

~~Distribution of Colloidal Radioactive Chromic~~  
Phosphate After Intracavitary Administration<sup>1</sup>

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COLLOIDAL radioactive gold, Au<sup>198</sup>, has been used for several years in the palliative treatment of patients with recurrent pleural and abdominal effusions caused by malignant neoplasm. Its use has been reasonably well established and evaluated, but the results, although encouraging, leave much to be desired. The extent of the contribution of the gamma emission of the radioactive gold to the therapeutic result is not known, but this component of the radiation produces a total-body effect that is undesirable. An isotope which, like colloidal gold 198, remains largely in the region of the injected cavity, yet has only a beta emission, would have the advantage of producing less total-body irradiation. Since the need for protecting the operator against gamma radiation complicates the use of the gold, a beta emitter would also be easier to administer. P<sup>32</sup> with an energetic beta emission of 1.72 mev, in the form of a colloid, chromic phosphate, should be useful for this purpose. The chromic phosphate can be made in a stable colloidal form so that there is little dissociation *in vitro*.

In evaluating the use of chromic phosphate for intracavitary injection, it is important that several questions be answered.

1. Is the material stable and not dissociated in the body following injection?
2. Is there a tendency for it to settle or plate out on pleural or peritoneal membranes, as does gold 198, or does it remain equally distributed throughout the fluid in the cavity?
3. Are there any significant hematological changes following its use in therapeutic amounts?
4. Finally, is there any advantage over colloidal gold 198 or deep x-ray therapy

in the control of recurrent effusions in patients with cancer?

The present report is not an answer to all of these questions but is concerned only with efforts to gather data on the metabolism and distribution of chromic phosphate after intracavitary injection. No clinical effects are considered.

METHODS

Four patients with pleural effusion and 6 with ascites, all of whom had cancer (proved histologically) were selected. The effusions were recurrent and necessitated paracenteses for relief. The chromic phosphate used was obtained from Abbott Laboratories. It takes several days to produce each batch, and this probably contributes to the present rather high cost.

In order to obtain data on the concentration of the chromic phosphate in the fluid of the abdominal and pleural cavities at various intervals after injection, two Polyethylene tubes were placed in the cavity. Through one of these the colloid was injected; through the other samples were drawn at intervals for radioassay. The patients were instructed to turn and move around in order that the colloid might be uniformly distributed. Following intracavitary administration, the levels of radioactivity in the blood, pleural and peritoneal fluids, and urine were measured at intervals. Samples were obtained frequently at first and then at longer intervals, since it was believed, from earlier studies with gold, that the initial disappearance from the fluid would be rapid. Blood samples usually were withdrawn at the same time that the fluid specimens were obtained. In 3 patients, who died at varying intervals after administration of

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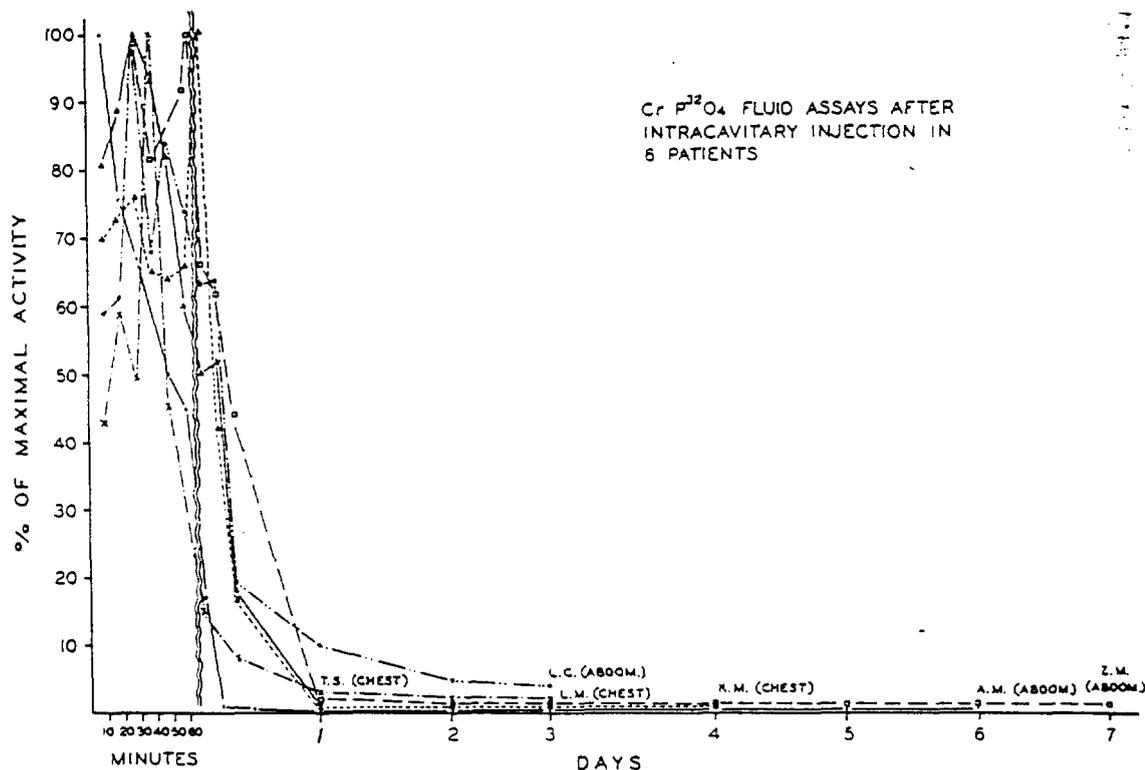


