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

BY

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Introduction.

Throughout the ages the metabolism of chloride in the body has aroused keen interest. A contributory factor has been the important part which common salt has played, and still plays, as a condiment and a preservative. The injurious or beneficial effects of salt have, since quite early times, been a subject for keen speculation. It has mainly been regarded as a valuable medicine, indeed as a sacred remedy, but the following book-title affords evidence that there were, even in early times, those who opposed the use of salt: *„Salz, die verbotene Frucht oder Nahrung und die Hauptursache von körperlichen und geistigen Krankheiten von Menschen und Tieren, wie es von den ägyptischen Priestern und von der heiligen Schrift gelehrt wird, in Uebereinstimmung mit des Autors langjähriger Erfahrung"* (Howard 1830) (1). It has long been known that the presence of chloride could easily be determined by means of precipitation with silver nitrate, and it was possible in quite early times to establish by balance investigations that in certain conditions (especially in diseases with a tendency towards acidosis) chloride might be retained in the body (Redtenbacher 1850 (2), Bohne 1897 (3), Ambard and Beaujard 1905 (4)). In these researches a distinction was

drawn between wet and dry chloride retention. In the former, water was retained at the same time, the general weight increased and oedema developed. In the latter—*"retention chlorurée sèche"*—chloride was retained without any simultaneous increase in the general weight or any retention of water. However, this last-mentioned form has been more or less forgotten; thus for instance Berglund (1946) (5) rightly says of the so-called dry chloride retention: "there is, however, no evidence that a healthy person retains more common salt than he needs". As it has been presumed that dry chloride retention takes place in cartilage, connective tissue, tendons and skin (Berg (6)), it occurred to me that the degeneration of these tissues might possibly be attributable to dry chloride retention. Proceding from this working hypothesis, I have investigated the chlorine content of various human tissues, particularly parallel series of normal and degenerated cartilage. I have found in studying the abundant literature on the metabolism of chloride in the body that probably all the methods used for estimating chlorine allowed a proportion of the chlorine to be lost, and consequently the values obtained for chlorine content have generally been too low. I have, therefore, considered that there is no need to give an exhaustive account of earlier publications—beyond the discussion on various previous methods of analysis to be found in Chap. III and the account of earlier analytical results given in Tables 2-5—especially as the questions studied in this paper have hardly been discussed at all in earlier literature.

Chapter I.

THE MATERIAL

For the purposes of this investigation, material has been obtained from the routine necropsies at St. Göran's Hospital, the Söder Hospital, and The Crown Princess Lovisa's Children's Hospital. The necropsies were done 12-24 hours after death. The following tissues have been examined: cardiac muscle, skeletal muscle, tendons, ribs, patellar bone, costal

cartilage, patellar cartilage, and intervertebral disc. In addition, specimens of malacic cartilage from the patella, of disc prolapses and disc hernias removed at operations for sciatica, and of various bones from orthopaedic operations were obtained from the Orthopaedic Department of St. Göran's Hospital. Disc hernias were also obtained from operations in the Neuro-surgical Department of the Söder Hospital. The various specimens were obtained and treated in the following manner:

Cardiac muscle: from adults the papillary muscle of the front mitral valve, and from embryos or infants the apex of the heart was taken.

Skeletal mucle: a deep part of the psoas muscle was taken.

Tendons: the purely tendinous part of the Achilles tendon adjacent to the calcaneus was taken.

Ribs: the portions of the 2nd and 4th ribs adjoining the cartilage were divided with rib-resection forceps; the periosteum scraped off, and thin slabs of bone were taken and cut into thin slices.

Patellar bone: the cartilage and a superficial layer of bone were taken off; the subjacent bone was then sawn out and finely sliced.

Costal cartilage: the 2nd and 4th costal cartilages between the sternum and the ribs were removed and thinly sliced.

Patellar cartilage: From post-mortem specimens the entire patella was removed, and specimens of the cartilage were taken from it and finely sliced; where there was malacia a macroscopically normal specimen was removed in addition to the malacic focus. The term "paramalacic" has been used for this macroscopically normal specimen. From operation cases the excised malacic specimen has been collected.

Intervertebral discs: the discs lying caudad to the 4th and 5th lumbar vertebrae were taken. From embryos and infants, all the lumbar discs were removed in order to provide sufficient material. As specimens of nucleus pulposus, the central parts of the nucleus were used, except in embryos and infants, where the entire nucleus was used. For the annulus fibrosus specimens, 1-2 mm of the outer layer of the annulus was first removed, and then the underlying parts were used.

Disc hernias and disc prolapses: these were taken at operations for sciatica, in all cases caudad to the two lowest lumbar vertebrae. The classification into prolapse and hernia respectively has been carried out in accordance with the statements made in the operation records. Only the softer parts of the specimens were used, while any more solid or tendinous parts that may possibly have belonged to the annulus fibrosus or longitudinal ligament were discarded.

Chapter II.

METHODS OF INVESTIGATION

The specimens thus obtained were all treated uniformly. They were carefully cut into small slices or pieces and weighed immediately. They were then dried to a constant weight in an electric furnace at a temperature of 115-120° C. and weighed once more, the difference in weight being taken to represent the water content of the specimen. This would appear to be the method most commonly accepted in the literature for determining the water content of biological material (Skelton (7), Close (8), Eichelberg & Bibler (9), Darrow et al. (10, Brown & Eichelberger (11). The possibility has to be reckoned with of minor errors arising owing to volatile substances other than water evaporating (too high values for the water content) and, in spite of the long drying period, of minor quantities of colloidally bound water not evaporating, or even of the specimen's increasing in weight through oxidation (too low values). However, under ordinary drying conditions the error is presumed to keep within a few per cent and to be constant if the procedure is uniform (Miller (12), Benedict & Manning (13, 14), Rimington (15), Nelson & Hulett (16)).

After the drying, the chlorine content of the specimens was determined by Berg's method (17). The principle is as follows: In a system closed by a "trap" (Fig. 1) the specimen was completely disintegrated by boiling in a mixture of concentrated chromic acid and sulphuric acid. The mixture of hydrochlorid acid, chlorine and lower oxides of chlorine thus produced was distilled into the trap, containing a solution of silver nitrate and arsenic trioxide. The amount of chlorine was then determined by the Volhard method (18). For the purposes of the present investigation, the procedure of determination has been evolved and the analyses carried out by the chemist, Dr. R. Berg.

