JOURNAL OF NUCLEAR MEDICINE 5:462-470, 1964

The Metabolism and Excretion of Co^{57} Tetraphenylporphinesulfonate in Cancer Patients[1]

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INTRODUCTION

The detection of malignant tumors by external scintillation scanning has been limited by the lack of a labelled compound which specifically localizes in tumors. Tetraphenylporphinesulfonate (TPPS), a synthetic porphine derivative, attained a higher concentration in an experimental rat tumor than in any of the other tissues of the host (5). Its concentration in tumor was more than 4 times greater than in liver, spleen or kidney and more than 10 times greater than in all other organs. These concentration ratios would be adequate for the detection of an incorporated gamma emitting radioisotope in a tumor, even if it were superimposed over liver. TPPS attained a tumor concentration 50-100 times greater than hematoporphrin (8). Unlike the naturally occurring porphyrins, TPPS is of known composition, has chemical stability and derivative formation is readily obtained. The Co^{57} chelate of TPPS was synthesized and enabled sharp delineation by scintillation scanning of a subcutaneously growing Walker 256 carcinosarcoma in a rat (7). The tissue distribution of Co^{57} TPPS was compared with five other labeled compounds in mice bearing subcutaneous ependymomas (3). The absolute tumor concentrations of this substance were as high as those of albumin I^{131} (which provided the best concentration of all gamma-emitting localizing agents in common use). The tumor to brain concentration ratios from 24 hours to one week were 60 to 1, compared with only 30 to 1 for I^{131} albumin. Furthermore, the blood levels of TPPS were lower.

In this report, the localization of Co^{57} TPPS in the tumors of patients with cerebral and visceral malignant tumors was evaluated. Five patients were studied in detail to determine the effective and biologic half life of Co^{57} TPPS in humans, and its metabolism and excretion patterns. Forty-six patients with a variety of tumors and myocardial infarctions were scintiscanned after administration of adequate amounts of the labelled compound.

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[1]This investigation was supported in part by Grant #IN-14E from the American Cancer Society, Inc., and Public Health Service Training Fellowing #2G-127C6.

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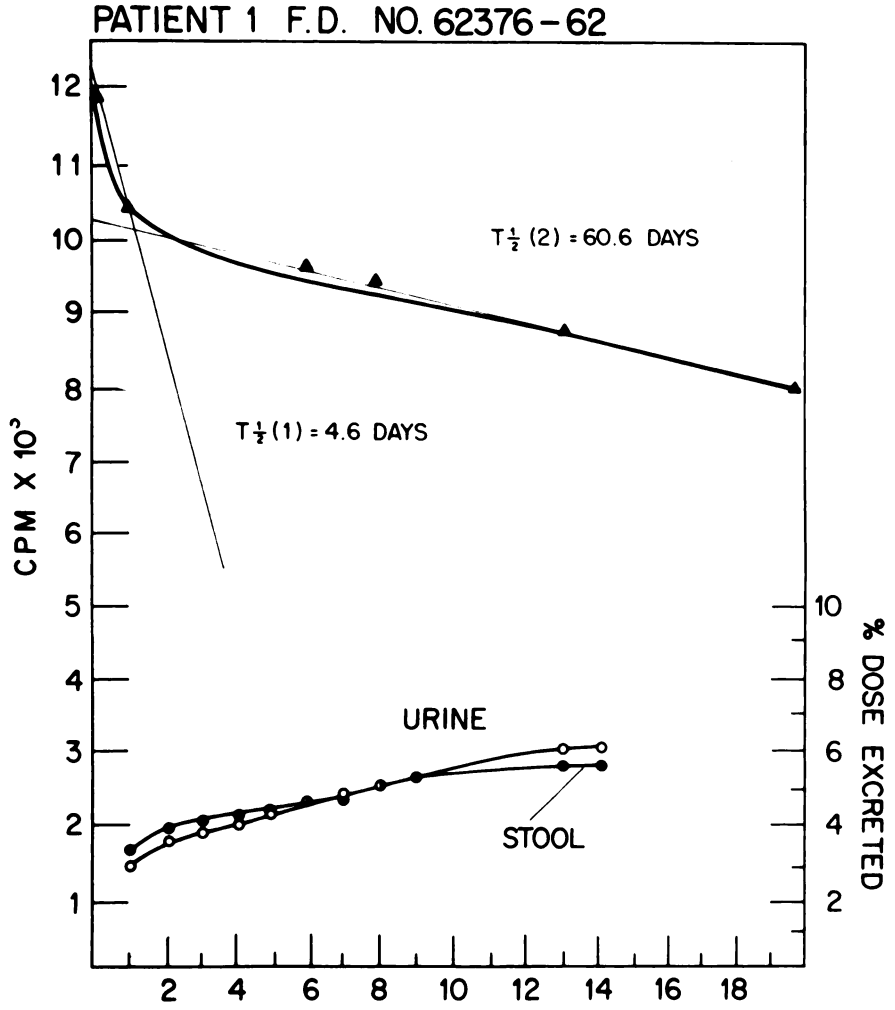
MATERIALS AND METHODS

