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UPTAKE AND DISTRIBUTION OF TECHNETIUM IN SEVERAL MARINE ALGAE

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ABSTRACT

The uptake or chemical form of technetium in different marine algae (Acetabularia, Cystoseira, Fucus) has been examined and a simple model to explain the uptake of technetium in the unicellular alga, Acetabularia, has been conceptualized. At low concentrations in the external medium, Acetabularia can rapidly concentrate technetium. Concentration factors in excess of 400 can be attained after a time of about 3 weeks. At higher mass concentrations in the medium, uptake of technetium by Acetabularia becomes saturated resulting in a decreased concentration factor (approximately 10 after 4 weeks). Approximately 69% of the total radioactivity present in ^{95m}Tc labelled Acetabularia is found in the cell cytosol. In Fucus vesiculosus, labelled with ^{95m}Tc, a high percentage of technetium is present in soluble ionic forms while approximately 40% is bound, in this brown alga, in proteins and polysaccharides associated with cell walls. In the algal cytosol of Fucus vesiculosus, about 45% of the ^{95m}Tc appears to be present as anionic TcO_4^- and the remainder is bound to small molecules.

1. INTRODUCTION

Technetium-99 is a long-lived radionuclide (half-life 2.1 x 10^5 years) produced during nuclear fission. Irradiated fuel from pressurized water reactors, as well as other types of nuclear reactors, contains Tc-99 which may be released to aquatic environments during nuclear fuel reprocessing or the disposal of aqueous wastes from other nuclear facilities. For example, Tc-99 has been detected in samples of algae and marine biota collected in the vicinity of a discharge area for low level radioactive liquid effluents from a nuclear fuel reprocessing plant at La Hague, France (Jeanmaire et al., 1981). In this case, Tc-99 has been measured in low concentrations in brown algae (Fucus) at distances greater than 100 km from the discharge at La Hague (Masson et al., 1983) confirming earlier suggestions that brown algae are a good indicator species for Tc-99 present in the marine environment (Jeanmaire et al., 1981).

Algae are important intermediaries in the marine food chain leading from aquatic contamination to people. Since algae contain substances which can potentially bind radionuclides and since they have a pronounced ability to accumulate heavy metals, which differs ~~between~~ various species, algae represent useful models to study the general characteristics of uptake and release and can under certain conditions serve as indicators of radioactive contamination. In the present study, the uptake of technetium by different marine algae was investigated and it was attempted to model its behavior in the giant unicellular alga, Acetabularia. In certain respects, Acetabularia may not be typical of other marine green algae since it concentrates technetium more than could be expected based on prior studies (Jeanmaire et al., 1981 ; Fisher, 1982). However, the physiology and metabolism of Acetabularia is already well known and thus the behavior of technetium can be more easily modelled in this organism. We have also characterized the chemical form of technetium in a marine brown alga, Fucus vesiculosus, because of the apparent high affinity of this genera for technetium in seawater (Masson et al., 1981).

2. MATERIAL AND METHODS

The unicellular green alga Acetabularia acetabulum (= mediterranea) was cultivated, under sterile conditions, in the laboratory (Lateur and Bonotto, 1973). The brown macroalgae Fucus vesiculosus and Cystoseira compressa were collected along the coast of the North Sea and the Mediterranean, respectively. The plants were kept at 21°C in sea water with addition of chloremphenicol (10 µg/ml) to inhibit bacterial growth.

The intracellular distribution of technetium in ^{95m}Tc labelled Acetabularia was determined by homogenizing washed cells in the culture medium and then subjecting the homogenate to differential centrifugation according to the methods of Shephard and Levin (1972). The chemical form of technetium in ^{95m}Tc contaminated Fucus vesiculosus was characterized in both fresh (frozen) and freeze-dried plant material by chemical extraction, extractions with artificial seawater, differential centrifugation, and gel permeation chromatography. First, samples of brown algae were successively extracted with various chemical treatments according to the methods of Bowen et al. (1962). Next, Fucus vesiculosus fronds were extracted with artificial seawater (30 g sea salt/liter), after thawing, to determine the amount of Tc-95m in the plant material that was removable by three successive overnight extractions at 4°C.