Fig. 1. Concentrations of radioactivity in the pleural and abdominal fluid of 6 patients after intracavitary administration of CrP<sup>32</sup>O<sub>4</sub>.

the colloid, autopsies were performed and tissue distribution data were obtained. Microscopic and gross autoradiograms were prepared from the tissues of these patients. The tissues were assayed for radioactivity by treating them with nitric acid and counting them in liquid form by means of a thin end-window Geiger tube.

#### RESULTS

Figure 1 shows the concentration of radioactivity plotted against time in the pleural and abdominal fluid of 6 patients. An erratic variation of concentration in the first hour probably represents mixing. Following this, there is a decrease in radioactivity that is too rapid to be a result of dilution. Since there is little absorption from the cavity, as will be shown later, this decrease must result from the settling out of the colloid on the serous membranes. This uniform pattern of decreasing concentration was not seen in 3 patients who

showed fluctuating high levels for several days. The reason for these high variable levels is not clear. It seems likely that they are related to the tendency of the colloid to settle out with gravity. There may have been inadequate mixing in the cavity before samples were drawn, or failure to assay representative aliquots of fluid. Figure 2 shows the concentration of radioactivity plotted against time, in pleural fluid before centrifugation and in the supernatant layer after centrifugation. There is about ten times as much activity in the fluid before centrifugation as there is in the supernatant layer. Microscopic autoradiograms of the sediment show aggregates of radioactivity associated with cellular material but do not show whether it is adsorbed on the cells or actually incorporated within them as ionic P<sup>32</sup>.

Figure 3 shows the averages of radioactivity levels determined on whole blood, washed red blood cells, and plasma in 3 patients. Most of the radioactivity is

TABLE I: TISSUE DISTRIBUTION OF CrP<sup>32</sup>O<sub>4</sub> AT AUTOPSY. DAR\* IN THREE PATIENTS WHO RECEIVED INTRACAVITARY CrP<sup>32</sup>O<sub>4</sub>

Patient and Days After Injection →	T. S. 14	J. H. 14	J. E. H. 3
Liver	0.51	0.39	5.74
Spleen	0.54	0.08	Surgically removed
Marrow	0.24	0.05	0.82
Lung	0.18	0.10	0.41
Adrenal	0.24	B.G.	0.86
Kidney	0.24	0.07	0.33
Lymph nodes distant from cavity	0.19	0.08	0.95
Lymph nodes adjacent to cavity	65.17	5.93	4.76
Serous membrane of cavity	451.7 (pl)	7.38 (pn)	41.47-1.37 (pn)

\* DAR =  $\frac{\mu\text{c./gm. in organ corrected for decay}}{\text{dose, } \mu\text{c./body wt., gm.}}$