Tetraphenylporphine (TPP) was prepared from pyrrole and benzaldehyde by the method of Rothemund and Menotti (4) and sulfonated by reaction with concentrated sulfuric acid. The chelation of Co^{57} was done by a modification of the method previously described (7) that eliminated elution from an alumina column, evaporation to dryness and a redissolving step. Twenty mgm of TPPS was dissolved in 1.0 ccH_2O to which 4 drops of concentrated ammonium hydroxide was added. Four cc of a 4:1 solution of concentrated acetic acid: saturated sodium acetate was added. Seventy-five millicuries of $Co^{57}Cl_2$[1] in 10.5 cc

[1]Obtained from Nuclear Science and Engineering Corporation, Pittsburgh, Pa.



Figs. 1-5. Total body counts and measurement in urine and stool of Co^{57} administered as Co^{57}TPPS. The extrapolated thin lines from the total body counting are of the effective half life of the first, T½ (1), and second, T½ (2) phases.

of 0.5N hydro-chloric acid was then added. The molar ratio of Co^{57} to TPPS was 1:625. The reaction volume was brought to 20.0 cc with water. The reaction flask was immersed in a boiling water bath for 60 minutes. This preparation was brought to pH 7.4, autoclaved, and administered to patients in aliquots diluted with physiologic saline.

The Co^{57} that had dissociated from the TPPS ligand was determined, at the time of use, by its elution from Dowex-2. The samples were placed on a 2 cm I. D. scintered glass filter column, containing 5.0 gm Dowex-2-× 8, 200-500 mesh, medium porosity, in the Cl^- form and eluted with 10 volumes of water. Dissociated Co^{57} was measured in the eluate. Of the Co^{57} TPPS used for injection, 95.2-100 per cent was undissociated. Urine and watery stool specimens could be placed directly on similar columns for the determination of dissociated Co^{57} in those specimens. Some stool specimens were dissolved in nitric acid, then diluted and neutralized before being placed in the column.

Co^{57} was counted in a Nuclear Chicago well-type scintillation spectrometer.

Scintillation scanning was done with the equipment in the Johns Hopkins Hospital Radioisotope Diagnostic Laboratory and the Bellevue Hospital Isotope Laboratory.