Samples of fresh (frozen) and freeze-dried Fucus vesiculosus, labelled with ^{95m}Tc, were also homogenized at 4°C in 0.2 M tris-HCl buffer (pH 7.2), containing 0.5 M sucrose and 0.01 M $MgCl_2$, then filtered through cheesecloth, and centrifuged. Cell debris, nuclei, plastids, and mitochondria were collected at 10,000 g for 20 minutes and the microsomal pellet was separated from the "postmicrosomal supernatant" by centrifugation at 100,000 g for 70 minutes. The chemical form of ^{95m}Tc in the postmicrosomal supernatant (cytosol) was chromatographed using Sephadex G-50 and G-25. The column dimensions were 79 x 2.6 cm for the G-50 and 95 x 2.6 cm for the G-25. Both columns were equilibrated with the elutant (0.02 M tris-HCl buffer, pH 7.2) and housed in a refrigerated room at 4°C. The flow rate was approximately 0.4 ml/minute for all fractionations. Elutant from the columns was monitored continuously for UV absorbance at 280 nm and fractions were collected by an automatic sample collector. The amount of ^{95m}Tc in the various fractions was analyzed by gamma spectrometry.

3. RESULTS AND DISCUSSION

When technetium of high specific activity (2 nCi/ml ^{95m}Tc or 90 fg/ml) is added to the culture medium containing Acetabularia, some technetium, corresponding to 78 ± 4 nCi (3.5 pg) is taken up almost instantaneously. Subsequent to this, the uptake is linear with time for a period of 18 days (16 ± 0.4 times the concentration of the surrounding medium per day) (Fig.1). Under these conditions ^{95m}Tc in the culture medium is soon exhausted and has to be replenished at regular intervals in order to ensure a continuous rate of uptake. Corresponding to the initial rapid uptake by Acetabularia, the radioactivity initially added to the culture medium disappears at first with a half-life of about 3 hours. Later, and for the following additions, the half-life of ^{95m}Tc in the medium is 3.70 ± 0.23 days. Data in the figures are usually presented as the ratio of concentration in the cell to the concentration in the medium and these concentration ratios, as shown in fig.1, have been corrected for the changing activity in the culture medium.

Labelled Acetabularia transferred to a non-radioactive medium looses about 80 nCi/g (4 pg) rapidly with a half-life of 1.8 ± 0.2 hours. This amount corresponds approximately to that initially taken up. However, the residual ^{95m}Tc, which is approximately 55% of the starting concentration, is tenaciously retained and apparently is partially lost only when the vitality of the algae in the culture diminishes after 10 to 14 days.

When higher mass concentrations of technetium are used, uptake by Acetabularia becomes saturated. This is evident when comparing low concentrations of ^{95m}Tc (0.1 nCi/ml or 4.5 fg/ml) with much higher concentrations of ^{99}Tc (1 nCi/ml or 59 ng/ml). In the case of ^{95m}Tc, the maximal concentration ratio is greater than 300, but with ^{99}Tc, the maximal concentration ratio is only 9.7 ± 0.7 after approximately 30 days (Fig.2).

The intracellular distribution of total radioactivity in ^{95m}Tc labelled Acetabularia, as determined by differential centrifugation, shows that most

(68.8 %) of the technetium is present in the cell cytosol. Next in importance are the chloroplasts which contain 26.6% of the total ^{95m}Tc in the cell. Only a small part of the total technetium in Acetabularia is associated with the cell wall material (3.6%) and the mitochondria (0.6%). Most of the technetium taken up by Acetabularia enters directly into the cell and is not fixed by the cell walls.

From these data, a model for the uptake of technetium by Acetabularia has been devised (Fig.3). Three types of binding seem to exist in this alga. A small part of the available technetium is very rapidly bound and released, but the binding mechanism is quickly saturated at very low concentrations of technetium (4 pg or 80 nCi of ^{95m}Tc). The second mechanism of binding also has a high affinity for technetium and seems essentially irreversible. Thereby, concentration ratios in excess of 400 can be attained after a time of 2 to 3 weeks. When concentrations of technetium exceed 600 ng (about 10 nCi ^{99}Tc), the second mechanism alsobecomes saturated and, presumably, non-specific binding (a third mechanism) takes over which allows concentration ratios of only 0.1 to 0.2.

The kinetics of technetium uptake by other algae have not been studied as well as Acetabularia. The brown alga, Cystoseira compressa, like Acetabularia, instantaneously takes up a small amount of ^{95m}Tc (1.72 $\pm$ 0.25 nCi or 76 fg) from the culture medium. Cystoseira shows an approximately linear uptake with a daily concentration ratio increase of 1.32 $\pm$ 0.15 on the first day and of 4.99 $\pm$ 0.46 thereafter (Fig.4). The decrease in activity in the medium corresponds to a half-life of 2.9 $\pm$ 0.2 days. As in experiments with Acetabularia the medium has to be regularly replenished with ^{95m}Tc.