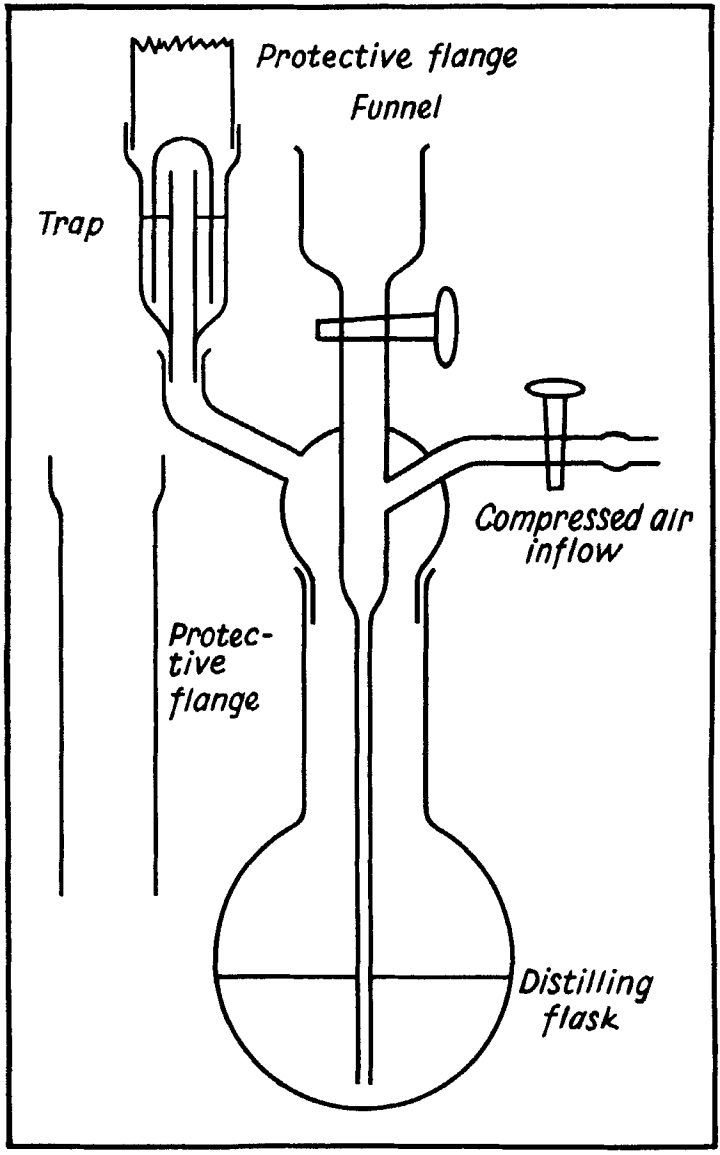
Protective flange
Funnel
Trap
Compressed air inflow
Protec-tive flange
Distilling flask

Fig. 1.

Analytical procedure:

Chemicals: concentrated sulphuric acid, chemically pure, *pro analysi;* distilled water, free from chlorine; a mixture of chromic acid and sulphuric acid (chromic acid 500 g, 50% sulphuric acid 500 g); arsenic trioxide, chemically pure, *pro analysi.* All the chemicals were tested prior to use and were found to be free from chlorine.

Solutions for titrimetric analyses: 0.1 N silver nitrate solution; 0.1 N ammonium thiocyanate solution; indicator: saturated ferric-ammonium alum solution. The solutions were obtained through the St. Görans Hospital dispensary from the chemist's shop, "Stenbocken". Permitted margin of error for the solutions in accordance with pharmaceutical requirements ± 0.3%. The solutions were checked each time a fresh batch was obtained and they were found to have an exact strength within a margin of 0.01 ml. Standardization against sodium chloride solution of known concentration was also performed.

The estimations were carried out in the following manner: the dried specimen (usually 100-300 mg of dry substance) was placed in the distilling flask. The trap was filled with 5-10 ml 0.1 N silver nitrate solution (the amount proportional to the weight of the sample), and as many ml of chromic-sulphuric acid mixture were added as was the weight of the specimen in decigrammes. (200 mg of specimen = 20 dg gives 20 ml of chromic acid-sulphuric acid mixture.) Compressed air was allowed to bubble through the distilling flask and the trap, after which the chromic acid-sulphuric acid mixture was brought to the boil over a Bunsen burner. The boiling must be carefully watched for the first 10-15 minutes as an abundance of carbon dioxide is formed during the first phase of combustion. The mixture was allowed to boil for a further 60-70 minutes, it being necessary sometimes to add more chromic acid-sulphuric acid if the fluid showed any tendency to assume a green colour (reduced chromium compounds). The boiling was continued until vapours of concentrated sulphuric acid began to escape. The fluid in the trap was then titrated with ammonium thiocyanate, ferric alum being used as indicator according to Volhard.

During the boiling, two phases of chlorine distillation could be distinguished, when biological material was used. The first phase occurred within the first 10-20 min. boiling, after which some time elapsed, during which no chlorine was distilled into the trap. Then, within 40-55 min., there followed a second phase in which fresh turbidity arose from the silver chloride. Apparently these two different phases in which chlorine is distilled are due to different linkage types. Thus during the

first phase sodium chloride or hydrochloric acid is distilled completely, while during the second the chlorine in e.g. purified casein (free of chlorides) is recovered. This point, however, has not been more closely investigated.

Control determinations:

The following substances were examined for the purpose of checking the method:

Hydrochloric acid, 2 ml 0.1 N. Five determinations. Upon final titration the amount of 0.1 N silver nitrate solution consumed was consistently 2.0 ml (A 3-A 7).

Toluenesulfonyl chloride, chemically pure, *pro analysi*. Four determinations made with the following percentage rates of chlorine: 18.5 18.4 18.5 18.6 (A 8-A 11).

Calculated value 18.64% chlorine.

Mixture of toluenesulfonyl chloride 0.500, methionine 5.000, glucose 3.500, calcium triphosphate 1.000 g. Three determinations made with the following percentage rates of chlorine: 0.93 0.88 0.92 (A 12-A 14). Calulated chlorine content 0.93%.

Analytical test (A) from the Svenska Träforskningsinstitutet, containing 1.05% chlorine in the form of dichlorosuccinic acid. (Chlorine content unknown when the analysis was carried out.) Two determinations made with the following percentage rates of chlorine: 1.06, 1.01.

Analytical test (B), as the preceding, but containing 0.101% chlorine. Two determinations made with the following percentage rates of chlorine: 0.12, 0.12. (A 55, A 56.)