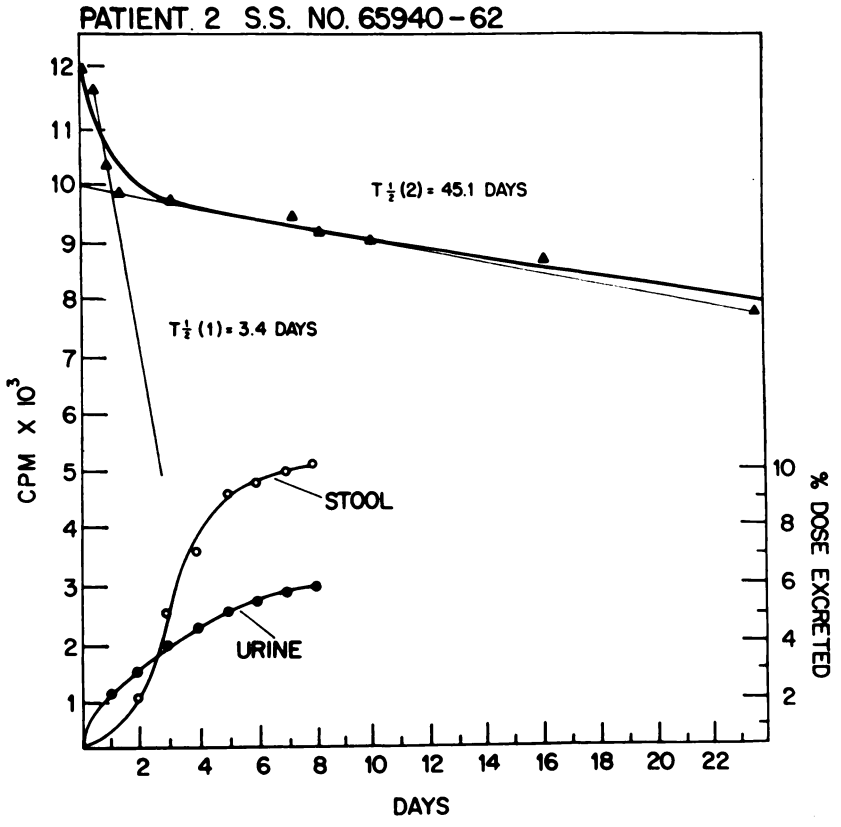


Fig. 2.

The New York University Total Body Counter, a low background gamma detecting facility made specifically for the measurement of radiation in humans, was utilized. It consists of a 6 inch steel walled room, measuring 5 × 7 × 6 feet. It contains an 8 × 4 inch NaI crystal whose output is connected through 5 photomultipliers to a 400 channel pulseheight spectrometer.

Five patients with inoperable malignant tumors were studied in detail, before or during a course of Co^{60} radiation therapy. Two had carcinoma of the lung, one of the bladder, one of the uterus and one of the esophagus. They had total body counting once or twice a day for the first few days, then periodically for between 14 and 28 days following the intravenous administration of 1.0 or 10.0 μc Co^{57} TPPS. The total amount of Co^{57} excreted in the urine and stool, and its separation into free and TPPS bound forms were determined on daily specimens.