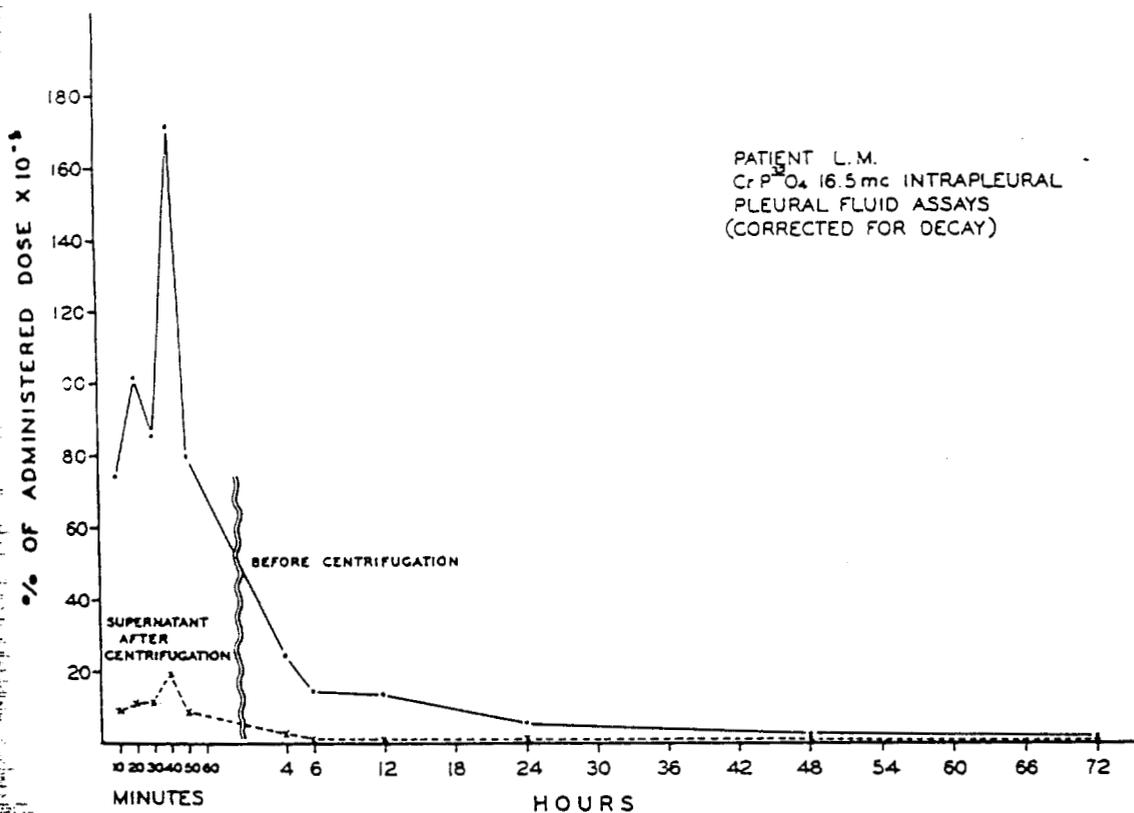


Fig. 2. Concentration of radioactivity in pleural fluid containing CrP<sup>32</sup>O<sub>4</sub>, before and after centrifugation. Most of the radioactivity is found in the sediment.

