TOXICOLOGY OF TRANSPORTABLE URANIUM: A CRITICAL CONTRIBUTION TO HEALTH PROTECTION OF WORKERS

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SUMMARY

The chemical toxicity of uranium has a remarkable importance in the evaluation of the risk of internal contamination due to transportable compounds of such element. The risk becomes most relevant when one has to deal with natural uranium or long-lived uranium isotopes. The maximum permissible concentrations recommended by ICRP and the other limit values proposed are based on the results of experiments that, though complete and valid in their general outline, are affected by the limited technical possibilities available when effected. Since now the possibilities of detecting and evidencing renal lesions are remarkably increased; it could be useful to review the problem of the chemical toxicity of uranium both on the basis of the advanced techniques available and on the experience gained in the field of health protection in nuclear environments. In fact modern techniques allow a relatively easy detection of even slight modifications and of their characteristics; at the same time they permit to carry out the analysis of very small biological samples. In the present work it is also stressed that studies carried out on other renal toxic substances, like cadmium, are giving very interesting results.

The occupational exposure of workers to the hazards of radioactive contamination involves health protection problems which still deserve further studies and research. It is well known that since the first definition of protection criteria suggested by international and national qualified organisms, several variations and advances have been introduced in the successively published recommendations[1,2,3,4,5,6].

Among the different nuclides taken into consideration to

control the risk of internal contamination, particular attention must be given to natural uranium and to long-life isotopes of this element: in fact their incorporation in man and higher animals is remarkably important as far as their chemical toxicity is concerned. Such a risk becomes of primary importance when we have to deal with those compounds of U-235, U-238 and natural uranium which are easily absorbed[3,4,6]. These toxicological assumptions represent, therefore, the basic reference for the definition of limit levels in radiation protection: their recognition is the result of a great bulk of studies and experiments considering both the biochemical and the morphological aspects[7].

However, it should be observed that, despite the precision and the extension of such investigations, not negligeable perplexities still exist on the actual physiopathological significance of the proposed limit levels. Just to quote an example, it is rather difficult to evaluate, on a histological level, findings described as "slight or extremely mild renal-tubular changes"[7]. Similarly, it is sometimes far from easy to use the available protection criteria to solve real problems, such as the correct evaluation of a possible renal damage in the case of a continuous contamination when the incorporation rate widely varies in time[8] and consequently to give in this case a right and reliable interpretation of the excretion data.

The problem is worth while a more modern evaluation made on the basis of recent knowledge in the field of renal physiology and physiopathology. First of all it is necessary to take into consideration one of the most sensitive indicator of uranium intoxication, "albuminuria", which, together with catalasuria and aminoaciduria, permits the detection of an early renal failure (in the form of tubular lesions) due to the introduction of such heavy metal in a soluble form[7,10].

Albuminuria has pratically been considered one of the most important reference symptoms, that is to-say a kind of "threshold" manifestation used in the definition of protection parameters. Now, the modern knowledge on the structure and origin of urinary proteins, as well as on the relationships existing between the different types of proteinuria and the morphological and functional nephropathic modifications, brings about a series of complex problems of interpretation emerging from analyses such as the electrophoresis, the immunoelectrophoresis, gel-filtration, ultracentrifugation, chromatography, immunodiffusion, etc.[11].

Obviously the simple quantitative evaluation of "albuminuria" in many cases results nowadays semeiologically inadequate. For instance in chronic pielonephritis, a quantitative description of proteinuria, often not too high, does not always describe a severe renal damage[12]. Consequently the importance of "albuminuria" on a physiopathogenetic level appears to be decreased: an evaluation must be made in quantitative and qualitative terms of "pro-

teinuria", or, better, of "proteinurias". More recently, in fact, on the basis of their electrophoretic behaviour, the fractions forming the "proteinuria" have been accurately detected and a morphological distinction has been made possible as far as "pathologic proteinurias" are concerned, in "glomerular" and "tubular" proteinurias[11,13,14,15,16,17].