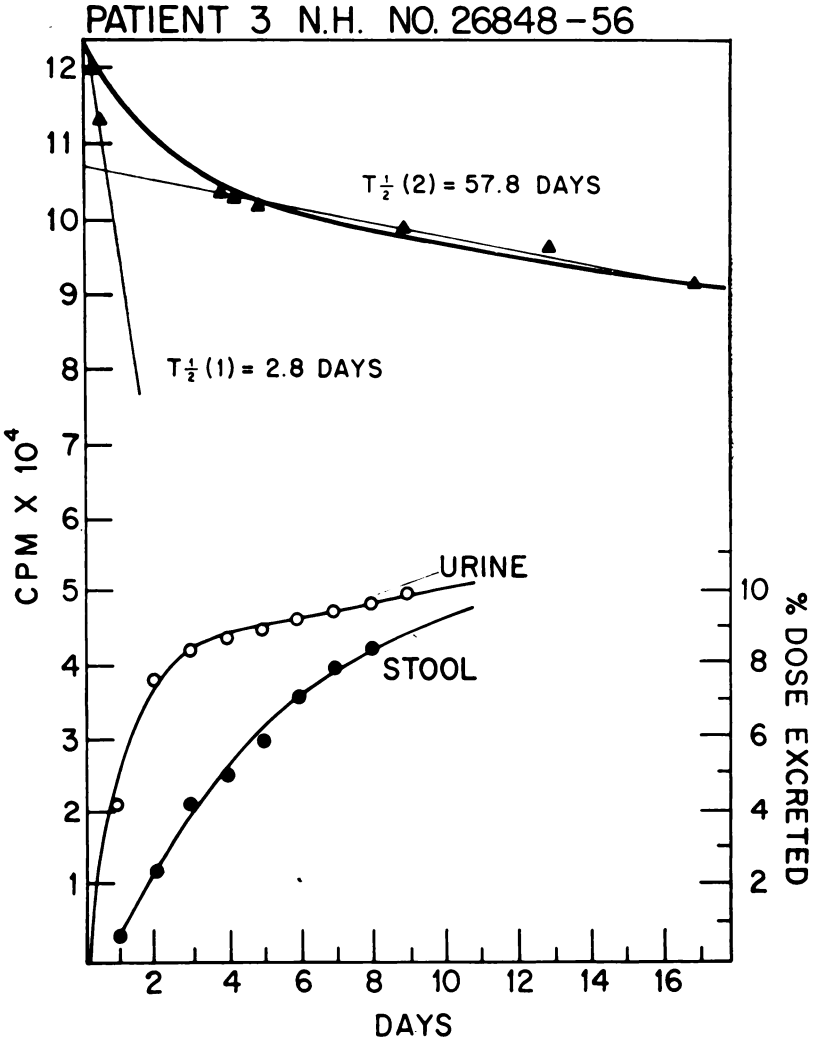


Fig. 3.

A series of 46 patients with malignant cerebral and visceral tumors and other conditions received between 100 and 500 μc intravenously for scinti-scanning at varying intervals following injection.

RESULTS

Figures 1-5 summarize some results with the 5 patients who had total body counting and Co^{57} excretion studies. The total body counter provided a reliable measure of the effective half life [T(½)] of the radioisotope. There was an apparent diphasic excretion. The effective T(½) for the first phase varied from

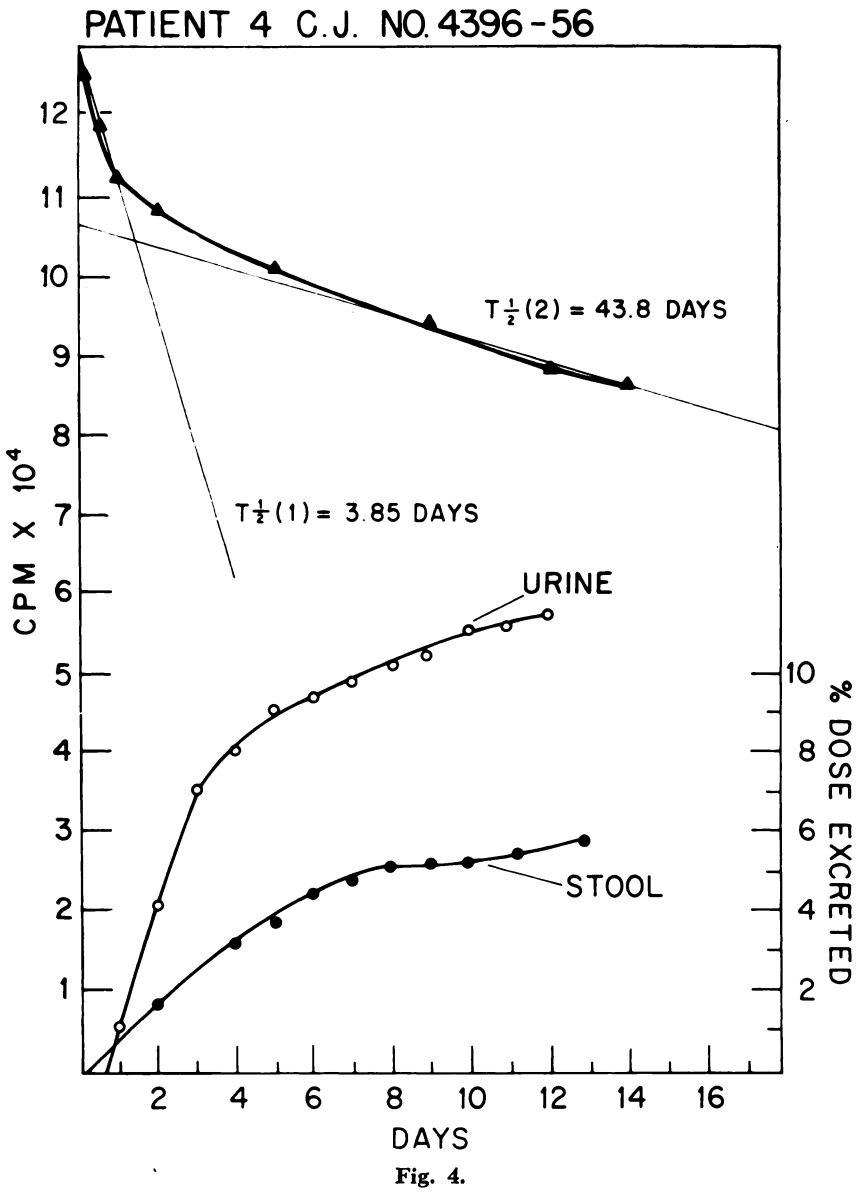


Fig. 4.