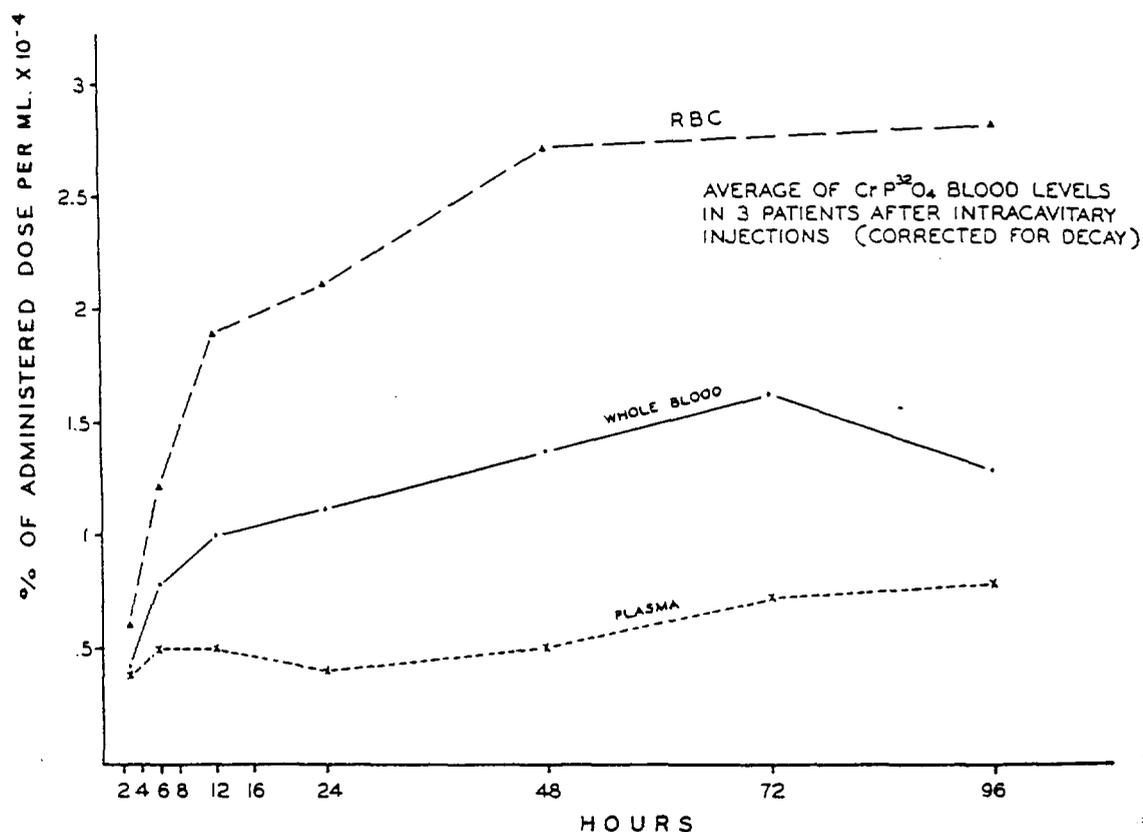
seen to concentrate in the red blood cell portion. Upon hemolysis and dialysis of these red blood cells, 41 per cent of their radioactivity passes into the dialysate, which indicates that they contain ionic phosphate. One must assume, then, that much of the radioactivity in the blood results from ionization of the chromic phosphate. This concentration of radioactivity is quite different from that seen after intracavitary injection of gold.

which shows very little concentration in the red blood cells. It is important to realize that the total amount of P<sup>32</sup> circulating in the blood at any time is actually quite small. In the 3 patients shown, the maximum amount, obtained by multiplying the concentration of P<sup>32</sup> per milliliter of blood by the estimated blood volume, was slightly less than 1 per cent of the administered dose.

Urinary excretion of the chromic phos-

TABLE II: APPROXIMATE DISTRIBUTION OF  $\text{CrP}^{32}\text{O}_4$  IN ORGANS AT AUTOPSY AFTER INTRACAVITARY INJECTION

Patient	Dose (mc.)	Cavity of Injection	Days After Last Dose	Per Cent in Liver	Per Cent in Spleen	Per Cent in Marrow
T. S.	19.5	Pleural	14	1.57	0.14	1.19
J. H.	2.2	Peritoneal	14	0.97	0.02	0.23
J. E. H.	4.3	Peritoneal	3	12.3	Surgically removed	4.09

Fig. 3. Averages of levels of radioactivity after intracavitary injection of  $\text{CrP}^{32}\text{O}_4$ , determined on whole blood, washed red blood cells, and plasma.

phate in 10 patients is shown in Figure 4. The average is approximately 5 per cent of the administered dose over a period of eleven days. This is considerably more than the amount of gold 198 excreted over a similar period of time and suggests that much of the excretion is of ionic phosphate.

Autopsy distribution data for 3 patients are shown in Table I, where the relative concentrations in different organs are compared. Two items are of particular interest. The highest concentration of radioactivity is on the serous membranes of the injected cavities, and the uptake in lymph nodes adjacent to these cavities is relatively good. We have not seen

gold 198, similarly administered, concentrate to such an extent in lymph nodes. Table II shows the percentage of the administered dose concentrated in the liver, spleen, and marrow in the same 3 patients. The percentage in the marrow can be only a rough approximation. The distribution in these reticulo-endothelial organs is very similar to that of gold 198. One would not, however, expect to find such a high concentration as 12 per cent in the liver (shown for patient J. E. H.) three days after an injection of gold.

If there is ionization of the chromic phosphate, one might expect to find a higher concentration in neoplastic tissue.