A previously demonstrated very high affinity of brown algae (Fucus vesiculosus and F. serratus) for technetium in the marine environment (Masson et al., 1981 ; Masson et al., 1983) leads to questions regarding the chemical form of technetium in this alga. Table 1 shows the percentage of ^{95m}Tc extracted from contaminated Fucus vesiculosus by successive treatments with ethanol, weak mineral acids, and NaOH. The higher percentage of ^{95m}Tc extracted from the fresh alga using ethanol, compared to the dry alga, may include some ionic forms due to water soluble contents of the fresh tissue being diluted into the ethanol fraction. About 50% of the ^{95m}Tc in dried Fucus is extracted by

HCl and $HClO_4$ indicating a major part of the technetium is present in the alga in ionic forms. More than one third of the ^{95m}Tc in Fucus is associated with "insoluble" forms which are extractable only with NaOH or which remain in the final residue.

Interpretation of the previous chemical extractions is complicated by the possibility that chemical changes in the algae early in the procedure may lead to later artifacts in extraction. However, a simple extraction of a ^{95m}Tc contaminated Fucus vesiculosus frond with artificial seawter supported the prior results. A majority (51%) of the ^{95m}Tc was extracted from the brown alga by a single overnight extraction at 4°C. Three successive overnight extractions removed approximately 60% of the ^{95m}Tc from the alga while approximately 40% remained unextractable.

Differential centrifugation studies using ^{95m}Tc labelled Fucus vesiculosus showed a high percentage of technetium present in the postmicrosomal supernatant. Approximately 79% of the ^{95m}Tc present in the centrifuged filtrate was in "soluble" forms in the cytosol. On a total algae basis, this soluble fraction corresponds to about 48% and may be TcO_4^- or technetium associated with organic molecules. A smaller portion of ^{95m}Tc in the alga (13%) is associated with cell debris, cell organelles, and the postmicrosomal pellet.

Gel permeation chromatography, with Sephadex G-50, of the postmicrosomal fraction from Fucus vesiculosus labelled with ^{95m}Tc showed that most of the radioactivity eluted in a single peak at an elution volume (Ve) to void volume (Vo) ratio of about 3 indicating a major association with molecules less than 1500 MW. Less than half of the ^{95m}Tc chromatographed diffusely on the G-50 column with a range in Ve/Vo ratio of 1 to 2.8 indicating some lesser association of ^{95m}Tc with organic molecules in a MW range of 1500-30 000.

There were no major differences between the chromatographs of fresh and freeze-dried brown algae when chromatographed on Sephadex G-25. The chromatograph of the post microsomal fraction from freeze-dried Fucus vesiculosus showed that approximately 45% of the ^{95m}Tc eluted from the column in a single peak with a K_{av} value of 1.4 (Fig.5). The K_{av} (equals $(Ve-Vo)/(V_t-Vo)$, where $V_t = 3\ V_o$) defines the solute behavior on the gel independently of the bed

dimensions and column packing. In Fucus, this peak was not associated with significant UV absorbance at 280 nm. When a solution of $(NH_4)^+ TcO_4^-$, in water, was chromatographed on the G-25 column, the ^{95m}Tc eluted with a K_{av} value of 1.4 (Fig.5). Based on the equivalent K_{av} values of the technetium present in the brown alga and the TcO_4^- in aqueous solution, as well as the minimal UV absorbance at $K_{av} = 1.4$, it appears that about 45% of the technetium in the algal postmicrosomal fraction was present as anionic TcO_4^- while the remainder was bound to small organic molecules having a MW between 1000 and 5000.

4. CONCLUSION

There are several findings from this study which could have implications for the use of algae as biological indicators of technetium pollution in the marine environment. First, contrary to suggestions that green marine algae concentrate technetium less than brown algae (Jeanmaire et al., 1981 ; Fisher, 1982), Acetabularia, a giant unicellular green marine alga, has been shown to accumulate technetium far above concentrations present in the surrounding medium when concentrations in the medium are low. Most of this technetium has penetrated directly into the cell and little is associated with the cell wall. However, the accumulation mechanisms in Acetabularia, and probably in other algae as well, can become saturated when mass concentrations of technetium in the surrounding medium are high and this causes a decrease in the concentration factor. This dependence of algal accumulation of Tc on the mass concentration of Tc in the medium has significance for the experimental determination of concentration factors used for risk assessment of the transfer of Tc in the marine food chain. Experiments with ^{99}Tc for determination of concentration factors in algae will typically require higher mass concentrations of Tc in the culture medium than experiments with ^{95m}Tc. Consequently concentration factors for algae based on radioactivity (e.g. nCi per g algae/nCi per ml water) can differ greatly for the two isotopes due to differences in the mass of technetium per curie. Finally, a high percentage of technetium present in ^{95m}Tc labelled Fucus vesiculosus is present in soluble ionic forms while approximately 40% is irreversibly bound in the brown alga in proteins and polysaccharides chiefly associated with cell walls. In the algal cytosol, which contains "soluble" technetium, about 45% appears to be present as anionic TcO_4^- and the remainder is bound to small molecules that are the size of peptides.