Blank determinations for checking purposes without any specimen were made before the analyses were started and before using fresh batches of chromic acid or sulphuric acid. (A 1, A 2, A 231, A 232, A 439, A 440). In no case was it possible to discover any trace of chlorine in the receiving fluid. Nor was any turbidity due to silver chloride discernible macroscopically. Two chlorine determinations were made on abdominal fat prepared by melting, no trace of chlorine being discovered (A 15, A 16). Nor could any chlorine be found on analysing methionine (A 195, A 196), glucose (A 248, A 249) or calcium triphosphate (A 211).

For calculating the variation coefficient of the method, repeated determinations of the chlorine content in casein were made. (Supplied by the Kebo Co., free of vitamins. It was not possible to ascertain the method of preparation of this casein, as the article was imported.) This specimen was also used for

purposes of comparison with other methods of chlorine analysis. In a control series using arbitrarily weighed quantities of casein the following rates were obtained, expressed in mg chlorine to 100 g (mg%): 1161, 1261, 978, 991, 1080, 1026, 1086, 1070, 1090, 1092, 1123, 1079, 1040, 1042, 1170, 1117, 1058, 1057, 1091, 1136, 1195. (A 17-A 37.) Twenty-one determinations with a mean of 1093 ± 15 mg% = 1.09% ± 0.01, and with the variation coefficient 6.1%. In another series with a constant weighed amount of casein (500 mg) the following figures were obtained: 1136, 1015, 1072, 1122, 1143, 1108, 1108, 1079, 1115, 1058, 1065, 1079, 1115. (A 38-A 50.) Thirteen determinations with a mean of 1093 ± 10 mg% = 1.09% ± 0.01, and with the variation coefficient 3.3%. Combining the two series, we obtain the mean 1093±10 mg% = 1.09±0.01%, with the variation coefficient 5.2%. The degree of accuracy attained by the method was thus satisfactory.

When using Berg's method of determining the chlorine content of casein, but using a gravimetric, instead of a titrimetric, determination of silver chloride, we obtained 1.05 and 0.99 percentage rates of chlorine (A 51-A 52), that is to say, the same values with a gravimetric as with a titrimetric determination.

Chapter III.

COMPARISON WITH OTHER METHODS OF CHLORINE DETERMINATION

It is common to practically all methods that they are based on the principle that bromine, iodine and chlorine alone produce with silver nitrate a precipitate insoluble in nitric acid. Moreover, it is essential that the specimens to be examined do not contain, or cannot be suspected of containing, any appreciable amounts of other halogens. Comparisons with some of the most commonly employed methods of chlorine analysis have been made by analysing casein:

Van Slyke (1923) (19) has described a method of determining chlorine in the blood and tissues. His method, or any one of the numerous slight modifications of it (McKittrich & Schmidt (20), Wilson & Ball (21),

Eisenman (22), v. Slyke & Sendroy (23), Wilkins & Jones (24), Sunderman & Williams (25), Fiske & Sokhey (26)) is still probably the most commonly used in medical investigations (Russel (1926) (27), Cameron & Wilton (1928) (29), Close (1933) (8), Winter (1934) (28), Muntwyler et al. (1940) (30), Eisele & Eichelberger (1945) (31), Broch (1948) (32)). The procedure for the macro-method is as follows: the specimen is boiled in an open flask in a water bath for 1-12 hours with concentrated nitric acid (sp. gr. 1.4), in the presence of silver nitrate. The surplus silver nitrate is then titrated according to Volhard, after which the quantity of chlorine can be calculated. When a control estimation was made on a solution of potassium chloride of known strength and on blood serum with this solution added, v. Slyke found that the method yielded satisfactory values, though they tended to be somewhat low.

Using casein (which, according to preceding analyses, contained 1.09% chlorine) the following examinations were made by v. Slyke's method. The experiments were performed using Berg's apparatus for chlorine analysis, in order to ascertain whether, with v. Slyke's procedure, any loss arises through the formation of volatile chlorine or chlorine compounds. In these experiments a trap containing only a solution of sodium hydroxide was used with Berg's apparatus. It was subsequently found that with this receiving fluid the chlorine determination of purified casein (chlorine content 0.96%) gave highly varying and too low values (0.48, 0.43, 0.24, 0.14, 0.43, 0.59%), and consequently that a receiving fluid with sodium hydroxide alone is not sufficient for a quantitative chlorine determination. This explains the varying total values for the chlorine content in subsequent experiments, though it does not affect such conclusions as are to be drawn from the v. Slyke method:

(*1*) Boiling by v. Slyke's method for 4 hrs: Titration of the solution in the distilling flask according to Volhard showed no chlorine in the specimen. Examination of the trap (sodium hydroxide only) showed an amount of chlorine equivalent to 159 mg% chlorine. (2) Boiling by v. Slyke's method for 4 hrs: Titration gave the value 33 mg% chlorine. Volatile chlorine in this test 132 mg%. (3) Boiling by v. Slyke's method for 4 hrs: Titration showed no chlorine in the distilling flask. Volatile chlorine 184 mg%. The chlorine determination according to Berg (only sodium hydroxide solution in the trap) yielded the value 88 mg% for the remainder.

It was evident, therefore, that with the v. Slyke method volatile chlorine or chlorine compounds escaped and could, partially at least, be collected in a trap (containing in these experiments only 0.1 N sodium hydroxide solution). It might even be suspected that upon the combustion of an organic substance (casein) with concentrated nitric acid, certain non-disintegrated organic (and chlorine-containing) products

remained, and accordingly the following experiments were made: (4) Boiling by v. Slyke's method, but without silver nitrate, for 4 hrs: During this process volatile chlorine was given off, viz. 107 mg%. The remainder of the solution in the distilling flask was examined by Berg's method (only sodium hydroxide solution in the trap) and found to contain 268 mg% of chlorine. Silver nitrate had to be excluded from this experiment as chlorine cannot be completely recovered from silver chloride by means of chromic acid-sulphuric acid. Large amounts of chromic acid-sulphuric acid (70 ml) had to be added before combustion was complete, demonstrating that the oxidation with nitric acid was not complete. (5) Boiling by v. Slyke's method, but without silver nitrate, for 12 hrs. During this process volatile chlorine equivalent to 472 mg% escaped. The rest was examined by Berg's method (only sodium hydroxide solution in the trap) and contained 82 mg% of chlorine. Here, too, large amounts of chromic acid-sulphuric acid mixture (50 ml) were required to obtain complete oxidation.

These experiments, therefore, clearly showed that, although v. Slyke's method was suitable for determining inorganic chlorides, it did not yield satisfactory results when used for determining chlorine in casein. In the first place, considerable amounts of volatile chlorine escaped during digestion, and in the second place the combustion was not complete, chlorine in a bound form being left after digestion, although this was carried out for the maximum period indicated—12 hrs.