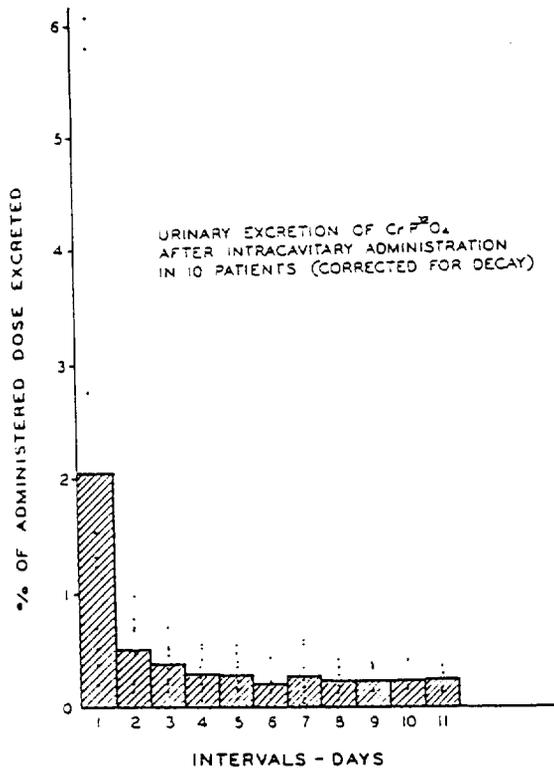


Fig. 4. Urinary excretion of  $CrP^{32}O_4$  after intracavitary administration.



Fig. 5. Autoradiogram of liver (containing metastatic neoplasm) following intraperitoneal administration of colloidal radioactive gold,  $Au^{198}$ . There is no concentration in the neoplastic area.

than if it all remained as a true colloid. Figure 5 shows an autoradiogram prepared from the liver of a patient given gold 198 intraperitoneally. No radioactivity is seen in the areas of metastatic neoplasm, which involves the liver extensively, but there is a good concentration in the liver parenchyma. Figure 6 shows a photograph and autoradiogram of liver from a patient with lymphosarcoma given ionic  $P^{32}$  intravenously. Here, there is a higher concentra-

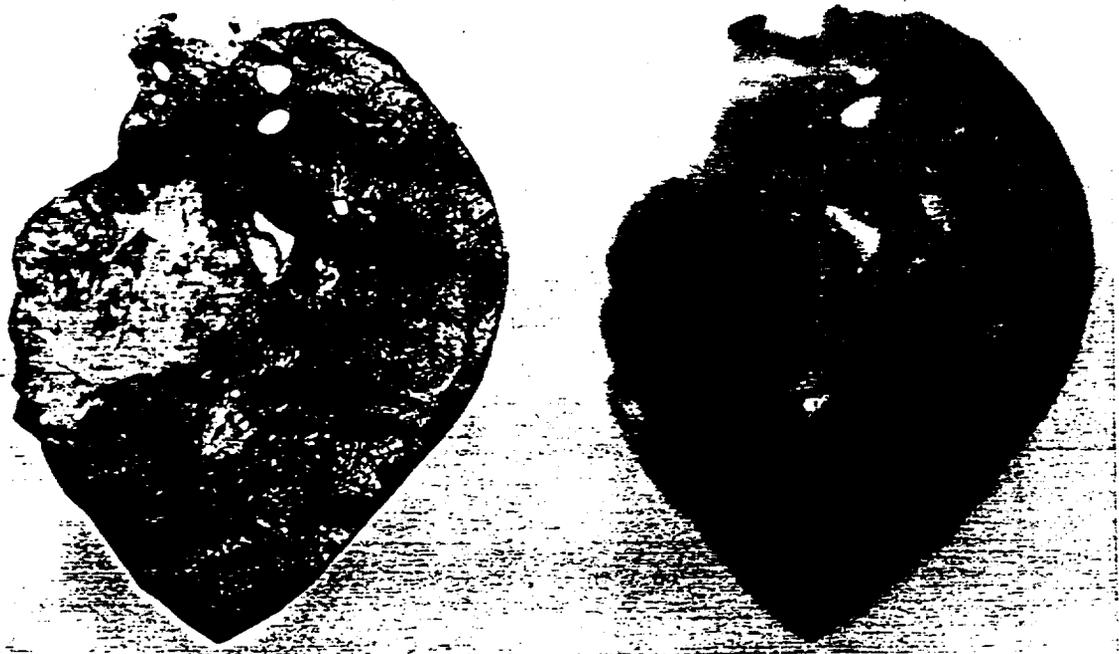


Fig. 6. Photograph (left) and autoradiogram of liver (containing metastatic neoplasm) following intravenous administration of  $P^{32}$  as sodium phosphate. There is a higher concentration of  $P^{32}$  in the neoplasm than there is in the liver parenchyma.