It is presently unknown if the anionic TcO_4^- in Fucus represents externally adsorbed technetium or technetium incorporated into algal cells.

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Table 1. Fractionation of ^{95m}Tc in the labelled brown alga, Fucus vesiculosus.

Extractants[a]	Fractions containing[b]	% of quantity in frond[c]	
		Fresh	Freeze-dried
95% Ethanol	Lipids, amino sugars, free amino acids, lipophyllic pigments	25 ± 6	10 ± 1
0.2 M HCl			
Supernatant	Ionic forms incl. salts of organic acids, phosphates, carbonates	29 ± 1	40 ± 1
Precipitate with acetone	Proteins, pectates	< 1	< 1
0.5 M $HClO_4$			
Supernatant	Remaining ionic forms	7 ± 1	9 ± 4
Precipitate with acetone	Nucleic acids and some proteins	< 0.1	< 0.1
2 M NaOH	Most proteins and some poly-saccharides	37 ± 5	38 ± 4
Residue	Cell wall material	2 ± 1	3 ± 1

[a] According to method of Bowen et al. (1962)

[b] Based on Myttenaere and Masset (1966)

[c] Mean ± standard deviation based on replicate samples

Figure legends

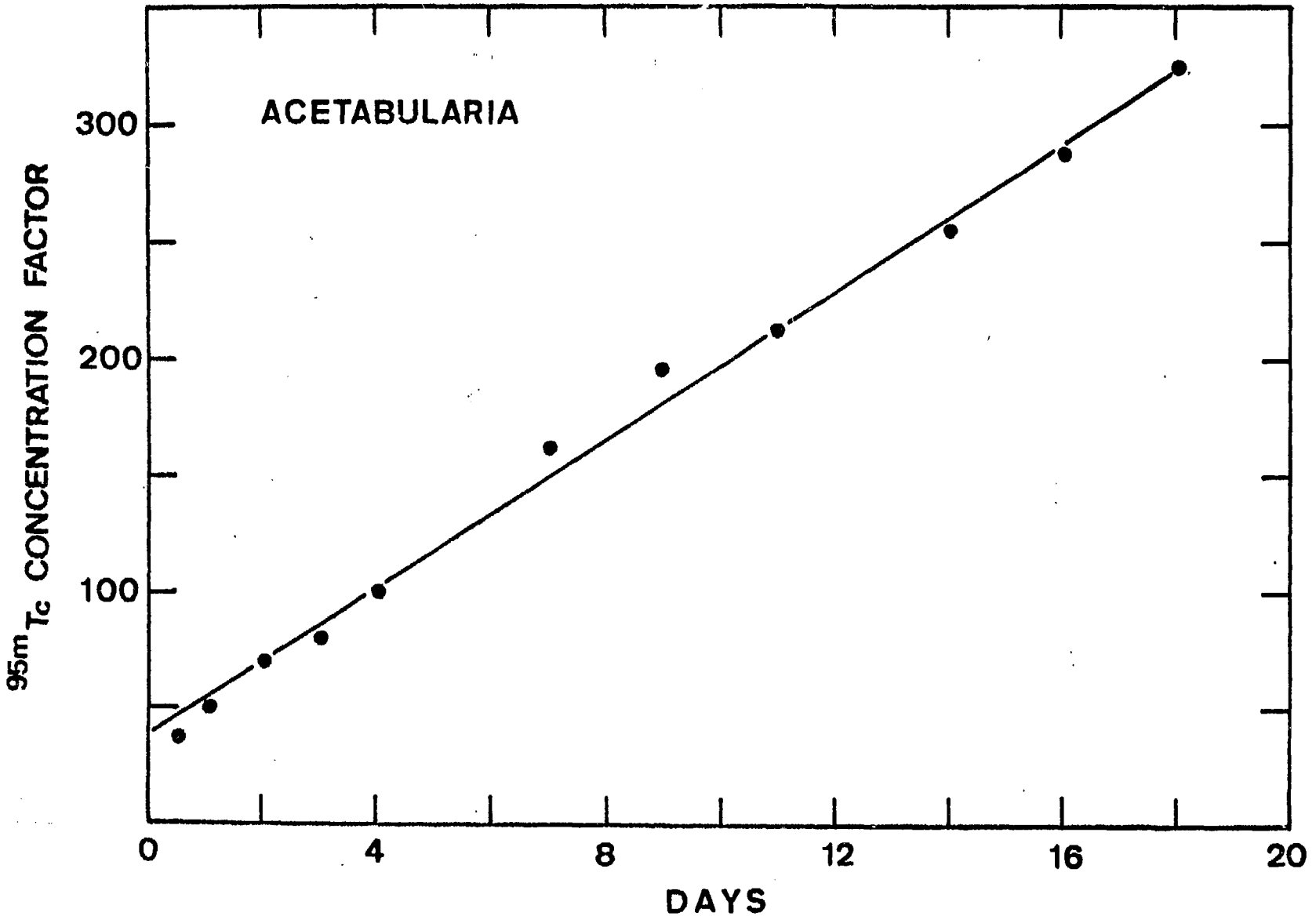
Figure 1. Uptake of ^{95m}Tc (pertechnetate) at a level of 2 nCi/ml (90 fg/ml) by Acetabularia acetabulum as a function of time. The activity in the medium was regularly readjusted, and the concentration ratios have been corrected for mean activity levels during the exposure.

