to the active, living cells there is a large organ which certain writers have thought fit to call by a collective name: the collagen system (Klemperer et al. (98, 99), Klemperer (100), Duff (101), Evans (102)). This differs from the active cells by offering merely a more passive resistance to any

displacements of ions that may occur. The collagen system is regarded as comprising, apart from the connective tissue in other organs, subcutaneous connective tissue, tendons and cartilage—in fact, the very tissues that have been studied in this investigation. Here we find a tissue exceedingly poor in cells (i.e. not very active), built up mainly of collagen and the closely allied proteins elastin and chondromucoid. The collagen system also shows substantial agreement with Close's (8, 103) third group of tissues. He divided the tissues of the body according to their water content, into three groups: (1) body fluids, (2) cellular tissues, (3) supporting tissues. The last group, which has the lowest water content, contains protein of the type of gelatine derivates, is incapable of acting as a buffer, is poor in cell-nuclei, and has a slow metabolism. To this group may be referred connective tissue, tendons, cartilage, bone and white nerve substance.

Compared with other proteins, collagen is unusually resistant to various solvents, such as water, saline mixtures, diluted acids and bases, and it has the property of becoming hydrated and of swelling in diluted acid (acidosis (104)). The collagen system is characterized clinically by the fact that it forms a patho-anatomical basis for a whole range of allergic or para-allergic diseases, polyarthritis, lupus erythematodes, periarteritis nodosa etc., and also for the degenerative senile diseases: degeneration of articular cartilages and intervertebral discs, myocardial fibrosis, atherosclerosis of the vascular system, etc. The histological picture of the allergic diseases in particular, and also of the degenerative senile diseases, is characterized partly by fibrinoid degeneration, and partly by an alteration in the staining behaviour of the collagen, viz. the tissue has a stronger affinity to acid dyes: eosin (102, 103, 104), orange G (Mallory), picric acid (v. Gieson) as has been later shown to be the case with lowered pH: acidosis (Singer & Morrison (105)). That the collagen system is also peculiar as regards its buffering capacity has been demonstrated by Rous & Beattie (106, 107), who were able to show by administering acid (acidosis) in in-vivo experi-

ments with rats that different tissues responded quite differently. The collagen system (connective tissue, cartilage, subcutis, vessels, tendons) changed its pH according to the administration of acid to the extent that the pH fell in cases of acidosis, while tissues with a high cell content, such as glandular parenchyma, maintained their pH right up to the death of the animal. This, then, must be interpreted as indicating that the acid (HCl) introduced is retained in the connective tissue and gives rise to local acidosis.

The collagen system may therefore be described as an organ which is not actively capable of protecting itself against the influence of its environment, and which, when acidosis occurs, retains chloride. Since many writers have shown that chloride is retained in cases of acidosis, and the present direct investigation has shown that a substantial increase in the chlorine content is associated with degenerative processes, chloride retention may easily be conceived of as a factor contributing to the origin of degeneration. To this it might be objected that degeneration is a separate phenomenon and that chlorine retention is a factor secondary to it. This, however, is refuted by the fact, which has already been pointed out above, that in patellar cartilage we find that there is already an increase in chlorine in the premalacic cartilage, that is to say, primary to the macroscopical degeneration. A further argument is the fact that the chloride retention in the tissue is a process that may be induced externally (through factors giving rise to acidosis), i.e. it is not secondary to anything that takes place in the tissue. So long as nothing definite is known about the pathogenesis of the degenerative diseases in cartilage, there may be some justification for stating, as a working hypothesis, that when chlorine is found in increased amounts in degenerated tissues and chloride can be retained in the presence of acidosis , anything that induces acidosis must be a contributory factor to degeneration. It is worth noting in this connection that in conditions accompanied by a tendency towards acidosis, such as diabetes mellitus and nephropathy, collagen diseases, such as osteo-arthritis and

atherosclerosis, are particularly common (Millard & Root (108)).

Summary: As a working hypothesis the following scheme is given for the pathogenesis of degeneration of cartilage tissues:

(1) a chronic condition of acidosis in the body;
(2) a passage of chloride ions into cartilage and other tissues within the collagen system;
(3) a change in the colloid-osmotic properties of the tissue protein and a decrease in pH;
(4) a lowered resistance to mechanical strain;
(5) a degeneration of the parts most exposed to mechanical strain.

Chapter VIII

SO-CALLED "BOUND" CHLORINE

In the preceding pages the writer has on several occasions come to the conclusion that, in a number of tissues, notably those that have undergone pathological changes, chlorine is found to be bound to protein. This conclusion has been based on the comparatively high chlorine rates that have been obtained—in comparison with earlier investigations. The following calculation was made: it was assumed that all the water in the tissue contained chloride in a concentration equivalent to that in the extra-cellular fluid, viz. 0.41%; the surplus chlorine was then assumed to be bound to protein and to be osmotically inactive.

