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Radioactive Chromic Phosphates: Comparative Study of the *in vivo* Distribution and Feasibility of Preparation

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Chromic radiophosphate has been used extensively for irradiation of tumors (1) (2), palliation of malignant effusions (3) (4) and for prophylaxis, following surgical tumor removal (5) (6).

Several methods of preparation are currently in use and the final product can have different chemical, as well as physico-chemical properties, which could produce a different biological behavior.

The present work is a comparative study of the chromic phosphate *in vivo* distribution, of the possibility of its hydrolysis in the tissues and of the feasibility and radiochemical yield of the method of preparation.

The radiopreparations assayed were: Type I) a particulate chromic phosphate prepared according Dobson et al (7), which is a modification of Jones's original method (8) based on the precipitation of chromic phosphate, dehydration by heating and grinding. Type II) a particulate preparation according to the author's method (9) which, through an oxidation-reduction reaction and a posterior olation and oxolation, gives a basic chromic phosphate. Type III) the same as preparation Type II, but dehydrated and ground as for the preparation Type I. Finally, a true colloidal solution (Type IV) which is prepared as the Type II, but avoids the precipitation through the formation of a formation of a complex with an organic ligand (10).

METHODS

All the preparations tested were double labeled with ^{32}P and ^{51}Cr. Type I was prepared according to Dobson et al (7). For the preparation of Type II the following technique was used: 2.5 ml of CrO_3 solution (10 mg/ml), containing ^{51}Cr as $CrO^2{}_4$-, were added to 1 ml of H_3PO_4 solution (10 mg/ml), also containing ^{32}P as $PO^3{}_4$-. The mixture was heated for five minutes in a boiling water-bath.

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Then 4 ml of fresh Na_2SO_3 solution (50 mg/ml) were added. After boiling for 10 more minutes and cooling with cold water, the suspension was centrifugated. The precipitate was washed three times with distilled water and finally resuspended in normal saline.

For Type III the preparation was the same, but in addition it was treated by heating, grinding and screening as like Type I. The true colloidal solution Type IV was prepared as follows: The same volume of CrO_3 and H_3PO_4 solutions as described for Type II were treated with the same amount of Na_2So_3, but dissolved in 1 ml of 6% gelatin solution. After boiling, the clear blue greenish colloidal solution was dialyzed against distilled water, changing the water at least eight times during four to five hours. The final dialysis was performed against normal saline and practically no activity was in the saline.

All these preparations present a continuous spectrum of particle sizes. For Type I, the major portion are from about 0.5 to over 1 μ in diameter, for type II with a diameter between 0.6 and 2μ (Fig. 1) and for the true colloidal solution a particle size between 0.1 and 0.3 μ (Fig. 2).

Groups of adult albino rats were injected into the tail vein with approximately the same dose (25-50 μC in 2 mg of solid chromic phosphate) of each type of cromic phosphate. Five animals from each group were sacrificed at different intervals: one day, seven days and 14 days and after evisceration, liver, lung and spleen were pooled separately. The skeletons were separated from the remains by digestion with boiling 10% Ba $(OH)_2$ during five hours. In this way, the muscle proteins were hydrolized leaving the bones completely free of other