Fig. 7. Photograph (left) and autoradiogram of liver (containing metastatic neoplasm) following intraperitoneal administration of colloidal  $\text{CrP}^{52}\text{O}_4$ . There is concentration in both liver parenchyma and neoplasm, with the greater amount in the liver.

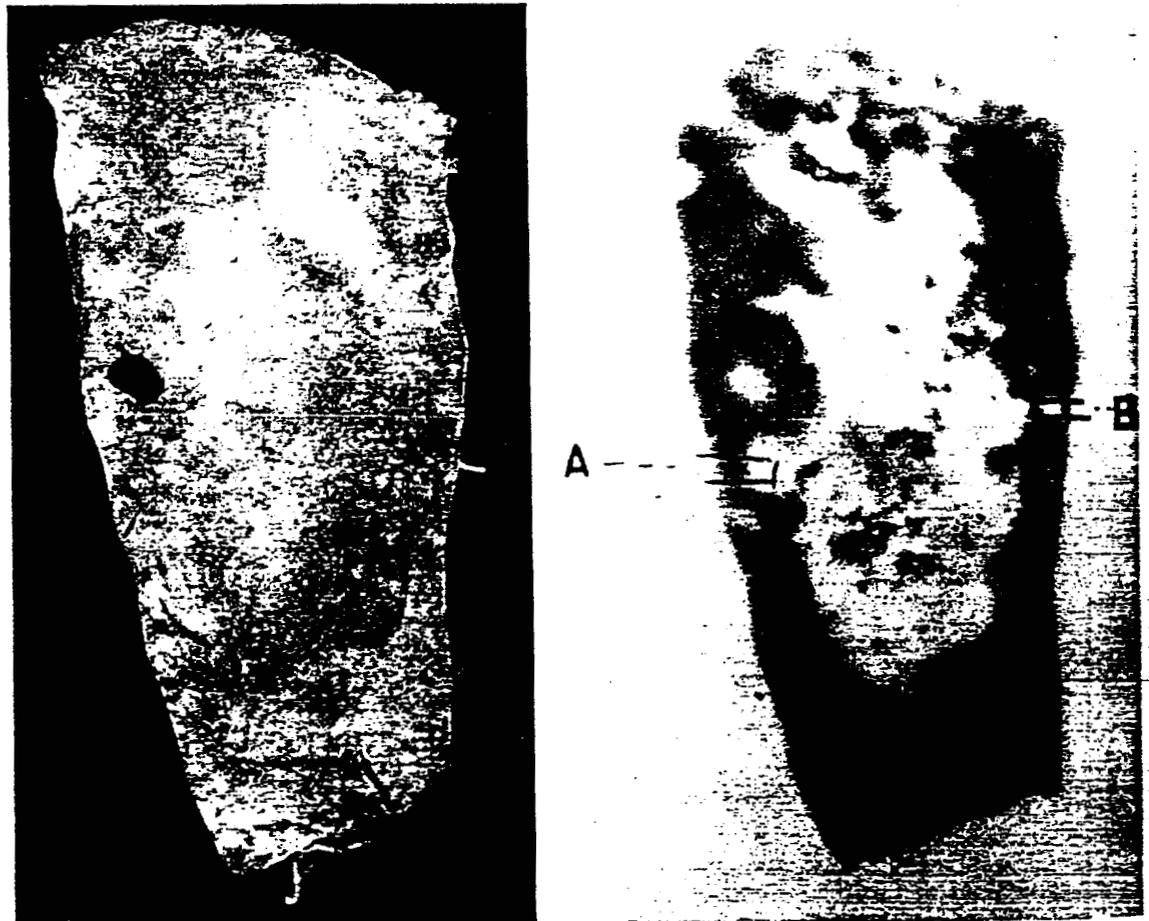


Fig. 8. Photograph (left) and autoradiogram of parietal pleura following intrapleural administration of colloidal  $\text{CrP}^{52}\text{O}_4$ . The central area that is invaded by neoplasm shows little concentration of the isotope.

tion in the tumor than in the liver. Figure 7 is a photograph and autoradiogram from the liver of a patient given chromic phosphate intraperitoneally. Deposition of radioactivity is seen in both tumor and liver, suggesting that the chromic phosphate is distributed like both phosphate ion and a colloid.