The electrophoretic behaviour of proteinurias in the different possible combinations has often shown to be remarkably consistent with the different pathogenetic and clinical phases of several nephropathies. The observation of an electrophoretic picture indicating a renal damage, confirmed at the biopsy, in the presence of very poor, or even not detectable, urinary proteins, shows the great possibilities given by modern methods.

We did not find in the current specialized literature studies of this kind, related to the characterization of proteinuria caused by uranium. However, it might be interesting to try to outline some diagnostic possibilities even if on a theoretical basis.

Taking into account the capacity of soluble natural uranium of causing lesions to proximal tubules, the hypothesis that the "expected" proteinuria might essentially be of a tubular kind (simple or mixed) appears to be justifiable.

Though considering the incertitudes connected to the origin and formation processes of the tubular proteinuria, the latter presents morphological and structural features which allow a rather exact differentiation: it is made by micromolecular aggregates (molecular weight: 20,000 - 50,000) detectable by means of an ultracentrifuge; it often shows an anomalous behaviour in the presence of precipitating factors, boiling included; its content in urines is generally poor and sometimes not even detectable[11]. Consequently diagnostic difficulties may arise owing both to the commonly low values and to the irregular reactivity to precipitation also when the content is high.

However, these difficulties can be overcome by the electrophoretic examination (on cellulose acetate or on gels of acrylamide or of starch). In fact the morphological picture permits a satisfactory characterization also when the quantitative determination, carried out with methods commonly used in clinical research, gives scarce results or no results at all. The electrophoretic morphology of tubular proteinuria is characterized (on cellulose acetate) by the prevalence of globulins as compared to albumin; by a low intensity of the transferrinic fraction; and by the presence of homogeneous bands with differing mobility in the gamma zone.

The "glomerular" proteinuria presents a rather different picture; the albumin fraction is remarkably wide and intense, and the transferrinic band is very sharp. Obviously mixed forms also exist and are often encountered; they appear as the result of

various overlappings of the pure forms.

The first studies on tubular proteinurias have been carried out by Butler and Flynn in 1958[13]. But we would like to stress that at the same time Luessenhop and coll. established the minimum dose of hexavalent uranium able to cause, when injected intravenously, albuminuria in man[10].

The evaluation criteria used in this study to detect the "albuminuric threshold" were based on methods which are no more adequate to give an evaluation of the kidney functionality, especially at the level of the "intravenous tolerance dose of hexavalent uranium for man"[10]. It is also necessary to observe that a correct interpretation of this symptom is sometimes made more difficult by the frequent presence of proteinuria in patients affected by neoplasia[18], as the examined subjects actually were[10].

In 1959 Publication 2 of ICRP[3] reported, among others, the MPC in air and water for natural uranium, U-235, and U-238 in soluble form. These values, already contained in the 1954 "Recommendations"[2] are still substantially unchanged. In fact the modifications appearing in Publication 6 for the value of MPC in water derive from the different hypotesis accepted for intestinal absorption of uranium[4]; while the consideration of its ability in producing renal lesions remained the same. In any case, it should be observed that the MPC values for soluble uranium have been subjected to some criticism[19] since their very first appearance.

In urines of experimental animals intoxicated with hexavalent uranium, the presence of plasma-albumin and of various plasma-globulins was shown by electrophoresis (on paper?)[7]. It is interesting to compare these results to those obtained in the case of tubular proteinuria.

In fact, the tubular proteinuria (in its pure form) shows on paper electrophoresis an albumin/globulins ratio lower than 1, and the presence of a fraction alpha-2 of a remarkable entity, a fraction beta relatively high and, in some cases, a fraction with anomalous mobility in the cathodic sense (post-gamma). By means of electrophoresis on starch gel the globulinic fractions show a pre- and post-albuminic, alpha-2, beta and post-gamma mobility[11,14,16,17] (we already described the electrophoretic results on cellulose acetate).