Figure 2. Uptake of ^{99}Tc (pertechnetate) at a level of 1.0 nCi/ml (59 ng/ml) by Acetabularia acetabulum as a function of time.

Figure 3. Model for the uptake of technetium in the green marine alga Acetabularia acetabulum, based on experiments with ^{95m}Tc and ^{99}Tc, showing three mechanisms of uptake : 1) rapid and reversible , 2) irreversible, and 3) low binding affinity (non-specific adsorption to surfaces).

Figure 4. Uptake of ^{95m}Tc pertechnetate at a level of 6 nCi/ml by the brown alga Cystoseira compressa as a function of time.

Figure 5. Chromatographs of ^{95m}Tc activity in the elutant from a Sephadex G-25 column for a sample of the postmicrosomal supernatant from a labelled brown alga, Fucus vesiculosus (above), and for a sample of ^{95m}Tc (pertechnetate) in water (below).


ACETABULARIA
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200
100
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4
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12
16
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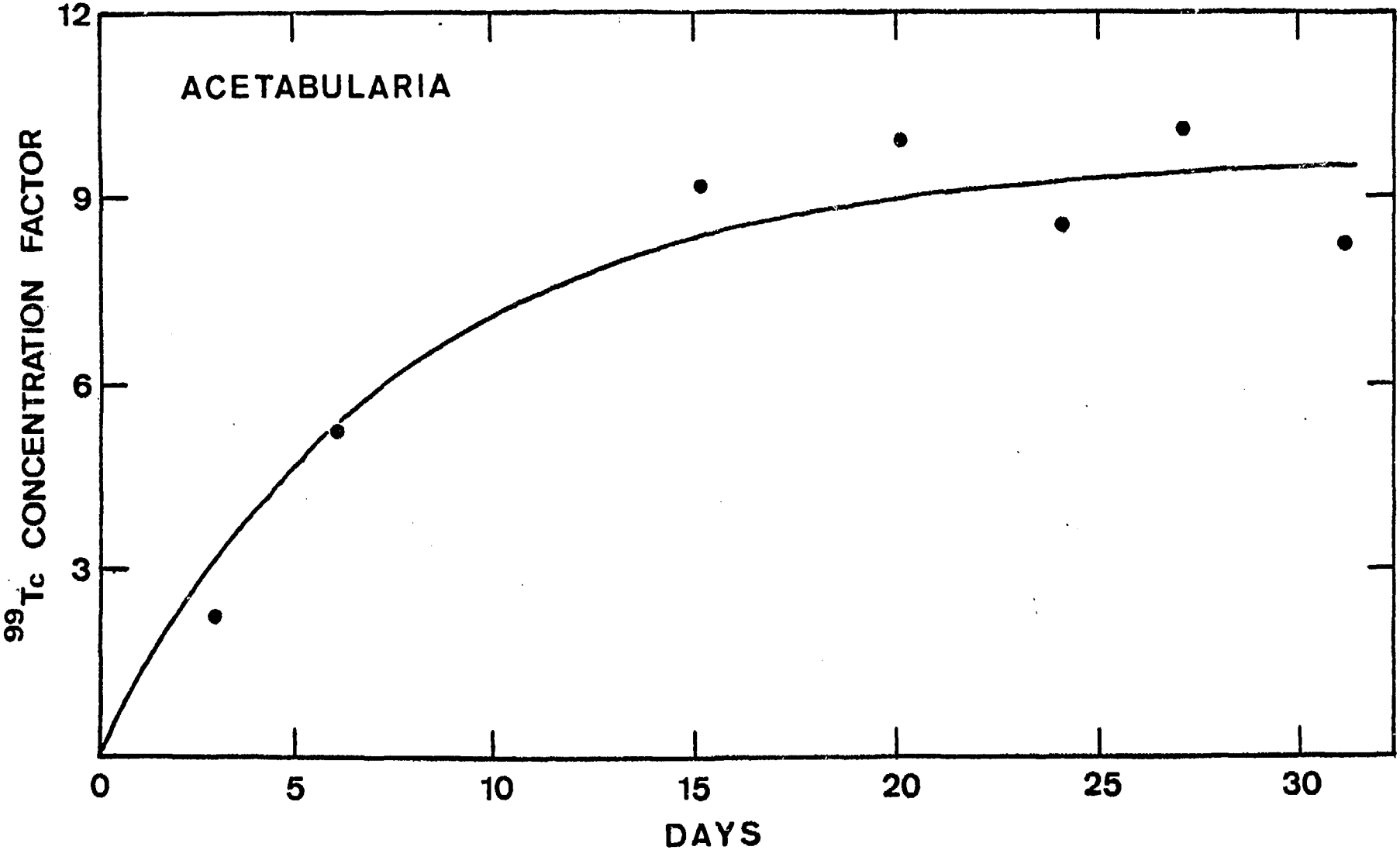
DAYS
95m Tc CONCENTRATION FACTOR


