# On the Chlorine Content in Human Muscle and Skeletal Tissue

## A rectification

OLOV LINDAHL

*Department of Orthopaedics, St. Göran's Hospital, Stockholm, Sweden*

In *Acta Orthop. Scand.* 1949 [1] the present writer published an article with the above title. As Friberg [2] and Mutt [3] have already pointed out, the method employed for the chlorine determination embraces a source of error, which renders the results duly obtained, unreliable. The author wishes to express regret for the unfortunate occurrence, and at the same time give an account of the error and its effect on the final conclusions.

When ascertaining the quantitative determination of chlorine, it is usual for practically all methods to determine this element as silver chloride. This procedure, of course, is under the presumption that the investigated preparation contains no appreciable quantity of other halogens or cyanides as, obviously, cyanide as well as bromide and iodide, in association with silver, forms insoluble precipitations in nitric acid. In the material investigated by the writer none of these substances were present. The possibility of the formation of cyanide during the analysis itself, is a source of error which has not been reported in earlier literature and to which, hitherto, attention has not been paid. In spite of the same, it has been revealed in tests relating to biological material that cyanide is formed during the determination when performed with the Berg method. The precipitation of silver chloride and silver cyanide achieved by means of silver nitrate has since been looked upon as silver chloride, with the result that too high values have been obtained. When Berg's method is used for organic compounds containing chlorine but not nitrogen, the values are fully correct, whereas in determinations relative to preparations which, in addition to chlorine contain protein, cyanide is formed during the analysis and consequently the values attained, are too high. The same source of error is met with when determining the chlorine content, at least of casein, according to the Grote-Krekeler method. Analyses performed at different laboratories, have given values ranging from 0.11 to 1.1 % chlorine in casein. When the Grote-Krekeler method was applied as a control in order to verify values acquired by Berg's method, this source of error remained undetected. The possibility of cyanide formation and of too high values must moreover be counted with in respect to chlorine analysis according to Thompson and Oakdale, and also Robertson (see Chap. III of the foregoing work [1]).

In order to determine the magnitude of this error, it has been necessary to work out a chlorine determination method in which the error has been eliminated, and then compare the values with both the analytic methods. In doing so the writer used a modification of the chlorine determination method employed by Zacherl and Krainick [4]. With this procedure the combustion of the dried material is brought about with concentrated sulphuric acid and chromium trioxide in the presence of silver chromate, at which, as repeated tests have shown on the combustion of different protein substances (casein, cartilage, connective tissue and muscular protein), cyanide is not formed. As a further precautionary measure a receiver was used (potassium arsenite and acetic acid) which selectively retains chloride and eliminates cyanide. The chloride determination was afterwards made in the usual

manner according to Volhard. A more detailed account of this method for determining chlorine will be published later. On employing this new method (Zacherl and Krainick), in comparison with the foregoing (Berg) for the analysis of different preparations, the following values have been obtained:

| Substance | % Cl according to Berg Cyanide formation | % Cl according to Zacherl and Krainick No cyanide formation |
|---|---|---|
| Liquefied subcutaneous fat | 0.0 | 0.0 |
| Toluenesulfonyle chloride (18.64 % Cl) | 18.5 | 18.5 |
| Dichlorosuccinic acid+glycose (1.05% Cl) | 1.02 | 1.01 |
| As above (0.10 % Cl) | 0.12 | 0.10 |
| Tyrosine "Merck" | 4.03 | 0.08 |
| Crude casein | 1.09 | 0.04 |
| Purified casein | 0.96 | 0.04 |
| Dialysed muscular protein | 1.29 | 0.07 |
| Connective tissue protein | 2.50 | 0.97 |
| Cartilage from intervertebral disc | 1.0—2.5 | 0.4—0.6 |

Hence, it is apparent that although Berg's method when applied for determining the chlorine content in nitrogen-free organic chlorine compounds and in inorganic chlorine compounds gives correct values, the values given when the organic material contains nitrogen are too high, due to the formation of cyanide. The cyanide formation is then constant for the same preparation (casein. See the foregoing work, Chap. III [1]), but varies with different preparations. The method is therefore not applicable for determining the chlorine content of biologic material. The conclusions published in my foregoing work [1] can therefore not be adhered to, and the working hypothesis, based on study of pertinent literature relevant to the connection between so-called dry chloride retention and degeneration of the collagen system, is not supported by the analyses. Regarding the question as to whether any difference exists between the chlorine content of healthy and degenerated cartilage tissue, it can be said that the difference obtained with Berg's method (difference in chlorine content + the cyanide formation capacity) can either depend on an existing difference in chlorine content (in which event the cyanide formation should be identical in the compared series), or variableness in the cyanide formation (thus involving similarity of chlorine content in both series). Investigations on this point are continuing and will be published later. The "bound chlorine" detected by the author might probably be explained by the too high values which occurred on account of the cyanide formation. Hence, from the investigations made, there is no longer any reason to assume the presence of organic chlorides in the human body. The writer hopes to return to this question also in a later work.

1. Lindahl, O. *Acta Orthoped. Scand.* **18** (1949) 346.
2. Friberg, S. *Ibid.* **19** (1949) 1.
3. Mutt, V. *Ibid.* **19** (1949) 2.
4. Zacherl, M., and Krainick, H. *Mikrochem.* **11** (1932) 61.

Received June 2, 1950.