This topic shows many difficulties in any case. Several questions have been posed, since the first experiments, concerning the passage of plasmatic albumin into urines, especially in the frequent absence of appreciable glomerular lesions in the case of small doses of uranium[7,10]. On the other hand the genesis of tubular proteinuria involves many aspects which are still to be explained[20,21,22].

It is also very interesting to compare the preceding observations with analogous aspects supplied by toxicological studies of another heavy metal, cadmium, also responsible of renal lesions mainly at the tubular level. The proteinuria evidenced by Friberg in 1950 in workers exposed to cadmium dusts and vapours[23] was deeply studied; the characters distinguishing it from proteinurias found in nephritis and nephroses[24] are now well known.

The cadmic protein (molecular weight ranging from 20,000 to 30,000; sedimentation constant of about 2.2 S) does not precipitate when boiling, or when treated with picric acid; it is evidenced by a precipitation with nitric acid, with 25% trichloroacetic acid, with 3% sulfosalicylic acid. The electrophoretic spectrum includes four or five fractions with a low albumin/globulins ratio; 50% approximately of the components behave as gamma-globulins in electrophoresis, immunoelectrophoresis, and DEAE-cellulose chromatography.

By means of specific antisera it was possible to demonstrate that about 30% of cadmium proteins is constituted by L chains of gamma-glubulins[25].

It could be wise to recall here that also recently the difficulty of evidencing the peculiar micromolecular proteinuria of subjects exposed to cadmium by means of conventional methods has been stressed; while electrophoretic methods permit to recognize such proteinuria with reliable results[26].

The reported aspects of cadmium toxicology are intended as an example showing the difficulties and complexity of protection studies and demonstrate that these kind of problems do not exclusively concern uranium.

The observations made on the electrophoretic behaviour of proteinurias, represent a partial view of the problem, but they are in any case sufficient, according to our opinion[27], to select the importance of some controls not only at the damage level, but rather at the level of "contact" of uranium with the kidney. On the other hand, the concept of threshold is being critically reviewed, as evidenced during the Symposium of the American Accademies of Industrial Hygiene and Occupational Medicine held in Cincinnati in February 1970[28].

The definition of dose without effect and of acceptable reaction advanced on that occasion undoubtedly involves aspects of biological phylosophy extremely delicate and, in any case, the necessity of having investigation means of high sensitivity and specificity.

Summing up, on the basis of recent knowledge in the field of physiopathology and diagnostics a check appears to be auspicable of the validity of the values established for uranium years ago and still used: in order to confirm them or, if necessary, to

adequate them to more modern criteria. On the other hand, the methods available today could permit to carry out an adequate observation in man too, in the range of the present "admissibility", and at very low levels without involving undue risks.

As far as, in particular, the electrophoretic multifractionation of urinary proteins is concerned, the present availability of techniques which can be carried out also on not concentrated urines[29] can permit wider experiments on small animals, such as rats for which it has already been established that the lethal dose for uranium relatively to the body weight is well comparable to that for man[8,10].

The aim of these observations will be reached if the above discussions show the necessary requirements for a translation into a useful research program.