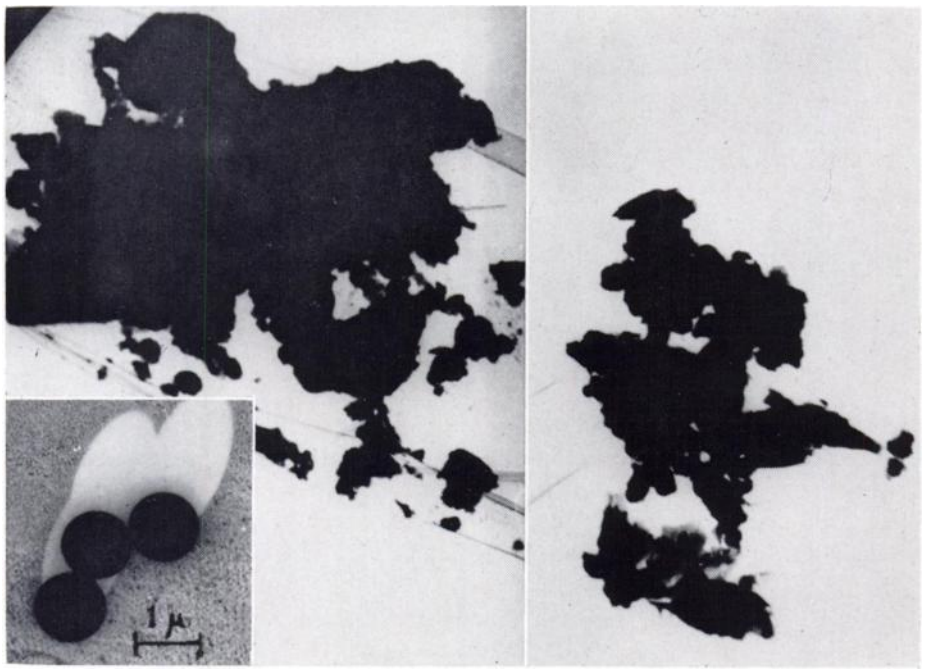

Fig. 1. Electron-micrograph of particulate chromic phosphate (type II).

tissues. The pooled skeletons, as well as the organs, were ashed at 600-700°C and the ashes dissolved in concentrated HCl and then diluted to 1 N. Finally, aliquots of each mineralized fraction were counted for ^{32}P with a beta counter and for ^{51}Cr with a scintillometer. The beta counting was corrected for self-absorption, using a self-absorption curve, obtained by measuring samples containing different weights of mineralized material with the same added ^{32}P activity.

RESULTS

Table I shows the values of the ^{32}P activity found in the organs and skeleton, as percent of the injected dose. The activity accumulated in liver shows a fairly similar pattern for both particulate phosphates treated thermically (Types I and III). With the other two types, a decrease is observed with the time. Since it seems logical, because of its smaller particle size, the true colloidal solution (Type IV) is less retained by the lungs and the spleen. The opposite happens with the bones, where the smaller particles are quickly trapped. In all these cases, the behavior of the Type II is between the thermally treated and screened Types I and III, and the colloidal Type IV.

TABLE I

		24 hours		*7 days*		*14 days*	
	Type	*β Activity* %	*Ratio* β/γ	*β Activity* %	*Ratio* β/γ	*β Activity* %	*Ratio* β/γ
Liver	I	81.1	1.1	84.5	1.1	92.1	1.1
	II	53.9	1.1	60.3	1.8	47.0	0.9
	III	69.7	0.9	77.1	0.9	87.0	1.0
	IV	78.5	0.9	71.8	0.8	59.4	0.8
Lung	I	5.2	1.1	4.0	1.4	2.0	0.9
	II	6.8	0.8	1.4	1.1	2.0	0.6
	III	2.6	0.6	1.8	0.7	4.2	1.4
	IV	0.3	1.7	0.2	1.4	0.1	1.4
Spleen	I	7.7	1.2	9.0	1.1	7.8	1.0
	II	4.6	1.0	5.3	1.4	5.3	1.1
	III	6.1	1.0	5.1	0.9	7.7	1.2
	IV	2.4	0.9	2.4	1.0	1.2	1.0
Bone	I	0.4	0.2	2.0	1.2	2.2	1.5
	II	4.8	1.2	8.8	1.7	5.5	2.3
	III	0.8	0.6	0.3	0.7	2.0	0.8
	IV	5.7	1.8	3.6	4.1	6.6	7.3

Distribution of the radioactivity in different organs (as percent of the injected dose).

TABLE II

Type	*Radiochemical yield* %	*Time required* hs	*Special equipment*
I	50–65[1]	2–3	———
	30–40[2]	12–24	Furnace and mill
II	85–95	½	none
III	60–75	12–24	Furnace and mill
IV	70–85	6–8	none

[1]Yield after precipitation.
[2]Yield after heating, grinding and screening.