ACETABULARIA
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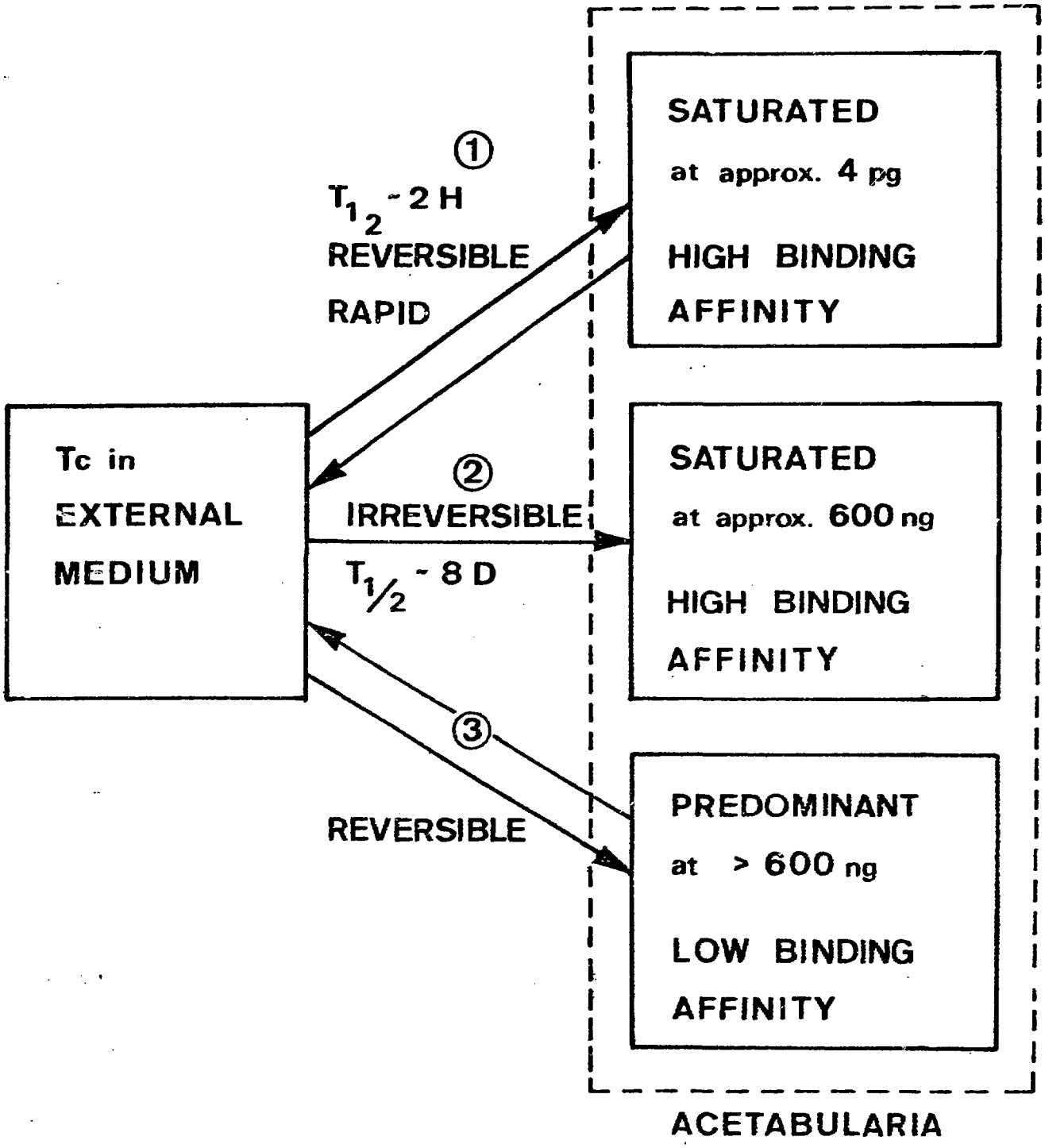
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