REFERENCES

1. Maximum permissible amounts of radioisotopes in the human body and maximum permissible concentrations in air and water. USA National Bureau of Standard, Handbook 52, 1953.
2. Recommendations of the International Commission on Radiological Protection (Revised December 1, 1954). Brit.J. Radiol., Supplementum n. 6, London, 1955.
3. Report of Committee II on permissible dose for internal radiation. ICRP Publication 2, Pergamon Press , New York, 1959.
4. Recommendations of International Commission on Radiological Protection. ICRP Publication 6, Pergamon Press , Oxford, 1964.
5. Recommendations of International Commission on Radiological Protection. ICRP Publication 9, Pergamon Press, Oxford, 1966.
6. Report of Committee 4 on evaluation of radiation doses to body tissues from internal contamination due to occupational exposure. ICRP Publication 10, Pergamon Press , Oxford, 1968.
7. Voegtlin C., Hodge H.C., eds.,: Pharmacology and toxicology of uranium compounds. Books 1 to 4. McGraw-Hill Book Company, Inc., New York, 1949-1953.
8. Eve I.S.: Some suggested maximum permissible single intakes of uranium. Health Physics, 10, 773, 1964.
9. Sunderman F.W., Sunderman F.W.Jr., eds.: Laboratory diagnosis of kidney diseases. Warren H. Green, Inc., St. Louis, Missouri, 1970.
10. Luessenhop A.J. et al.: The toxicity in man of hexavalent uranium following intravenous administration. Amer. J. Roentgenol., 79, 83, 1958.
11. Maiorca R., Scarpioni L.: Le proteinurie. Fisiopatologia e diagnostica. Il Pensiero Scientifico editore, Roma, 1968.
12. Lenti G., Vercellone A.: La pielonefrite cronica. Aggiornamenti clinicoterapeutici. Ed. Minerva Medica, 1960.

13. Butler E.A., Flynn F.V.: The proteinuria of renal tubular disorders. Lancet, 2, 978, 1958.

14. Butler E.A., Flynn F.V.: The occurrence of post-gamma protein in urine; a new protein abnormality. J.Clin.Path., 14, 172, 1961.

15. Traeger J. et al.: Analyse électrophorétique des protéinuries des glomérulonéphrites et des syndromes néphrotiques. J.Urol. Néphrol. (Paris), 71, 1056, 1965.

16. Traeger J. et al.: Analyse électrophorétique des protéinuries rencontrées au cours des atteints tubulaires rénales. J.Urol. Néphrol. (Paris), 71, 728, 1965.

17. Traeger J. et al.:Les protéinuries des atteintes tubulaires rénales. Path.Biol., 14, 5, 1966.

18. Leyton G.R. et al.: Chemical studies and physical characteristics of urinary proteoses in cancer. Clin.Chim.Acta, 9,157, 1964.

19. Hursh J.B.: Suggested urinary tolerance levels for enriched uranium. UR-515, 1957.

20. Butler E.A. et al.: A study of urine proteins by two-dimensional electrophoresis with special reference to the proteinuria of renal tubular disorders. Clin. Chim.Acta, 7, 34, 1962.

21. Creeth J.M. et al.: An ultracentrifuge study of urine proteins with particular reference to the proteinuria of renal tubular disorders. Clin. Chim. Acta, 8, 406, 1963.

22. Harrison J.F., Northam B.E.: Low molecular weight urine protein investigated by gel filtration. Clin. Chim.Acta, 14, 679,1966.

23. Friberg L.: Health hazards in the manufacturing of alkaline accumulators with special reference to chronic cadmium poisoning. Acta Med. Scand. 138, suppl. 240, 1, 1950.

24. Patty F.A. ed.: Industrial hygiene and toxicology. Cap.XXVII, Interscience Publishers, Inc., New York, 1967.

25. Vigliani E.C., Pernis B., Amante L.: Etudes biochimiques et immunologiques sur la nature de la protéinurie cadmique. La Med.Lavoro, 57, 321, 1966.

26. Pujol M. et al.: Tubulopathie des intoxications chroniques par le cadmium. Arch. Mal. Prof., 31, 637, 1970.

27. Righi E., Arzano S.: Il protidogramma elettroforetico su urine non concentrate nella valutazione della idoneità al lavoro con rischio di contaminazione radioattiva. CNEN Report LNF-72/82, Frascati (Rome), 1972.

28. Hatch T.F.: Thresholds: do they exist? Arch. Environ. Health, 22, 687, 1971.

29. Brancaccio D.: Multifrazionamento elettroforetico di proteine urinarie non concentrate. Arch. Ital. Urol. Nefrol., 42, 315, 1969.