Figure 8 shows a photograph and an autoradiogram prepared from a flattened sheet of pleura invaded by neoplasm, from a patient given colloidal radioactive chromic phosphate intrapleurally. There is little activity in the tumor compared with a good deposition on the surrounding surface. This is only an isolated case, and we have no evidence that it represents a pattern of distribution.

#### DISCUSSION

The information presented here is based on only a small number of patients, but the results seem fairly consistent. There is good evidence that after the injection of chromic phosphate most of the radioactivity remains on the walls of the injected cavity. Only a small percentage of the injected dose is present in the blood at

any one time, but it is a larger proportion of the dose than is seen after injection of gold 198. Three points of interest in which the behavior of the chromic phosphate differs from that of gold 198 after its intracavitary injection are the high concentration of radioactivity in the red blood cells, the higher urinary excretion, and the greater deposition in neoplastic tissues. These results suggest strongly that some of the radioactive phosphorus has broken away from the colloidal complex and is distributed like phosphate ion.

#### SUMMARY

Preliminary studies indicate that the distribution of chromic phosphate after intracavitary injection is similar in most respects to that of colloidal radioactive gold. There is evidence, however, for some ionization of the chromic phosphate and this ionized portion follows the distribution expected for phosphate ion. From the point of view of distribution, chromic phosphate appears to be suitable for intracavitary therapeutic use.

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#### SUMARIO

#### Distribución del Fosfato Crómico Radioactivo Coloidal después de la Administración Intracavitaria

Estudios preliminares indican que la distribución del fosfato crómico radioactivo coloidal a continuación de la administración intracavitaria es semejante en su mayor parte a la del oro radioactivo coloidal. Tres puntos de interés en que el comportamiento del fosfato crómico discrepa del oro son: (1) la alta concentración de radioactividad en los glóbulos rojos; (2) la mayor excreción urinaria; y (3) el mayor depósito en el tejido neoplásico. Esos resultados sugieren que

parte del fósforo radioactivo se desintegra del complejo coloidal y se distribuye según sería de esperar para el ion fosfático. Desde el punto de vista de la distribución, el fosfato crómico radioactivo parece ser apropiado para empleo terapéutico.

La serie en que se basan estas conclusiones comprendió a 4 enfermos con derrame pleural y a 6 con ascitis, todos ellos con cáncer, comprobado histológicamente.

(For Discussion of this paper, see following pages.)

## DISCUSSION

Raymond L. Libby, Ph.D. (Los Angeles, Calif.): It is indeed a pleasure to open the discussion on a paper such as that presented by Dr. Root and co-authors, which adds to our basic knowledge of the properties of various radioactive materials used in the treatment of cancer—in this instance, intracavitary radioactive chromic phosphate.

The authors have evaluated four factors in the use of  $\text{CrP}^{32}\text{O}_4$ :

*First*, they have shown that  $\text{CrP}^{32}\text{O}_4$  does plate out on the walls of the cavity. This probably accounts for most of its effectiveness. Our own studies would confirm this observation.

*Second*, they have not observed any alarming hematological effects which would preclude the use of  $\text{CrP}^{32}\text{O}_4$ .

*Third*, they have pointed out certain obvious advantages of  $\text{CrP}^{32}\text{O}_4$  over colloidal gold for intracavitary use. To these observations I might add that a  $\text{P}^{32}$  beta particle of average energy will affect a volume of tissue fifty times that of the average gold beta particle.

*Fourth*, and finally, they have studied the stability of  $\text{CrP}^{32}\text{O}_4$  *in vivo* and have presented some evidence for its dissociation or ionization, *i.e.* (a) a greater concentration of  $\text{P}^{32}$  activity in red cells than in plasma; (b) the deposition of  $\text{P}^{32}$  activity in liver metastases; (c) the urinary excretion of  $\text{P}^{32}$  activity.

This extracavitary radioactivity is of course undesirable. I would like to comment briefly on this problem. Our own studies would indicate that most, if not all, of the  $\text{P}^{32}$  activity found in the blood and urine and other organs after an intracavitary injection of  $\text{CrP}^{32}\text{O}_4$  may be accounted for by the presence of a readily dialyzable  $\text{P}^{32}$  fraction in the  $\text{CrP}^{32}\text{O}_4$ . We have observed such a fraction in our own  $\text{CrP}^{32}\text{O}_4$  preparations as well as in Abbott preparations. This dialyzable fraction represents anywhere from 1 to 4 per cent of the total  $\text{P}^{32}$  activity and is enough to account for the extracavitary  $\text{P}^{32}$  activity observed by the authors.