2.8 to 5.0 days equivalent to a biologic T(½) of 2.82 to 5.1 days. The effective T(½) for the second phase varied from 43.8 to 60.6 days equivalent to a biologic T(½) of 47.3 to 77.3 days. These extrapolated values are indicated by the thin lines in the figures. The pattern of excretion was similar in the urine and stool, although the cumulative recovered Co^{57} was only about one-half that excreted as indicated by total body counting. This discrepancy was probably due to collection losses. Geometrical factors in total body counting, and in urine and stool measurements were carefully controlled.

The proportion of Co^{57} dissociated from TPPS in the urine and stool of these patients is shown in Table I. There was considerable variation between patients and in one patient from day to day. More of the Co^{57} was found in the dissociated form in the urine than in the stool. The total amount excreted in the urine was generally greater than that in stool. The total amount of Co^{57} TPPS that was dissociated prior to excretion approaches 40 per cent.

There was no correlation between the dissociation of Co^{57} TPPS and the two phases of excretion. The proportion of free Co^{57} in the urine and stool was approximately constant during the period of observation.

Two patients received 10 μc Co^{57} TPPS by mouth at the completion of their other studies. The percent dissociation of the Co^{57} in the stool was the same as in the orally administered doses, less than 5%, indicating that Co^{57}TPPS is not further dissociated during passage through the gastrointestinal tract.

Thirty patients with suspected cerebral tumors were studied by scintillation scanning (using an intravenous dose of 5 μc/kg body weight.) The scans at 24 hours were similar in appearance but not superior to those obtained using I^{131} albumin. Slightly better definition was achieved at 48 hours. In one instance, a large frontal glioma with a slow rate of growth showed a much higher tumor-to-brain count rate ratio at one week than with either I^{131} albumin or Hg-203 chlormerodrin. In another patient, an area of focal encephalomalacia concentrated the isotope and was sharply delineated at 48 hours. Ten patients with tumors of the lung, breast, stomach, colon, bone and soft tissue of an extremity received 200 μc. of Co^{57} TPPS, but no tumor localization was seen by scanning. High concentrations were regularly detected in the liver. Six patients with myocardial infarction failed to show localization of the radioactivity in the precordium.

DISCUSSION

The failure to find localization of Co^{57} adequate for scintilation scanning in the extracerebral tumors of these patients could be due to the dissociation of Co^{57} from TPPS and its distribution as the free cobaltous ion, or to a different tissue distribution of the Co^{57} chelate of TPPS than of TPPS in the free base form.

A comparison of the tissue concentration of TPPS after its administration and of Co^{57} after intravenous Co^{57} TPPS administration to tumor bearing rats and mice, revealed several significant differences. The Walker 256 carcinosarcoma attained a TPPS concentration about 4 times greater than all other organs of the tumor bearing rat. However, the concentration of Co^{57} in the tumors of Walker 256 carcinosarcoma bearing rats and ependymoma bearing mice was only ⅓ to ½ that in the liver and ½ to ⅔ that in kidney (7). In the ependymoma bearing