Sodium peroxide method: This method is described in a number of modifications. Pringsheim (1904) (33) used sodium peroxide alone, and the combustion was carried out in a nickel crucible by ignition with a glowing platinum wire. Robertson (34) has reported unsatisfactory results from the use of this modification. Parr (1908) (35) employed, besides sodium peroxide, also potassium chlorate, boric acid, potassium nitrate, magnesium oxide and benzoic acid for effecting combustion, which took place in a nickel bomb. He stated that in some cases the combustion with sodium peroxide alone proved incomplete. Treadwell & Hall (1945) (36) used a surplus amount of sodium peroxide and potassium hydroxide for the combustion in a nickel crucible. All authors report satisfactory results on control with known chlorine compounds. For estimating the chlorine content in casein, a modification evolved by Berg (37) has been used in two analyses: 0.3 g specimen, 1.8 g potassium, 0.9 g sodium peroxide (chlorine-free reagents) were carefully mixed and melted in a nickel crucible. The melt was reduced with precipitated titanic acid ($Ti(OH)_4$) in the presence of finely dispersed

cadmium. Upon subsequent precipitation with silver nitrate, silver chloride was obtained, equivalent gravimetrically to 1.13 and 1.18% chlorine in casein.

The figures for the chlorine content in casein obtained by Berg's method (1.09%) could thus be verified by this means.

Grote-Krekeler (1933) (38, 39, 40) suggested a method of determining sulphur and chlorine. The specimens were for this purpose subjected to combustion in an air-current by heating to a white heat in a quartz tube. In order to ensure complete oxidation the gases have to pass through two similarly white-hot quartz filters with fine pores and are then collected in a receiver containing a sodium sulphite solution. Chlorine is then determined gravimetrically or titrimetrically as silver chloride. This is the most modern method and is regarded as one of the most accurate. The chlorine content of casein has been determined by this method in a number of laboratories:

In the Biochemical Department of Stockholm University, where a sodium sulphite solution was used as receiving fluid, the following values were obtained: 0.17, 0.12, 0.11, 0.11% chlorine. Thus the value was about 10 times lower than that obtained with Berg's method.

In the Department of Organic Chemistry of Stockholm University, where a sodium peroxide solution was used as receiving fluid, the following values were obtained: 0.35, 0.38, 0.37% chlorine. Seeing that here too the value were considerably below those obtained with Berg's method, a separate test of various receiving fluids was made in order to ascertain how far they influenced the results. Using the method and apparatus recommended by Berg, but with varying receiving fluids, determinations were made of the chlorine content of known organic chlorine compounds. It was then found that in a number of cases the values obtained with sodium sulphite and sodium peroxide in the receiving fluid were slightly too low, whereas correct values were obtained with the combination sodium hydroxide and arsenic trioxide. Two determinations by the Berg method were then made in the same laboratory with a receiving fluid consisting of a solution of sodium arsenite and with gravimetric determination of silver chloride. The following values were obtained: 1.1, 1.0%—in close conformity, that is to say, with the writer's own figures. There were then carried out, with Grote-Krekeler and the same receiving fluid (sodium arsenite), three determinations of casein, with the following results: 1.2, 1.1, 1.0%.

In the Analytical Department of the Svenska Träforskningsinstitutet, where various receiving fluids were tested (hydrogen peroxide in an alkaline solution, sodium arsenite solution, sulphur dioxide water) the values of the chlorine content in casein varied between 0.80 and 1.04%.

It was found that when casein was burnt it had a tendency to puff up, so that ash stuck to the combustion tube. If this happened the values were somewhat lower, whereas if a larger combustion vessel was used, in which the risk of losing ash was eliminated, higher values around 1% were obtained. It was also found that the ash into which the casein was converted formed a glassy film in the platinum vessel (this film could only be discovered by weighing the vessel), and that this ash was not soluble in water but only in nitric acid, viz. after having been exposed to the latter for 24 hours. The excessively low values previously obtained (0.11 and 0.35%) were thus amply accounted for, since in these cases the combustion vessel had been merely rinsed out with water. In this investigation no perceptible difference could be observed when different receiving fluids were used.

It was thus proved that the values obtained with Berg's method could be confirmed with other methods.

Upon a comparison of further methods of chlorine determination published in the literature we find one or two which in principle very closely resemble Berg's method, the chief differences being minor variations in apparatus, oxidizing material or receiving fluid.

Thompson & Oakdale (1930) (41) described a method of determining chlorine in organic material. The specimen was completely disintegrated in a flask and distilled into a receiving fluid. Concentrated sulphuric acid, combined with persulphate or chromic acid, was used as oxidiser, and an alkaline solution of arsenic trioxide as receiving fluid; the chlorine content was then determined titrimetrically or gravimetrically as silver chloride. When checked with known organic chlorine compounds, exact values were obtained except for a number of volatile compounds such as chloroform, in which the values tended to be too low.

Robertson (1915) (34), described a method of determining chlorine in organic material. The specimen was disintegrated by combustion with chromic acid and sulphuric acid, the receiving fluid used being an alkaline solution of hydrogen peroxide. Robertson reports satisfactory results with known organic chlorine compounds, though the more volatile compounds tended to give too low values.

Professor Karl Myrbäck, of the Biochemical Department at Stockholm University, where some of the control analyses have been made, has most obligingly examined the various

analytical results, using both the Berg method and the Grote-Krekeler method. He considers that the analytical results presented here show that Berg's method of determining chlorine in organic material is quite a suitable one to employ in the present investigation.

In principle, Berg's method of determining chlorine is, like the other methods referred to here, a method for determining the halogens. We must in that case assume that all the halogens present are determined. It is true that the silver salts differ somewhat in colour. Silver chloride is pure white, bromide is pale yellow, and iodine is bright yellow, but if bromine and iodine salts in small quantities are precipitated with silver chloride, this slight coloration can hardly be directly discernible. That the method may nevertheless be regarded as a method of determining chlorine is due to the fact that the material used here—tissues from the human motor-skeletal system—contains only extremely small quantities of bromine and iodine or none at all. Compared with the chlorine content, the iodine and bromine content is so small that this error may be entirely disregarded. The entire human body contains halogens in the following proportions (42): chlorine 175.0 g, bromine 0.10 g, iodine 0.03 g. The two latter together comprise 0.07% of the amount of chlorine. The iodine is chiefly present in the thyroid gland, which has not been used in this investigation.