I would like to make just one final comment—about the criteria that should be used to select the patients for intracavitary  $\text{CrP}^{32}\text{O}_4$ . In our own experience, the most successful results have been obtained on those patients who have exhibited free tumor cells in the pleural or ascitic fluid; we have seen little or no response in those patients with no free tumor cells.

Bernard Roswit, M.D. (New York, N. Y.): At the Veterans Hospital in the Bronx, New York, we have been utilizing a colloidal suspension of chromic phosphate containing  $\text{P}^{32}$  for more than two years for the interstitial treatment of advanced inoperable tumors of the head and neck and certain other inoperable tumors. It is of interest that in these investigative studies, the metabolic distribution was almost identical with that described by the Oak

Ridge group after intracavitary injection. We have carefully followed a patient with lymphosarcoma and pleural effusion, who benefited from intracavitary use of this radiomaterial. In a preliminary tracer study, we noted a prompt disappearance from the pleural fluid (a matter of minutes), suggesting deposition on the pleura itself. This is a very satisfactory event, for optimum pleural irradiation. From theoretical consideration alone,  $\text{P}^{32}$  would seem to be a better agent for this function than  $\text{Au}^{199}$ , by virtue of its longer half-life, more energetic beta particle, ease and safety of handling. We expect to proceed with this type of investigation. Incidentally, the interstitial use of colloidal chromic phosphate with  $\text{P}^{32}$  for advanced neoplasms of the head and neck appears promising.

Wm. G. Myers, M.D. (Columbus, Ohio): In view of the findings of the many workers on the toxicity of chromium, I am wondering whether the authors have had an opportunity to make any determinations of blood coagulation in these patients, because it is well known that chromium toxicity is very greatly prolonged, perhaps (thinking out loud) due to the effect on the liver.

I would like to ask also, in view of the toxicity of chromium, whether they have tried to use any of the phosphates of the physiological group. I am particularly thinking of calcium phosphate or iron phosphate. These are also soluble and probably could be used to get away from the toxicity of chromium. Chromium is known not only to be toxic in this respect but it seems likely, also, that it may have a carcinogenic effect.

Paul C. Aebersold, Ph.D. (Oak Ridge, Tenn.): In considering such matters we must definitely take into consideration the amount of material injected. When one considers that the amount of chromic phosphate used in these cases is on the order of a few milligrams, I don't think we need to be too concerned about toxicity or carcinogenic effect. Furthermore, these patients already have advanced cancer.

Perhaps some other phosphate could and should be used, such as iron phosphate, but considerable development would be necessary to test the preparation procedures, degree of solubility and distribution within the body.

Donalee L. Tabern, Ph.D. (Abbott Laboratories, North Chicago, Ill.): With respect to Dr. Myers' comments upon the possible physiological action of chromic phosphate, I think that we must very carefully keep in mind the amounts of material involved. When chromic phosphate of our preparation is administered, the actual amount of chromium is on the order of a fraction of a milligram or so, certainly far below any possible hazard level. As

for the potential action of chromium, I believe such studies are already under way at Ohio State and show no bad reactions in amounts anywhere near the order of those here involved.

I can further state that we have prepared other presumably colloidal insoluble phosphates and have studied them both chemically and in animals. None of them show anywhere near the valuable properties, as far as distribution and lack of absorption are concerned, as does properly prepared chromic phosphate of appropriate particle size and in a suitable menstruum. This work is being submitted for publication very shortly.

Dr. Root (*closing*): Although we have not made a special study of toxicity of chromium, we have not seen any gross evidence of it in the patients treated.

Certainly the few patients who have come to autopsy have showed no histologic evidence of liver damage, which would be expected in chromium toxicity.

In the present study we were not particularly concerned about any carcinogenic effect of the chromium because all the patients had widespread metastases.

We have not tried any other phosphates that are more insoluble, but, as Dr. Myers suggested, they might have some advantage.

I do not know of any very scientific reason for the dosages employed for the clinical studies. The 20 millicurie dose seemed to result in about the maximal safe blood level, and we have not exceeded this amount; whether it is the optimum dose or not, I do not know.