mouse, intravenously administered cobaltous $Co^{57}Cl_2$ attained a tumor concentration ⅛ that of liver and ¼ that of kidney (4). The tissue concentrations of Co^{57} after Co^{57}TPPS administration were between those of TPPS alone and Co^{57} alone. This suggests partial dissociation of the Co^{57}TPPS complex and independent localization of Co^{57} and Co^{57}TPPS although the observed disparity cannot be entirely accounted for on that basis. The direct measurement of the porphyrin component of the Co^{57}TPPS complex in the tissues was not possible because of the extremely small amounts. The findings of considerable Co^{57}free of the TPPS ligand in the excreta indicate that partial dissociation of the complex does occur *in vivo*. This is consistent with the suggestion made by Bases, Brodie and Rubenfeld, that Cu^{64} labelled naturally occurring porphyrins were dissociated in the liver, presumably by the same mechanism that converts heme to bilirubin (1). Such a mechanism would also explain the higher proportion of dissociated Co^{57} in the urine than in the stool. Free Co^{57} is much less tightly bound to serum protein than Co^{57}TPPS. Cobaltous Co^{57} is excreted mainly through the kidney. The tighter protein binding of the porphyrin bound Co^{57} of Co^{57}TPPS may contribute to its tendency to localize in some tumors, similar to the localization of the protein-bound radioisotopes RISA and I^{131} cholografin. Another factor that may be responsible for the smaller relative concentration of label in the tumor is slower catabolism of TPPS by tumor compared to the liver, kidney and other organs. Relatively rapid degradation of Co^{57}TPPS could lead to considerable accumulations of Co^{57} and little of intact TPPS in liver, for example. In the tumor, on the other hand, slow degradation would lead to relatively greater concentrations of TPPS molecules compared to Co^{57}. The spectrophotometric and fluorometric assays used to determine the 4:1 tumor: liver concentrations of TPPS (5) measured only the intact TPPS molecules in those tissues.

Finally, the incorporation of Co^{57} into TPPS may have altered the pattern of localization to some extent. The uniquely high concentration of porphyrins in tumor, compared to other tissues, the intracellular localization of both hematoporphyrin (6) and TPPS (9) in the cytoplasm of the tumor cells, and the unusually strong binding of hematoporphyrin to a cytoplasmic protein (6), suggest that some specific cellular process or material contributes to the accumulation

TABLE I

PER CENT DISSOCIATION OF Co^{57} FROM TPPS

Patient	*Number of Daily Samples*	*Urine*		*Stool*	
		Mean	*Range*	*Mean*	*Range*
1	9	36.8	26–48	28.2	23–49
2	8	51.2	36–79	10.0	8–13
3	14	57.3	48–75	30.7	2–39
4	14	47.6	37–56	25.3	19–34
5	13	55.2	42–69	28.0	18–40

of porphyrin in tumors. The results of this study indicate that the exploitation of this property will require the synthesis of a derivative with the label either incorporated directly into the TPPS molecule or in a more stable bond than that of the Co^{57} chelate.

On a clinical basis, Co^{57}TPPS may prove useful in selected cases. The definition of cerebral tumors by scintillation scanning at 72 or more hours is excellent. Consequently, this agent may succeed in demonstrating some gliomas that are not infrequently missed with the conventional agents, I^{131} albumin and Hg^{203} chlormerodrin. The detection of breakdown of the blood-brain barrier in small foci, such as in encephalomalacia, may sometimes be desirable. It is interesting to note that Co^{57}TPPS did not localize in myocardial infarctions as had the protein-bound Hg^{203} chlormerodrin reported by Carr *et al.* (2).