Chapter IV

RESULTS OF THE ANALYSES

Details are given below of the analytical results obtained in different tissue groups; the consecutive number of each analysis, the age of the patient, the dry-substance content, and the chlorine content of the fresh and dried specimens are shown. In a separate list entitled "Analytiska data" the follow-

ing additional particulars are given: the patient's initials, hospital, record number, diagnosis, description of the specimen, the quantity of silver nitrate solution used in the analysis. In view of its limited general interest, this supplement has not been included in the present paper but has been filed in the Library of the Karolinska Institutet, Stockholm, Sweden.

Heart muscle, children

Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried	Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried
341	4 embryo mths.	13.8	90	648	377	new-born	15.5	300	1932
					400	„	18.2	489	2685
352	4 „	12.5	252	2024	409	„	18.0	291	1620
240	5 „	14.2	153	1076	454	„	15.7	201	1283
407	6 „	18.2	334	1834	364	„	16.5	283	1719
370	7 „	15.7	253	1614	333	1 year	20.2	239	1183
394	9 „	17.9	313	1749	320	4 years	19.7	214	1085

Heart muscle, adults

353	23 years	20.0	237	1183	219	64 years	21.7	94	434
328	40 „	21.6	311	1437	345	67 „	20.2	309	1529
287	42 „	21.5	278	1295	349	67 „	20.9	237	1136
295	42 „	20.3	315	1547	198	68 „	19.4	207	1066
304	46 „	19.3	452	2343	202	69 „	19.6	354	1804
278	50 „	20.5	399	1941	237	69 „	26.3	222	845
318	51 „	23.7	274	1154	207	73 „	21.3	326	1532
257	54 „	21.8	224	1030	312	75 „	21.6	485	2248
261	60 „	29.1	227	780	472	76 „	22.0	218	991
268	60 „	19.7	209	1061	233	79 „	20.6	147	716
235	61 „	25.1	145	577	251	87 „	19.8	128	649
216	63 „	23.1	364	1575	224	93 „	21.5	188	877

Skeletal muscle, children

371	7 embryo mths.	18.2	340	1866	411	new-born	18.8	339	1803
					443	„	18.8	348	1845
395	9 „	16.4	341	2084	334	1 year	21.9	366	1675
379	new-born	18.8	288	1533	321	4 years	21.1	250	1181
401	„	20.2	354	1751					

Skeletal muscle, adults

354	23 years	19.9	243	1220	220	64 years	20.5	100	490
288	42 „	24.0	262	1089	189	65 „	23.0	333	1449
296	42 „	23.6	294	1244	474	76 „	19.3	193	1000
305	46 „	21.9	553	2525	234	79 „	28.3	480	1696
262	60 „	23.7	164	692	252	87 „	23.0	169	733
269	60 „	21.7	168	772	225	93 „	28.6	179	628

Achilles tendons, children & adults

Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried	Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried
399	9 embryo mths.	24.8	370	1490	270	60 years	35.8	669	1868
					236	61 „	39.5	483	1222
335	1 year	36.6	673	1841	215	63 „	30.8	778	2528
325	4 years	38.1	792	2077	188	65 „	36.1	527	1459
286	42 „	35.0	664	1900	238	69 „	46.5	483	1037
297	42 „	32.5	735	2261	243	74 „	32.8	513	1566
279	50 „	32.1	833	2596	313	75 „	38.0	652	1717
319	51 „	38.2	767	2008	253	87 „	31.6	472	1494
258	54 „	36.6	217	594	226	93 „	38.9	626	1609
263	60 „	33.1	566	1710					

Ribs, children

373	7 embryo mths.	41.1	307	747	403	new-born	47.7	423	888
					337	1 year	60.3	409	679
366	new-born	45.8	468	1022	323	4 years	47.0	353	751
382	„	52.1	539	1035					

Ribs, adults

77	28 years	44.6	468	1050	69	57 years	60.1	1174	1952
292	42 „	57.4	354	616	264	60 „	60.3	306	507
301	42 „	61.4	376	612	272	60 „	47.8	314	657
281	50 „	52.5	466	888	63	73 „	50.6	1039	2054
260	54 „	62.4	351	562	244	74 „	63.0	322	512
88	57 „	56.9	490	862	255	87 „	68.5	367	536

Patellar bones, adults

85	27 years	63.8	676	1061	81	66 years	82.1	645	786
76	28 „	68.7	667	971	62	73 „	73.1	494	675
57	48 „	60.7	402	662	65	73 „	78.4	370	472
68	57 „	71.0	381	537	60	86 „	77.8	502	645
90	57 „	82.1	477	581					

Various bones, children & adults

342	4 embryo mths.	37.4	304	813	femur
242	5 „	40.2	620	1543	„
58	6 years	71.7	1494	2082	radius
475	19 „	65.7	488	743	calcaneus
476	19 „	74.6	331	444	talus
477	29 „	73.4	415	565	phalanx of toe
480	36 „	69.5	319	460	„
478	43 „	67.9	172	253	metatarsal bone
479	47 „	82.5	577	699	„

Costal cartilages, children

Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried	Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried
374	7 embryo mths.	20.1	385	1920	404	new-born	21.3	485	2271
					412	„	22.9	455	1988
398	9 „	18.0	344	1915	444	„	22.3	400	1796
107	new-born	21.2	307	1449	338	1 year	30.0	575	1919
111	„	20.1	584	2905	104	2 years	25.3	316	1251
175	„	20.2	398	1972	166	2 „	28.7	854	2981
367	„	17.6	351	1992	322	4 „	29.8	530	1779
381	„	21.1	448	2118					

Costal cartilages, adults

74	28 years	39.0	435	1117	271	60 years	41.6	679	1631
332	40 „	37.4	603	1614	214	63 „	41.4	1840	4450
291	42 „	40.6	616	1518	221	64 „	41.3	748	1812
300	42 „	42.0	527	1257	187	65 „	37.9	337	890
280	50 „	39.8	628	1578	316	75 „	38.4	444	1157
160	51 „	38.4	747	1943	183	85 „	40.0	608	1519
259	54 „	41.2	640	1551	254	87 „	35.6	373	1047
265	60 „	43.6	492	1130	227	93 „	39.1	328	841