The study of the ratio beta activity/gamma activity in the tissue and the ratio beta activity/gamma activity in the injected chromic phosphate gives an indication of the hydrolisis that the compound can suffer *in situ*. This ratio (Ratio β/γ in Table I), when 1 or close to this value, indicates no change in the chromic

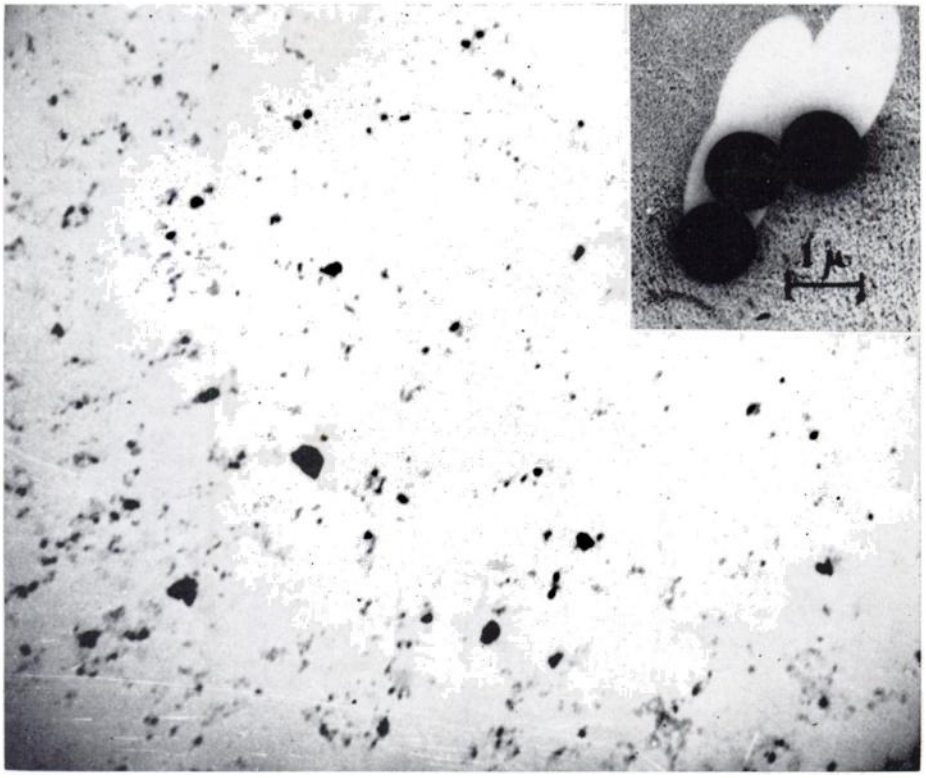

Fig. 2. Electron-micrograph of colloidal chromic phosphate (type IV).

phosphate molecule. The increase of this value can be related to an increased ^{32}P activity in the tissue as can be seen in the values corresponding to bone.

Table II gives an estimative picture of the feasibility and radiochemical yield of each preparation. Obviously, the simplicity, short time required and good yield are *sine qua non* conditions for its use at any hospital radioisotope service.

DISCUSSION

The decrease observed, with time, in the liver, for the preparations Type II and IV, could be related to the observed fact (10), that the amount eliminated through the bile is greater when the particle size is small.

In this experimental work the differences observed in the pattern of distribution are more related to the particle size than to the nature of the chromic phosphate. For instance, the behavior of Type II is generally between those of Types I and III, and Type IV which could be explained, because all the particle size spectrum is present in Type II.

The *in vitro* stability to both hydrolysis (11) and isotopic exchange (12) has already been studied and those results agree with the present findings, which indicate a fairly good stability. Only in the case of true colloidal solution (type IV), where actually the type of bond between the chromium and phosphorus is not yet known, the phenomenon of hydrolysis has a significative importance, especially concerning the bone accumulation. Anyway, the ratio β/γ does not exclude the possibility that the hydrolysis was done in another tissue with a later migration and uptake by the bone.

As a result of these findings, the time consuming thermal treatment of the chromic phosphate can be avoided and a simpler, faster and good yielding technique of preparation may be used.

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