JOURNAL OF NUCLEAR MEDICINE 5:920-928, 1964

The Uptake of Differentially Coupled ^{74}As-Arsano-Poly-L-Lysine in Tumor-Bearing Mouse Tissues[1]

John L. Mego, Ph.D. and J. Donald McQueen, M.D.[2]

Baltimore

In an experimental evaluation of scanning agents, Long *et al* noted that high tumor-to-brain ratios were obtained with substances which tended to become bound by proteins in the plasma, and that the highest concentrations in tumor were obtained with radioiodinated albumin (1). This macromolecule and polyvinylpyrrolidone-^{131}I (2) have been popular scanning agents, although the persistently high blood levels and long biological half-life are major disadvantages. We have studied the localization of macromolecules, coupled with ^{74}As-arsanilic acid, in tumor and other tissues (3,4). These materials are not suitable for clinical scanning because of prolonged retention in the liver and kidneys, although the tumor levels were approximately as high as those obtained with ^{131}I-albumin (3).

In an evaluation of the distribution of intravenously injected ^{74}As-arsonoazo-albumin in tumor-bearing mice, it was noted that the tissue levels varied with the amount of ^{74}As-arsanilate coupled per molecule of protein. In a similar study, ^{74}As-arsanilate was coupled to poly-L-lysine and injected into tumor-bearing mice (4). During the course of this work, it was also noted that inconsistent results were obtained if the ratio of arsanilate to lysine was varied. In

[1]Supported by Contract AT (30-1) 2182, U. S. Atomic Energy Commission, and Grant No. C-3265, National Cancer Institute, National Institutes of Health. Part of the material in this paper was presented at the American Chemical Society, Division of Biological Chemistry, 146th National Meeting, Denver, Colorado, January 19-24, 1964.

[2]Division of Neurological Surgery, Baltimore City Hospitals, and The Johns Hopkins University School of Medicine.

view of the differences in uptake by tissues according to the method of preparation of these materials, it was considered worth while to carry out a detailed study of the effect of adding varying quanties of ^{74}As-arsanilate to poly-L-lysine in order to ascertain if some consistent pattern could be detected which could be of value in the preparation and evaluation of other macromolecular scanning agents or similar materials.

MATERIALS AND METHODS

Poly-L-lysine hydrochloride of molecular weight 210,000 was obtained from Mann Research Laboratories and used throughout this study. ^{74}As-arsanilic acid was synthesized and coupled to polylysine by diazotization. Details of the synthesis and coupling procedures have been reported elsewhere (3, 4).

Diazotized ^{74}As-arsanilate in amounts ranging from 60 to 150 μmoles with specific activities of 300,000 to 75,000 cpm/μmole, was added to 6 samples of 5 mg (30 μmoles lysine moiety) polylysine dissolved in 1 ml 0.5 M, pH 10, carbonate buffer. The pH was carefully maintained at around 10 with small amounts of normal NaOH during the addition of diazotate. Coupling was allowed to take place overnight, and the solutions were then transferred to dialysis bags and dialyzed against 0.1 M NaCl with 5×10^{-3} M phosphate, pH 7, for at least five days with daily changes of the dialyzing solution. All procedures were carried out at 4° C. After dialysis, the solutions were quantitatively removed from the dialysis bags and the volumes measured. A 0.1 ml sample of each preparation was counted in a well counter and the amount of arsanilate coupled per 30 lysines (5 mg polylysine) was calculated from the specific activity of the ^{74}As-arsanilate. All counts were corrected for background and decay. A typical preparation of ^{74}As-arsono-polylysine solutions is illustrated in the Table. In this Table, the

TABLE

THE PREPARATION OF DIFFERENTIALLY COUPLED ^{74}AS-ARSONO-POLYLYSINE

Sample	*μmoles diazotate added to 5 mg polylys***	*Sp. act. of diazotate** (*cpm/μmole*)	*Total cpm after dialysis*	*μmoles arsanilate incorp per 30 lysines***
1.	60	226,284	2,459,445	11
2.	75	226,284	2,912,846	13
3.	90	113,142	2,082,697	18
4.	105	113,142	2,208,161	20
5.	120	56,571	1,479,918	26
6.	150	56,571	1,745,242	31

*Corrected for decay during 5 days dialysis.