Normal patellar cartilages, children

239	5 embryo mths.	11.0	105	961	410	new-born	18.6	439	2355
					442	„	17.4	451	2596
408	6 „	15.5	245	1584	453	„	15.0	447	2982
372	7 „	16.1	339	2100	459	„	16.3	396	2432
466	8 „	15.1	322	2130	460	„	15.3	318	2073
393	9 „	16.1	324	2019	461	„	18.5	280	1510
176	new-born	17.9	66	366	336	1 year	21.7	465	2142
108	„	18.0	319	1775	165	2 years	21.6	142	657
112	„	17.8	307	1723	167	2 „	21.4	306	1431
365	„	15.9	281	1775	106	2 „	18.5	79	428
378	„	17.6	374	2126	139	4 „	22.9	418	1824
402	„	18.5	446	2405	324	4 „	22.7	514	2260

Normal patellar cartilages, adults

357	23 years	23.9	496	2071	117	28 years	25.9	367	1418
84	27 „	23.7	344	1455	157	29 „	22.9	488	2133
159	27 „	22.2	574	2584	361	36 „	25.1	376	1494
75	28 „	24.2	388	1606	329	40 „	23.1	283	1226

Paramalacic patellar cartilages, adults

447	20 years	23.5	619	2630	303	42 years	24.1	844	3504
127	23 „	24.8	757	3054	456	46 „	25.7	581	2263
471	31 „	22.7	504	2224	418	47 „	23.5	610	2592
191	34 „	24.8	502	2022	103	50 „	23.7	526	2225
469	38 „	25.5	546	2138	283	50 „	24.2	677	2799
99	41 „	24.5	630	2568	119	51 „	28.0	586	2094
294	42 „	21.2	554	2619	391	51 „	23.7	440	1856

Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried	Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried
67	57 years	23.6	467	1974	201	68 years	22.3	408	1833
386	57 „	25.2	559	2223	206	69 „	24.5	800	3264
360	58 „	24.9	483	1941	363	70 „	23.7	515	2175
154	60 „	22.4	428	1909	92	73 „	27.6	555	2015
267	60 „	24.0	185	772	64	73 „	26.5	468	1769
277	60 „	24.6	623	2536	96	74 „	23.1	514	2227
136	62 „	26.1	1054	4034	246	74 „	23.6	339	1436
150	66 „	23.4	631	2699	148	87 „	23.5	1004	4277
347	67 „	21.2	511	2406					
			Malacic patellar cartilages, adults						
142	24 years	21.1	542	2577	467	59 years	18.6	468	2516
470	31 „	20.1	505	2512	153	60 „	20.5	415	2029
70	38 „	19.1	389	2037	266	60 „	19.0	265	1392
468	38 „	23.0	510	2213	276	60 „	18.1	612	3387
293	42 „	16.8	476	2840	135	62 „	19.3	1368	7100
302	42 „	20.7	650	3148	149	66 „	17.6	653	3706
455	46 „	22.5	404	1800	346	67 „	20.0	357	1787
417	47 „	20.5	607	2968	205	69 „	20.6	528	2566
102	50 „	20.9	626	3002	362	70 „	18.8	530	2823
282	50 „	18.1	533	2939	91	73 „	20.9	487	2332
118	51 „	21.7	322	1487	129	73 „	18.9	566	3005
390	51 „	19.6	405	2065	95	74 „	19.6	437	2226
66	57 „	18.9	546	2888	245	74 „	21.0	663	3154
385	57 „	20.4	531	2603	147	87 „	18.5	501	2715
			Patellar cartilages in osteo-arthritis, adults						
449	43 years	19.1	397	2076	452	70 years	23.4	670	2867
392	49 „	21.8	506	2316	384	71 „	22.0	579	2634
465	52 „	22.9	474	2068	441	72 „	26.3	701	2669
462	56 „	22.6	260	1152	61	73 „	23.6	599	2536
89	57 „	25.4	578	2275	210	73 „	24.1	879	3647
438	57 „	23.0	637	2771	451	74 „	23.7	527	2225
376	60 „	21.1	265	1254	126	74 „	22.0	526	2396
457	60 „	21.5	418	1943	317	75 „	25.3	496	1961
450	61 „	22.4	383	1705	463	75 „	23.5	408	1733
464	62 „	22.1	503	2278	415	76 „	20.4	514	2518
369	63 „	22.0	601	2735	124	79 „	24.1	664	2755
80	66 „	22.3	366	1638	458	82 „	26.1	360	1379
387	66 „	21.8	562	2584	181	85 „	23.5	448	1905
416	66 „	20.9	581	2777	59	86 „	25.7	998	3881
419	66 „	21.1	535	2532	436	86 „	19.9	540	2713
348	67 „	23.2	454	1960	256	87 „	23.5	348	1479
128	69 „	22.2	582	2619	230	93 „	24.5	257	1051
435	70 „	22.3	581	2612					
			Normal nucleus pulposus, children						
396	9 embryo mths.	12.3	297	2418	177	new-born	14.9	166	1109
					405	„	13.3	351	2642
109	new-born	14.0	125	888	413	„	14.0	261	1860

Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried	Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried
445	new-born	14.6	151	1034	168	2 years	12.8	378	2958
339	1 year	18.6	198	1065	140	4 „	11.4	92	807
105	2 years	12.1	142	1175	326	4 „	12.3	249	2029
				Normal nucleus pulposus, adults					
448	20 years	15.9	245	1542	163	51 years	16.8	250	1489
355	23 „	13.5	189	1395	86	57 „	21.4	219	1023
82	27 „	18.5	208	1124	273	60 „	21.1	182	864
72	28 „	15.2	120	789	145	61 „	19.1	282	1475
115	28 „	18.1	144	798	137	62 „	22.6	83	369
155	29 „	18.4	279	1516	185	65 „	22.0	135	614
330	40 „	21.4	332	1556	151	66 „	26.3	432	1644
97	41 „	18.4	186	1007	78	66 „	23.3	236	1014
289	42 „	21.9	318	1451	199	68 „	24.2	388	1602
298	42 „	19.2	283	1472	203	69 „	27.8	400	1440
306	46 „	18.8	347	1849	122	79 „	17.6	103	588
100	50 „	20.9	197	945	228	93 „	25.7	166	645
				Degenerated nucleus pulposus, adults (post-mortem specimens)					
284	50 years	25.3	464	1830	350	67 years	23.8	360	1512
161	51 „	22.1	466	1109	208	73 „	27.3	597	2185
437	57 „	19.7	331	1681	93	74 „	25.2	443	1756
358	58 „	23.9	380	1591	314	75 „	21.2	333	1568
217	63 „	21.8	947	4338	179	85 „	24.7	370	1495
222	64 „	21.7	355	1630					
				Disc prolapses, adults (operation specimens)					
431	28 years	26.0	425	1637	247	38 years	24.3	207	850
190	29 „	25.1	526	2099	308	38 „	22.1	463	2092
310	30 „	22.9	534	2326	420	38 „	24.3	353	1453
121	31 „	22.4	569	2541	427	38 „	27.1	505	1862
430	32 „	19.9	434	2182	388	41 „	25.3	528	2087
194	34 „	22.3	580	2599	132	43 „	23.1	411	1778
383	34 „	25.0	542	2168	193	45 „	24.3	379	1560
424	35 „	18.2	420	2310	144	50 „	24.9	387	1555
				Disc hernias, adults (operation specimens)					
422	19 years	22.4	440	1965	428	33 years	21.3	410	1924
130	24 „	20.3	527	2598	143	35 „	17.3	2141	12348
250	25 „	23.5	299	1271	184	36 „	23.9	434	1817
212	26 „	24.2	458	1891	389	36 „	20.4	495	2421
125	28 „	25.2	670	2656	421	36 „	22.2	616	2775
432	28 „	18.9	310	1640	172	38 „	22.8	285	1248
343	29 „	22.4	456	2034	425	39 „	18.3	455	2490
426	29 „	23.1	492	2134	423	39 „	21.6	396	1836
213	30 „	19.9	289	1456	134	39 „	29.1	583	2003
434	30 „	23.5	517	2202	173	40 „	24.1	502	2079
197	31 „	24.1	531	2201	114	41 „	19.2	479	2491
344	33 „	19.0	482	2543	309	43 „	27.4	458	1669

Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried	Anal. No.	Age	Dry substance, %	Chlorine mg% fresh	Chlorine mg% dried
158	43 years	22.5	523	2322	170	48 years	22.0	287	1303
433	44 „	26.0	478	1837	192	48 „	24.6	386	1572
131	45 „	22.0	484	2203	429	50 „	22.6	457	2023
120	46 „	22.5	1039	4607	71	52 „	20.2	579	2863
481	46 „	13.4	527	3919	174	53 „	16.0	366	2281
311	47 „	21.6	551	2548	171	57 „	25.5	392	1538
133	48 „	19.4	548	2817					

Normal annulus fibrosus, children

241	5 embryo mths.	13.9	229	1651	entire discs	
375	7 „	15.5	417	2690	„	„
397	9 „	16.6	402	2423		
368	new-born	15.7	350	2230	entire discs	
380	„	16.0	413	2573	„	„
113	„	16.7	317	1899	„	„
406	„	20.3	530	2605		
414	„	18.9	433	2286		
446	„	19.3	428	2213		
110	„	18.5	272	1472		
178	„	20.7	390	1888		
340	1 year	24.7	423	1715		
169	2 years	28.1	734	2616		
141	4 „	22.7	965	4251		
327	4 „	23.6	484	2050		

Normal annulus fibrosus, adults

356	23 years	33.2	643	1939	87	57 years	32.2	296	922
83	27 „	31.9	547	1707	274	60 „	30.2	610	2019
73	28 „	22.4	440	1966	146	61 „	32.0	815	2547
116	28 „	34.2	575	1678	138	62 „	30.7	717	2339
156	29 „	27.8	680	2442	186	65 „	29.2	323	1108
331	40 „	34.3	563	1645	79	66 „	27.9	382	1371
98	41 „	20.8	493	2369	152	66 „	25.8	625	2425
290	42 „	32.5	611	1879	200	68 „	30.2	485	1608
299	42 „	31.9	543	1702	204	69 „	30.9	572	1852
307	46 „	28.0	410	1461	123	79 „	27.9	568	2038
101	50 „	30.3	406	1342	229	93 „	27.8	201	721
164	51 „	26.2	607	2316					

Degenerated annulus fibrosus, adults

285	50 years	32.4	558	1722	351	67 years	27.7	448	1620
162	51 „	28.1	817	2910	209	73 „	34.5	621	1802
359	58 „	34.5	418	1210	94	74 „	28.8	616	2134
218	63 „	33.7	1412	4192	315	75 „	29.9	493	1645
223	64 „	28.3	469	1655	180	85 „	30.2	508	1683

TABLE 1

Summary Table of Results.

The Table below gives the following data:

Column 1 the number of analyses made of the tissue.

Column 2 the average percentage of dry substance in the tissue examined, with the mean error of that average. The average water content is obtained in each case by deducting the % of dry substance from 100.

Column 3 the average chlorine content in the fresh specimen expressed in mg of chlorine to 100 g fresh tissue (mg%), with the mean error of that average.

Column 4 the average chlorine content in the dried specimen expressed in mg of chlorine to 100 g dried tissue (mg%).

Column 5 a value, computed from the averages for the tissue examined, of "bound chlorine" (see Tendons, Chap. VI and Chap. VIII). This computed value thus indicates the bound chlorine content of an imaginary average tissue, so that it does not exactly correspond to an equivalent value computed from the primary material.

Tissue	1 No. of analyses	2 % dry substance	3 mg % chlorine fresh tissue	4 mg % chlorine dried tissue	5 bound chlorine
Heart muscle, children	13	16.6 ± 0.6	262 ± 27	1573	0
„ „ , adults	24	21.7 ± 0.5	265 ± 20	1240	0
Skeletal muscle, children	8	19.3 ± 0.6	328 ± 14	1717	0
„ „ , adults	12	23.1 ± 0.8	262 ± 39	1282	0
Tendon tissue, aggregate	18	35.4 ± 1.1	601 ± 38	1721	949
Ribs, children	6	49.0 ± 2.7	417 ± 34	854	424
„ , adults	12	57.1 ± 2.0	502 ± 84	901	643
Patellar bones, aggregate	9	73.1 ± 2.6	513 ± 41	710	551
Various bones, aggregate	9	64.8 ± 5.2	524 ± 130	845	586
Bone, aggregate	36	61.7 ± 2.1	496 ± 43	831	549
Costal cartilage, children	14	22.8 ± 1.1	459 ± 39	2018	623
	16	39.8 ± 0.5	628 ± 88	1566	957
Patellar cartilage, normal, children	23	17.8 ± 0.6	321 ± 26	1811	0
„ „ „ , adults	8	23.9 ± 0.4	415 ± 34	1748	431
„ „ „ , aggregate	31	19.4 ± 0.7	345 ± 22	1795	77
„ „ , paramalacic	31	24.2 ± 0.3	578 ±32	2390	1103
„ „ , malacic	28	19.8 ± 0.3	532 ± 36	2708	1025
„ „ , osteo-arthritic	35	22.8 ± 0.3	520 ± 26	2276	890
Nucleus pulposus, normal, children	11	13.7 ± 0.6	219 ± 29	1635	0
„ „ „ , adults	24	20.3 ± 0.7	239 ± 20	1175	0
„ „ „ , aggregate	35	18.2 ± 0.7	232 ± 16	1320	0
Nucleus pulposus, degenerated (post-mortem specimens)	11	23.3 ± 0.7	459 ± 54	1811	622
Disc prolapses (operation specimens)	16	23.6 ± 0.6	454 ± 24	1944	597
Disc hernias (operation specimens)	37	22.0 ± 0.5	523 ± 50	2474	923
Disc hernias & disc prolapses	53	22.4 ± 0.4	502 ± 36	2314	821
Annulus fibrosus, normal, children	15	19.4 ± 1.0	452 ± 47	2304	680
„ „ „ , adult	23	29.5 ± 0.8	527 ± 30	1822	807
„ „ „ , aggregate	38	25.5 ± 1.0	497 ± 27	1999	753
„ „ , degenerated	10	30.8 ± 0.9	636 ± 94	2057	1143