at > 600 ng
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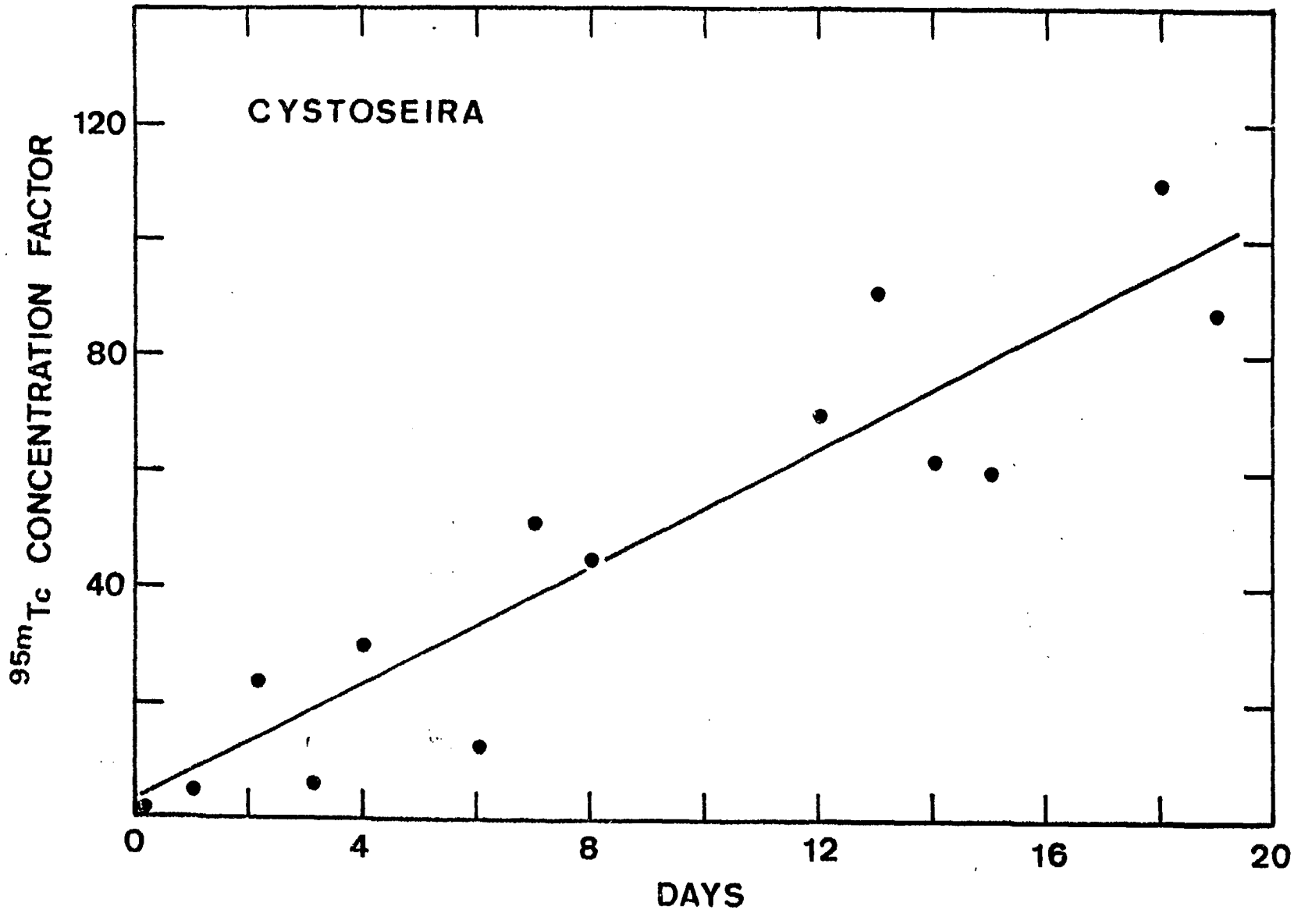