**5 mg polylysine is equivalent to 30 μmoles lysine moieties.

quantity of diazotized ^{74}As-arsanilate added to 5 mg (30 lysines) polylysine is shown in the second column. The specific activity, in counts per minute per μmole, of the added diazotate is shown in the third column. After dialysis, the solutions were quantitatively removed from the dialysis bags, measured, and a sample was counted. The total counts in the solution were calculated, and this number (fourth column) was divided by the specific activity (corrected for decay during the dialysis period) which resulted in the figure in the last column representing the number of μmoles arsanilate coupled per 30 μmoles lysine moieties.

0.1 ml containing 0.3 mg ^{74}As-arsono-polylysine was injected into the tail veins of C-57 mice. The tumor used in this study was a chemically-induced Zimmerman ependymoblastoma. The removal of tissues for counting, and other procedures were the same as those described previously (3). The total number of animals was 115, with 3 to 8 in each group.

RESULTS

Arsono-polylysine preparations containing less than 8 arsanilate groups per 30 lysine residues were extremely toxic when injected intravenously into mice. With 8 to 10 arsanilate groups, the material precipitated during dialysis (possibly a charge effect similar to the isoelectric precipitation of proteins). There was no evidence of toxicity with preparations containing more than 10 arsanilate groups.

Figure 1 shows the disappearance of ^{74}As-arsono-polylysine from the blood stream of tumor-bearing mice. Rapid clearance was found with the use of material containing 11 to 13 arsanilate groups (per 30 lysine residues). The blood levels remained higher with greater coupling ratios. There was some suggestion of decreasing clearance rates with each added increment of arsanilate although this was not clearly indicated with preparations containing more than 14 to 16 arsanilate groups. The clearance rates thus appeared to vary inversely with the coupling ratios which is opposite to the findings with differentially-coupled arsono-albumin. The blood levels with highly coupled arsono-polylysine were similar to those found with lightly coupled arsono-albumin (3).

The levels in tumor, shown in Fig. 2, rose when the number of conjugated arsanilate groups was increased above 11 to 13 and were correlated with the blood levels. The change in uptake with over 14 to 16 groups was not extensive. In terms of per cent of injected dose, the uptake at four hours of the preparation with 24 to 31 conjugated arsanilate groups was equal to that obtained with mouse serum albumin containing 1 to 2 arsanilate groups (3). There was a progressive increase in uptake with time with all preparations except the lightly coupled material (11 to 13 groups) which was also removed from the blood very rapidly.

The levels in the liver were correlated with clearance rates from the blood stream; the preparations which were most rapidly cleared from the blood were the ones which became most highly concentrated in the liver. As with the clearance rates from the blood stream, there was not a clear difference in uptake of the