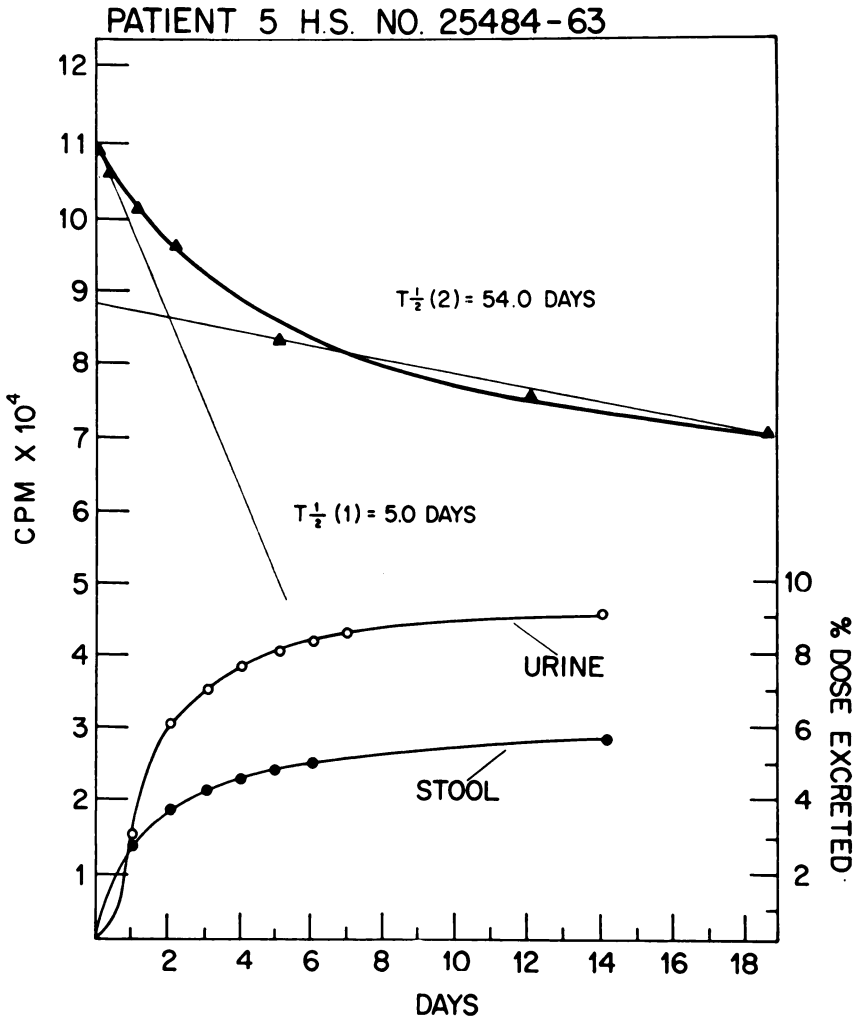


Fig. 5.

SUMMARY

Co^{57} tetraphenylporphinesulfonate was administered to patients with malignant tumors. Its effective and biologic half life, pattern of excretion in urine and stool, and *in vivo* dissociation was determined. Forty-six patients with cerebral and visceral tumors and myocardial infarctions were studied by scintillation scanning. Cerebral tumors were successfully demonstrated. Tumors in other locations, however, were not delineated. It was found that approximately 40% of the Co^{57} was dissociated. The failure of the radioisotope to localize in the tumors to the same extent as TPPS is explained at least partly by this dissociation.

REFERENCES

1. Bases, R., Brodie, S. S., Rubenfeld, S.: Attempts at Tumor Localization Using Cu^{64} labelled Copper Porphyrins. *Cancer* **11**:259, 1958.
2. Carr, E. A. Jr., Beierwaltes, W. H., Patno, M. E., Bartlett, J. D. Jr., and Wegst, A. V.: The Detection of Experimental Myocardial Infarcts by Photoscanning. A Preliminary Report, *Am. Heart J.* **64**:650, 1962.
3. Long, R. G., McAfee, J. G., Winkelman, J.: Evaluation of Radioactive Compounds for the External Detection of Cerebral Tumors. *Cancer Res.* **23, 1,** 98, 1963.
4. Rothemund, P., Menotti, A. R.: Porphyrin Studies IV. The Synthesis of Alpha, Beta, Delta, Gamma. Tetraphenylporphine. *J. Am. Chem. Soc.* **63**:267, 1941.
5. Winkelman, James: The Distribution of Tetraphenylporphinesulfonate in the Tumor-bearing Rat. *Cancer Res.* 22, **5**:589, 1962.
6. Winkelman, James: Intracellular Localization of "Hematoporphyrin" in a transplanted Tumor. *J. Nat. Cancer Inst.* **27**:1369, 1961.
7. Winkelman, James, McAfee, J. G., Wagner, J. N. Jr., Long, R. G.: The Synthesis of Co^{57} Tetraphenylporphinesulfonate and Its Use in the Scintillation Scanning of Neoplasms. *J. Nucl. Med.* **3**:249, 1962.
8. Winkelman, James and Rasmussen-Taxdal, D. S.: Quantitative Determination of Porphyrin Uptake by Tumor Tissue Following Parenteral Administration. *Bull. Johns Hopkins Hosp.* **107**:228, 1960.
9. Winkelman, James, Spicer, S. S.: Staining with Tetraphenylporphinesulfonate *In Vivo*. *Stain. Tech.* **37**:303, 1962.