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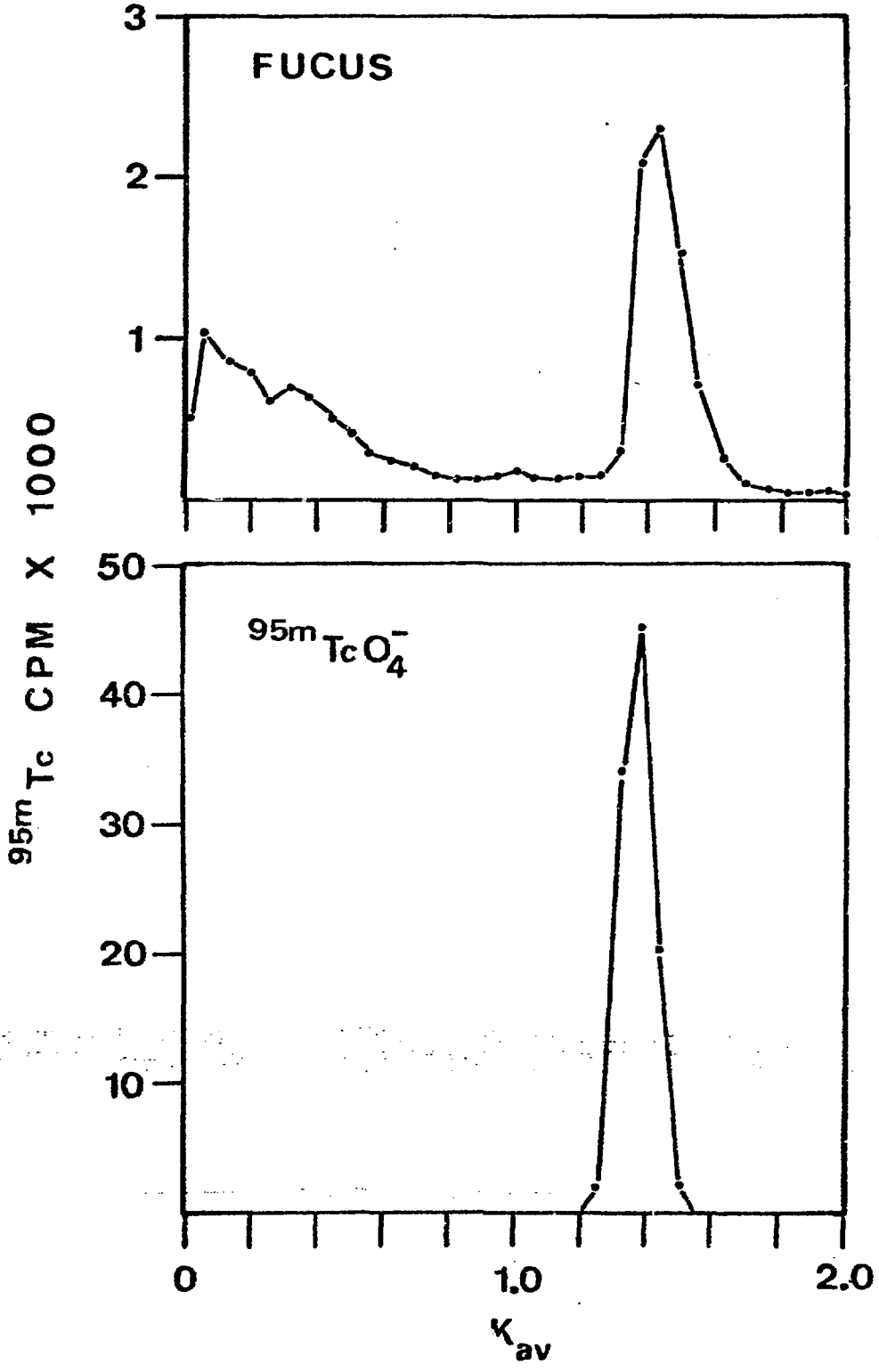
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