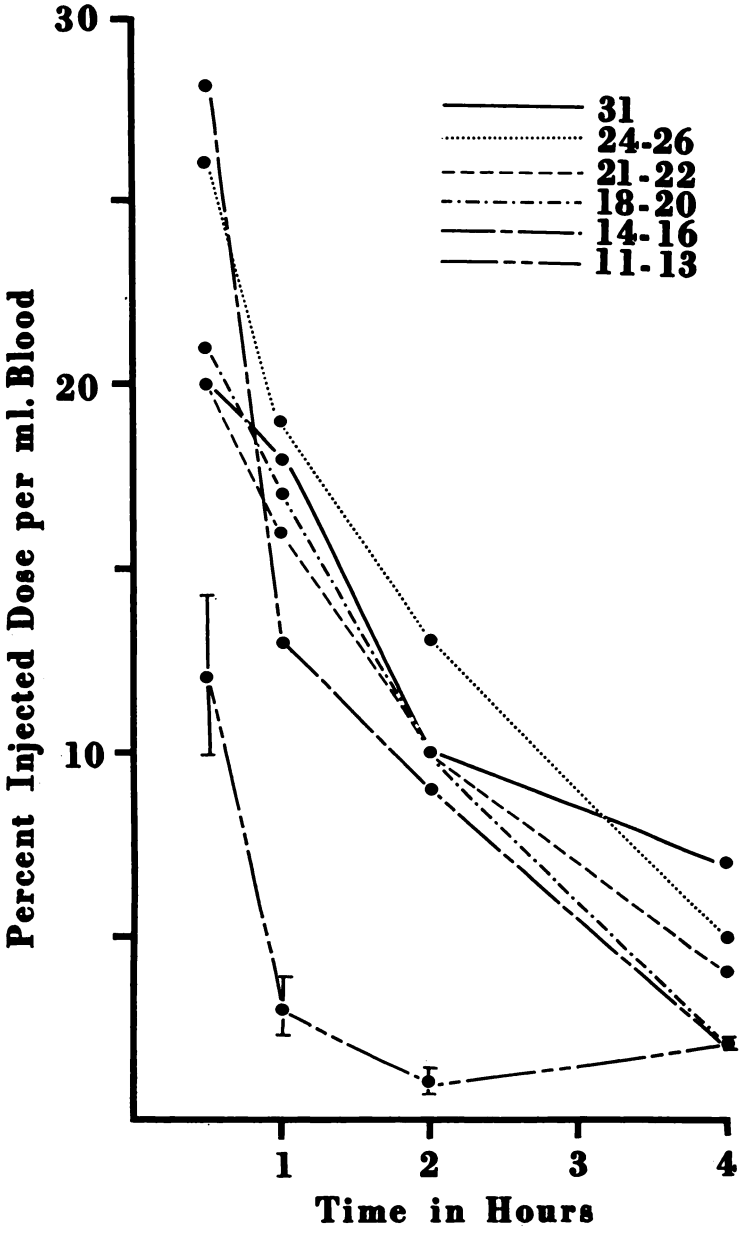

Fig. 1. Disappearance of ^{74}As-arsano-poly-L-lysine from the blood stream of tumor-bearing mice. Number of coupled arsanilate groups per 30 lysine moieties is indicated. Results in this and following figures are expressed as per cent of injected dose per ml of blood (or g of tissue) corrected to a 25 g mouse. 0.3 mg. ^{74}As-arsano-poly-L-lysine were injected intravenously and mice were sacrificed at indicated times.

preparations with greater than 14 to 16 arsanilates except at the four hour period. In general, the retention in liver was much the same as has been noted with various azoproteins (6-8).

The levels in kidney also varied inversely with the number of conjugated arsanilate groups (Fig. 4). These values were of particular interest since they demonstrated a graded effect which was not definite in blood clearance nor in liver or tumor levels. This apparent specificity was also found with azoalbumin (3). A constancy was noted in the values in each group in the interval between one-half and four hours which was quite different from the progressive rise in liver uptake.