To explain this linkage between protein and chlorine we have a choice of at least two possibilities: either the chloride may be bound as a salt to the protein in the form of acid proteinates, or else the chlorine may be organically bound.

It has long been a known fact, first as a result of investigations by H. Hammarsten and E. Hammarsten (109, 110), that in a salt-type linkage between large molecules (protein, nucleic acid) and small ions (chloride, sodium), the osmotic pressure

is not that which might be expected from Arrhenius' dissociation theory. H. Hammarsten showed that the chlorides of the basic proteins, protamin and histon, give a lower osmotic pressure than might be calculated merely from the concentration of chloride, that is to say, that some at least of the chloride ions are osmotically inactive. E. Hammarsten demonstrated a similar effect with albumin and the sodium salt of thymonucleic acid.

Since basic proteins exist only in very small proportions in the tissues investigated, a salt-type linkage between protein and chloride of the type described above can only account for a minor portion of the so-called "bound" chlorine.

The second alternative—chlorine in organic linkage in the proteins of the human system—has so far been rejected almost unanimously by those authors (111), who have studied problems of this nature. The existence of such compounds has certainly not been proved, although certain arguments have already been adduced in its favour. However, these views have been based on researches in which the methods of determining chlorine (v. Slyke or similiar methods) were not fully reliable. Fresh actuality is given to the question of the existence of organically bound chlorine in the human system by the higher analytical values now obtained. An examination of casein purified by the method of O. Hammarsten (112) showed that it contained 0.96% chlorine (an average of three analyses: 962, 912, 996 mg%). Seeing that practically all the chlorides are removed by Hammarsten's method of purification, the 0.96% might be organically bound. Whereas in biological material only a few compounds with organically bound chlorine are known—geodin, erdin (Raistrick (113, 114, 115)) and the dyes of certain purpuriferous molluscs: chloranthrachinon (Berg (116))—artificially produced proteins containing chlorine have been known for a long time. By treating casein with chlorine in the form of a gas or as chlorate, it has been possible to produce casein compounds containing 7-14% chlorine, the chlorine being retained even after various purifying processes. Corresponding compounds have been produced between casein

and iodine or bromine. In these processes it may be assumed that the halogens are linked to the aromatic amino-acids, tyrosine and tryptophane, in the casein molecule (117) (Millon's and Adamkiewitz' reactions with such chlorine-substituted products being consistently negative). The compound of iodine with tyrosine (di-iodo-tyrosine) is well known, but so far no corresponding chlorine compound has been demonstrated in man. From the chemical point of view, however, such a chlorine compound may well exist. Investigations on this point have already been started and indicate that amino-acids with a chlorine content can be isolated from serum albumin and casein after hydrolysis. However, it is intended to publish the results of these researches in a later paper.

SUMMARY

I. The writer has investigated the water content and the chlorine content of the following tissues: cardiac muscle; skeletal muscle; tendons; bone; costal cartilage; normal, malacic, paramalacic and osteo-arthritic patellar cartilages; nucleus pulposus; disc prolapses; disc hernias; and annulus fibrosus. The material was obtained from necropsies and operations.

II. The water content has been determined by drying to a constant weight, and the chlorine content by Berg's method. For the latter purpose the specimen has been disintegrated in a mixture of chromic acid and sulphuric acid, after which the chlorine has been distilled into a receiving fluid with silver nitrate and arsenic trioxide and determined by Volhard's method.

III. A comparison with other methods of determining chlorine, has shown many of them to be useless for specimens in which chlorine is bound to protein, and others to be usable only after certain precautionary measures have been taken.

IV. The analyses made on 481 specimens are reported. See Table 1, p. 365.

V. Statistical evaluation.

VI. The result of the analyses is discussed. An account is given of the average chlorine content of different tissues, and the writer comes to the conclusion that chloride exists in the muscles even intra-cellularly, and is probably bound to protein. The other tissues, with the exception of normal patellar cartilage in children and normal nucleus pulposus, contain chlorine bound to protein and osmotically inactive. A comparison of normal with pathological patellar cartilages and nucleus pulposus, shows that pathological cartilages have a greatly raised chlorine content, the increase being mainly due to the presence of chlorine bound to protein.

VII. The writer concludes that the increase in chlorine with degeneration may conceivably be due to a condition of chronic acidosis in the human system.

VIII. The so-called "bound" chlorine is discussed; it is assumed to be bound to protein partly in an osmotically inactive salt-type linkage and partly in an organic linkage.