Chapter V.

STATISTICAL EVALUATION

The statistical treatment of the material has been planned in consultation with B. Matérn, Ph. Lic., of the Statistical Research Group, Stockholm, who has also checked the calculations.

Averages, dispersion and the average's mean error for the different groups, the mean error for the deviation between two averages, and the correlation coefficient, have been worked out according to the usual formulae (Fischer 1941 (43)). The quotient between the deviation and its mean error has been computed. This quotient has been estimated in accordance with tables of Student's t-distribution, the degrees of allowance being made equal to the number of observations in the smallest group minus 1. If for this purpose the value of P has been found to be < 0.001, the deviation has been regarded as clearly significant; if $0.001 < P < 0.01$, the deviation has been regarded as significant, and if $0.01 < P < 0.05$, the deviation has been said to be probable. This method of calculating the value of t has been considered justified, seeing that in certain series there has been an abnormal distribution of the values, or else the dispersion in the two comparative groups has been palpably different (Welch 1947 (44)).

The various deviations within the material have been worked out below, the pertinent mean errors being given. Probable deviation is indicated by (*), significant deviation by (**), and clearly significant by (***).

Differences in chlorine content (mg%) between separate groups:

Heart muscle:
children—adults -2 ± 33
aggregate—skeletal muscle, aggregate -24 ± 30
Skeletal muscle:
children—adults 67 ± 42
Ribs:
children—adults -86 ± 90

Ribs: (cont.)
aggregate—patellar bones, aggregate — 39 ± 70
aggregate—various bones, aggregate — 51 ± 136
Patellar bones:
aggregate—various bones, aggregate — 12 ± 136
Costal cartilage:
children—adults — 168 ± 96
aggregate—normal patellar cartilage, aggregate 204 ± 56 (* *)
children—normal patellar cartilage, children 138 ± 47 (*)
adults—normal patellar cartilage, adults 213 ± 94 (*)
Patellar cartilage:
normal, aggregate—paramalacic — 233 ± 39 (* * *)
„ „ —malacic — 187 ± 43 (* * *)
„ „ —osteo-arthritic — 175 ± 35) (* * *)
normal, adults—paramalacic — 164 ± 46 (* *)
„ „ —malacic — 118 ± 50 (*)
„ „ —osteo-arthritic — 105 ± 43 (*)
normal, children—paramalacic — 257 ± 41 (* * *)
„ „ —malacic — 211 ± 45 (* * *)
„ „ —osteo-arthritic — 199 ± 37 (* * *)
„ „ —normal, adults + paramalacic + osteo-arthritic — 212 ± 33 (* * *)
„ „ —normal, adults — 94 ± 43 ($P < 0.1$)
paramalacic—malacic 46 ± 48
„ —osteo-arthritic 58 ± 41
malacic—osteo-arthritic 12 ± 45
Nucleus pulposus:
children—normal, adults — 19 ± 35
normal, aggragate—degenerated — 226 ± 57 (* *)
„ „ —disc prolapses — 222 ± 29 (* * *)
„ „ —disc hernia — 290 ± 52 (* * *)
„ „ —annulus fibrosus, normal, aggregate — 265 ± 32 (* * *)
normal, adults—degenerated — 220 ± 58 (* *)
„ „ —disc prolapses — 215 ± 31 (* * *)
„ „ —disc hernia — 284 ± 54 (* * *)
normal, children—degenerated — 240 ± 61 (* *)
„ „ —disc prolapses — 235 ± 38 (* * *)
„ „ —disc hernia — 304 ± 58 (* * *)
degenerated—disc prolapses 5 ± 60
„ —disc hernia — 64 ± 74
disc prolapses—disc hernia — 69 ± 56
Annulus fibrosus:
children—normal, adults — 75 ± 56
normal, aggregate—degenerated — 139 ± 98

Differences in dry-substance content (%) between separate groups:

Heart muscle:
children—adults — 5.1 ± 0.8 (* * *)
adults—skeletal muscle, adults — 1.4 ± 1.0
Skeletal muscle:
children—adults — 3.9 ± 1.0 (* *)
Patellar cartilage:
children—adults — 6.1 ± 0.7 (* * *)
normal, adults—paramalacic — 0.3 ± 0.5
„ „ —malacic 4.1 ± 0.5 (* * *)
„ „ —osteo-arthritic 1.0 ± 0.5
paramalacic—malacic 4.4 ± 0.4 (* * *)
„ —osteo-arthritic 1.4 ± 0.4 (* *)
malacic—osteo-arthritic — 3.0 ± 0.4 (* * *)
Ribs:
children—adults — 8.1 ± 3.4 ($P < 0.1$)
Costal cartilage:
children—adults — 17.1 ± 1.2 (* * *)
Nucleus pulposus:
children—adults — 6.7 ± 0.9 (* * *)
„ —annulus fibrosus, children — 5.7 ± 1.2 (* * *)
normal, adults—degenerated — 3.0 ± 1.0 (*)
„ „ —disc prolapses — 3.2 ± 0.9 (* *)
„ „ —disc hernia — 1.6 ± 0.9
„ „ —annulus fibrosus, normal, adults — 9.2 ± 1.1 (* * *)
degenerated—disc prolapses 0.2 ± 0.9
„ —disc hernia 1.4 ± 0.8
disc prolapses—disc hernia 1.6 ± 0.8
Annulus fibrosus:
children—adults — 10.1 ± 1.3 (* * *)
normal, adults—degenerated — 1.3 ± 1.2

To be continued in next issue.