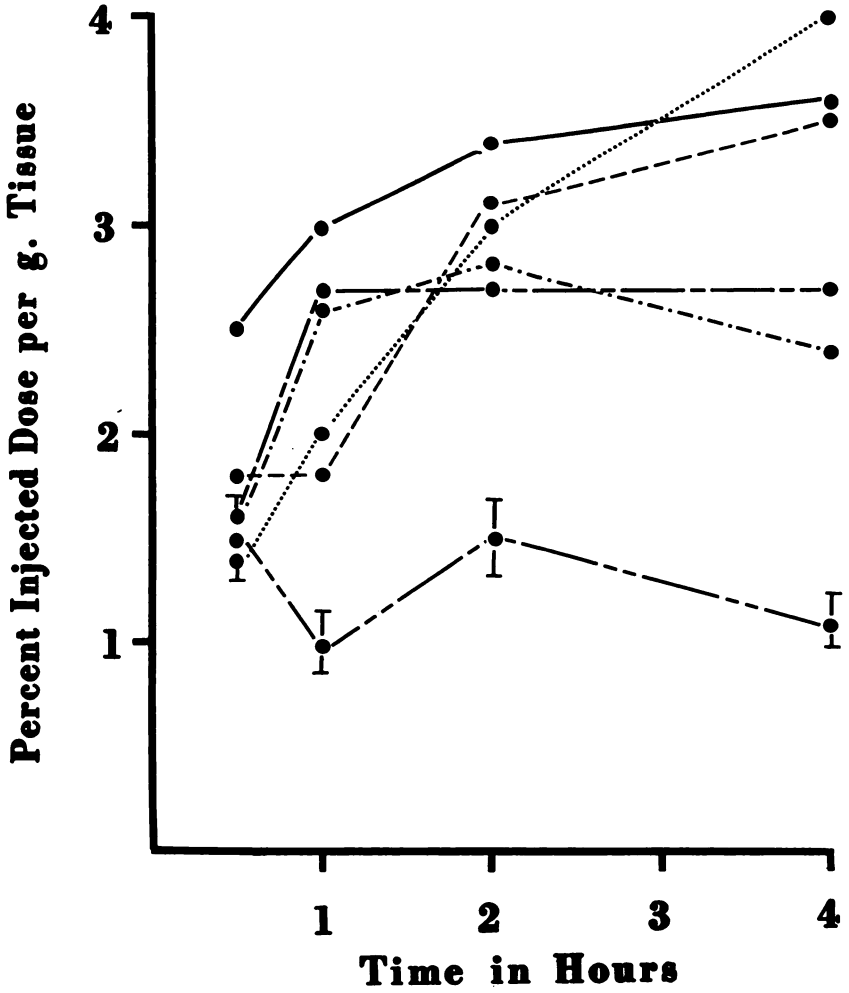

Fig. 2. Uptake of intravenously injected ^{74}As-arsano-poly-L-lysine in tumor (chemically-induced ependymoblastoma).