RESUME

I. L'auteur a examiné la teneur en eau et en chlore des tissues suivants : muscles cardiaques, muscles squelettiques, tendons, os, cartilages costaux, cartilages rotuliens normaux, malaciques, paramalaciques et ostéo-arthritiques, nucleus pulposus, prolapsus de disques intervertébraux, hernies de disques intervertébraux et annulus fibrosus. Le matériel examiné provenait d'autopsies et d'opérations.

II. La teneur en eau a été déterminée par séchage pour arriver à un poids constant et celle du chlore par la méthode de Berg. Les spécimens examinés dans ce but ont été désintégrés par un mélange d'acide chromique et d'acide sulfurique, après quoi le chlore a été distillé dans

un liquide récepteur avec du nitrate d'argent et de l'arsenic trioxide et déterminé par la méthodé de Volhard.

III. Une comparaison avec les autres procédés de détermination du chlore a montré que beaucoup d'entre eux étaient inutilisables pour les spécimens dans lesquels le chlore est fixé par la protéine et que, pour les autres, ils ne pouvaient être appliqués qu'après avoir pris certaines mesures de précaution.

IV. Il est rendu compte d'analyses de 481 spécimens. Voir tableau 1, p. 365.

V. Evaluations statistiques.

VI. Le résultat des analyses est discuté. La teneur moyenne en chlore des différents tissus est indiquée et l'auteur arrive à la conclusion que le chlore existe dans les muscles, même extra-cellulairement, et qu'il est probablement fixé par la protéine. Les autres tissus, à l'exception du cartilage rotulien normal chez les enfants et du nucleus pulposis normal, contiennent du chlore fixé dans la protéinte, inactif osmoticalement. Une comparaison entre les cartilages rotuliens pathologiques et le nucleus pulposus montre que les cartilages pathologiques ont une teneur en chlore fortement augmentée, cet accroissement étant principalement dû à la présence de chlore fixé par la protéine.

VII. L'auteur en conclut que l'augmentation de chlore dans les cas pathologiques doit être attribuée vraisemblablement à un était d'acidose chronique de l'organisme humain.

VIII. Le problème du chlore « fixé » par la protéine est discuté ; il est supposé être fixé par la protéine soit comme une combinaison de type salin inactive osmoticalement, soit comme une combinaison organique.

ZUSAMMENFASSUNG

I. Verf. hat Wasser- und Chlorgehalt in folgenden Geweben untersucht: Herzmuskulatur, Skelettmuskulatur, Sehnen-

gewebe, Knochengewebe, Rippenknorpel, normaler, paramalazischer und malazischer Patellarknorpel, Patellarknorpel bei Arthritis deformans, Nucleus pulposus, Zwischenwirbelscheibenprolaps, Zwischenwirbelscheibenbruch, Annulus fibrosus. Das Material wurde durch Obduktionen und Operationen erhalten.

II. Der Wassergehalt wurde bestimmt durch Eintrocknen zu konstantem Gewicht, und der Chlorgehalt nach Berg. Hierbei wurde das Präparat in einer Mischung von Chromsäure und Schwefelsäure verbrannt, wonach das Chlor in eine Silbernitrat und Arsentrioxyd enthaltende Vorlage überdestilliert und nach Volhard bestimmt wurde.

III. Von anderen Chlorbestimmungsmethoden erwiesen sich viele als unbrauchbar für Präparate, bei denen Chlor an Eiweiss gebunden ist, und andere erst nach gewissen Vorsichtsmassnahmen als brauchbar.

IV. Ergebnisse der Untersuchungen von 481 Präparaten. S. Tab. 1. S. 365.

V. Statistische Bearbeitung.

VI. Besprechung der analytischen Ergebnisse. Feststellung des durchschnittlichen Chlorgehalts in verschiedenen Geweben. Verf. kommt zu dem Ergebnis, dass Chlorid auch intrazellular in der Muskulatur und da wahrscheinlich an Eiweiss gebunden vorkommt. Übrige Gewebe ausser normalem Patellarknorpel bei Kindern und normalem Nucleus pulp. enthalten Chlor an Eiweiss gebunden und osmotisch inaktiv. Beim Vergleich von normalem und pathologischem Patellarknorpel und Nucleus pulp. zeigen die pathologisch veränderten Knorpel stark erhöhten Chlorgehalt, wobei die Erhöhung hauptsächlich auf an Eiweiss gebundenem Chlor beruht.

VII. Verf. kommt zu dem Schluss, dass die Chlorzunahme bei Degeneration wahrscheinlich auf chronische Azidose im Körper zurückzuführen ist.

VIII. Besprechung des Verhaltens des sog. gebundenen Chlors: es wird angenommen, dass dieses an Eiweiss gebunden

ist, teils in osmotisch inaktiver salzartiger Bindung und teils in organischer Bindung.

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