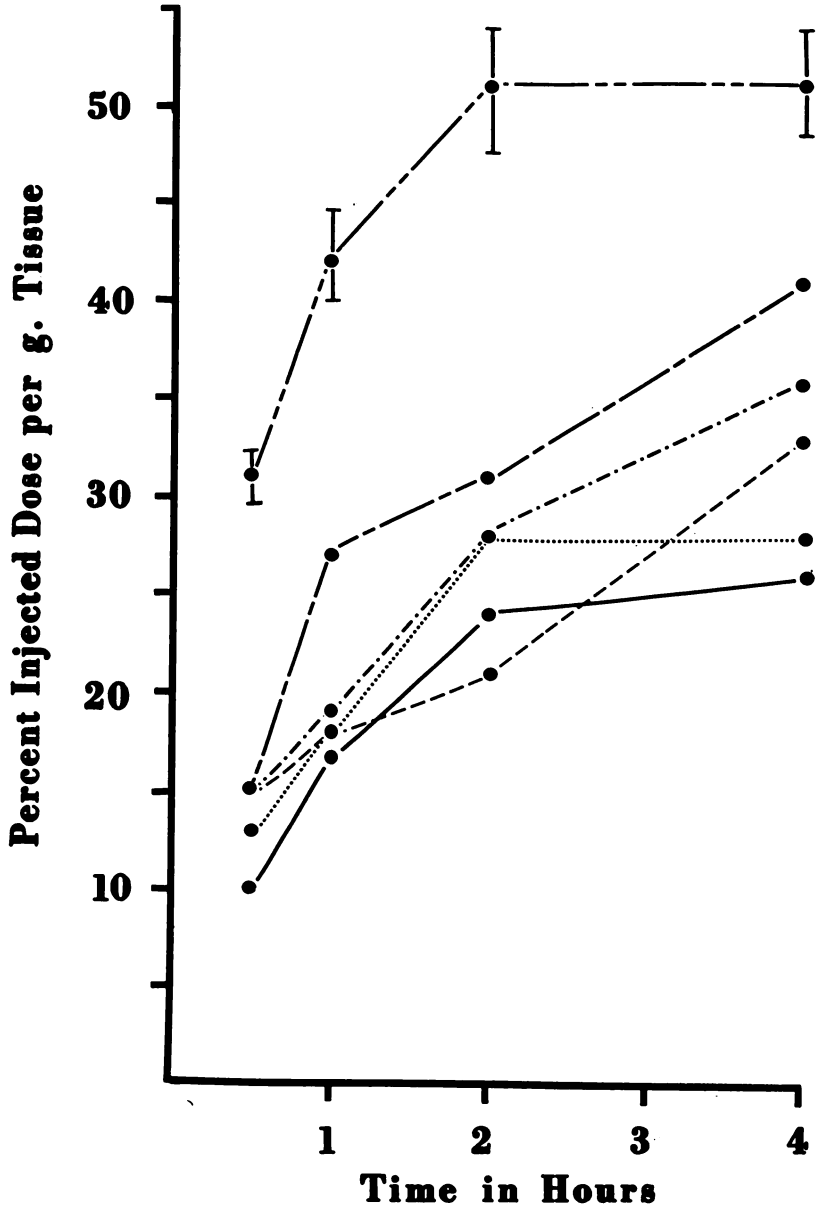

Fig. 3. Uptake of intravenously injected ^{74}As-arsano-poly-L-lysine in silver.

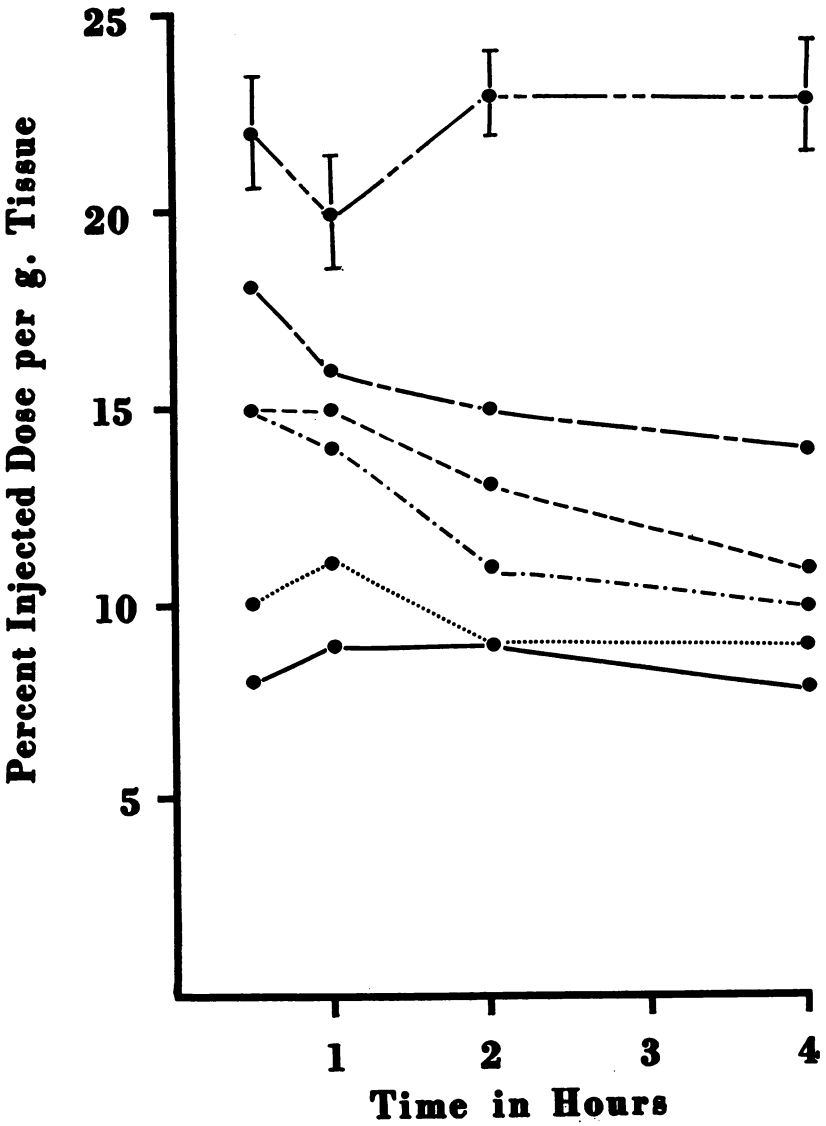

Fig. 4. Uptake of intravenously injected ^{74}As-arsano-poly-L-lysine in kidney.

DISCUSSION

In this study some striking differences were noted in the uptake of ^{74}As-arsono-poly-L-lysine in tissues of tumor-bearing mice which varied according to the ratio of arsanilate to free lysine groups in the final preparation. There appeared to be some consistent patterns which can be evaluated from these results. The uptake in tumor was directly related to the concentration and persistence of radioactivity in the blood stream which, in turn, depended on the rate and extent of uptake by the liver and kidneys. The relatively high uptake of ^{131}I-albumin (1) and ^{14}C-albumin (5) by tumors is also consistent with this observation since these materials are also retained in the circulation for prolonged periods with comparatively little uptake by kidneys and liver.

The preparations which were most rapidly removed from the circulation were those with the lowest ratio of arsanilate-to-lysine groups. In a similar study with ^{74}As-arsonoazo-albumin (3) the results were different in that the highest clearance rates were obtained with the more heavily coupled material. Our results with ^{74}As-arsono-poly-L-lysine do not permit any general theory concerning the mechanisms involved which would explain the variation in uptake patterns. However, poly-L-lysine is a highly basic material, and the addition of the negatively charged arsenate groups would tend to make the polylysine molecules more neutral or negative in net charge. Further work on this problem is in progress in this laboratory at the present time.

SUMMARY AND CONCLUSIONS

^{74}As-arsanilate was coupled to poly-L-lysine with various ratios of arsanilate to lysine groups in the reactions. The resulting compounds were dialyzed and injected into tumor-bearing mice; and the concentrations were measured in blood, tumor, kidney and liver up to four hours after injection. Lightly coupled preparations (11 to 13 arsanilate groups per 30 lysine residues) were most rapidly cleared from the blood stream and showed the highest uptake by liver and kidneys with relatively low concentrations in tumor. Increasing the number of arsanilate groups resulted in greater uptake by tumor and less rapid clearance from the blood stream with a corresponding decrease in uptake by kidneys and liver. The uptake into tumor, therefore, was directly related to the concentration in the blood stream. This finding appears to be of value in the preparation of potential scanning agents, macromolecular chemotherapeutic agents and similar materials.

ACKNOWLEDGEMENTS

We are greatly indebted to Mr. Harry Page for technical assistance.

REFERENCES

1. LONG, R. G.; MCAFEE, J. G. AND WINKELMAN, J.: Evaluation of Radioactive Compounds for the External Detection of Cerebral Tumors. *Cancer Res.* **23**:98, 1963.
2. PITLYK, P. J.; TAUXE, W. N.; KERR, F. W. L.; SEDLACK, R. E. AND SVIEN, H. J.: Location of Brain Tumors with Polyvinylpyrrolidone ^{131}I, *Archs. Neurol. Chicago* **9**:437, 1963.
3. MEGO, J. L. AND MCQUEEN, J. D.: The Uptake of As^{74}-labeled Arsonoazoproteins in Tissues of Tumor-bearing Mice. *Cancer Res.* **23**:523, 1963.
4. MCQUEEN, J. D. AND MEGO, J. L.: An Experimental Study of Tissue Concentrations Ratios of As^{74}-Arsenate and Labeled Organic Conjugates. *J. Neurosurg.* **21**:641, 1964.
5. BUSCH, H.; SIMBONIS, S.; ANDERSON, D. C. AND GREENE, H. S. N.: Studies of the Metabolism of Plasma Proteins in Tumor-bearing Rats. II. Labeling of Intracellular Particulates of Tissues by Radioactive Albumin and Globulin. *Yale J. Biol. Med.* **29**:105, 1956.
6. GITLIN, D.; LANDING, B. H. AND WHIPPLE, A.: Composition of Mitochondrial Preparations in Relation to Intracellular Localization of Azoprotein in the Mouse. *Proc. Soc. Exp. Biol. Med.* **78**:631, 1951.
7. KRUSE, H. AND MCMASTER, P. D.: The Distribution and Storage of Blue Antigenic Azoproteins in the Tissues of Mice. *J. Exper. Med.* **90**:425, 1949.
8. SABIN, F. R.: Cellular Reactions to a Dye-protein with a Concept of the Mechanism of Antibody Formation. *J. Exp. Med.*, **70**:67, 1939.