Metabolic Studies on the Accumulation of Tetraphenylporphinesulfonate in Tumors

Quantitative studies have established that parenterally administered tetraphenylporphinesulfonate (TPPS) localizes in transplantable animal tumors with a higher concentration ratio with respect to other tissues than any other known material[1]. Liver had $^1/_4$ the TPPS content of tumor and other tissues had still less. Radioactive chelates of TPPS have been administered to tumor-bearing animals and human cancer patients in the hope that diagnostic or therapeutic procedures could be developed[2–4]. Such applications have not been feasible because the isotopes failed to concentrate in tumors to the same extent as the parent compound. Co^{57} TPPS yielded lower Co^{57} tumor concentration ratios by a factor of 5.2[2]. Approximately 40% dissociation of Co^{57} TPPS was found in the excreta of cancer patients[3]. Assuming that this represented the degree of dissociation systemically and locally at the tumor, and knowing the distribution of free cobaltous Co^{57} cation[4], this could account for only 60% of the disparity between TPPS and Co^{57} from Co^{57} TPPS concentration ratios. These considerations led to the studies reported here, undertaken to determine the possible unique features of TPPS metabolism that determine its tissue concentration in tumor-host systems. It was hypothesized that if TPPS catabolism was rapid in the liver, TPPS fragments and high levels of incorporated radioisotope would accumulate. If the tumor were deficient in some enzyme, cofactor etc., more intact TPPS and relatively less isotope would accumulate.

Direct evidence for catabolism of TPPS was sought by following its disappearance from whole tissue homogenates. Conditions of incubation included variation of pH from 4.0–9.5, at intervals of 0.2 pH units between 5.5 and 8.5, with *Tris*, phosphate, bicarbonate and acetate buffer systems at several concentrations. Incubations with a final homogenate concentration of 0.1 g (wet weight)/ml in 0.15 *M* KCl or 0.25 *M* sucrose were carried out at 0, 25, and 37 °C with gentle shaking. The gaseous environment was both ambient and in some cases 95% O_2, 5% CO_2. Assays were performed on aliquots taken over at least 4 and as long as 20 h incubation, by a method that involved precipitation, extraction and optical density determination of the 413.5 nm Soret peak[1]. In none of these experiments was any consistent and significant loss of TPPS detected. Similarly, no diminution in TPPS fluorescence was seen in reaction mixtures continuously monitored using front fact optics, or in separately withdrawn aliquots. Other substrate preparations also were not metabolized under any set of experimental conditions. These included TPPS complexes with albumin, histone, protamine, poly-L-lysine, and denatured rat liver protein.

Liver slices were prepared and similarly incubated to determine whether TPPS metabolism had been lost with cellular disruption during the preparation of homogenates. Other animals received amounts of TPPS parenterally that gave readily detectable levels in tissue, and their liver slices were incubated without TPPS in the medium. This was designed to further simulate the in vivo conditions of TPPS with respect to physiologic concentrations, intracellular distribution, binding etc. With both preparations no disappearance of TPPS was detected under any conditions.

Specific enzymes that might act upon TPPS were assayed. Heme-α-methenyl oxygenase, a porphyrin-ring-opening enzyme active with pyridine-hemichromogen and several heme-haptoglobin complexes[5,6], was tested with TPPS and TPPS-pyridine complex. Problems with the assay system as described[5] included precipitation of the 0.5 *M* veronal buffer at pH 8.3, co-precipitation of hemin with protein on addition of trichloroacetic acid, and appearance of the 656 nm 'product' peak more often in boiled enzyme controls than experimental flasks. No changes were observed in the spectra of TPPS incubated with preparations that should have contained crude or partially purified enzyme. Variations of the experimental design that overcame the above-mentioned difficulties also failed to demonstrate ring-opening of either hemoglobin or TPPS substrate preparations. Sulfatase activity was determined using a microbenzidine method[7] modified by substitution of 25% acetic acid (v/v) for trichloroacetic acid, which was found to be contaminated with sulfate. The assay would have detected desulfonation of as little as 5% of TPPS. No such activity could be found. From all the above experimentation, it appears that the ring structure of TPPS is not opened, or the prosthetic groups removed, by enzymatic action in tissue.

The possibility that accumulation of TPPS by tumor reflected binding to an unusually abundant or unique cytoplasmic protein was assessed by equilibrium dialysis experiments. The binding of TPPS to soluble fractions of tumor, kidney, liver, spleen, muscle, and brain prepared by ultracentrifugation was determined. Conclusions from these experiments were qualified because the 'protein' preparations were not homogenous, they all showed great affinity for TPPS, bag binding was great, and equilibration was slow. However, no significant or consistent differences could be demonstrated.

From these studies it appears that differences in TPPS metabolism in tumor and other tissues cannot explain the earlier tissue distribution findings. This conclusion probably applies to the other porphyrins that have been tested or recommended as tumor localizing agents[8–12]. Thus far the incorporated radioisotopes have been chelated metals. Dissociation and independent distribution of the metals could not completely explain the discrepant distribution patterns of isotope and parent TPPS[3]. Why then did Co^{57} incorporated in Co^{57} TPPS distribute differently from TPPS alone? The most likely explanation is that metal porphyrins have a completely different distribution pattern than TPPS by virtue of the major change in electronic configuration conferred by the dz^2 orbital of the metal that is perpendicular to the otherwise flat planar molecule[13].

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It has been shown that oxidation-reduction reactions occur in orbitals belonging to the ring of free base porphyrins, but in metal centered orbitals of the metalloporphyrins[14]. These considerations suggest that attention be directed towards study of TPPS or similar compounds radioisotopically labeled directly within the ring structure[15].

Résumé. Des résultats antérieurs suggèrent que l'accumulation sélective de TPPS au niveau de certaines tumeurs est liée aux caractères particuliers de leur métabolisme. Au cours d'expériences utilisant des préparations tissulaires, il n'a pas été possible de mettre en évidence une dégradation du TPPS. D'autres mécanismes impliqués dans la distribution du TPPS sont discutés.

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Indigenous Microbial Flora and the Large Intestine in Tadpoles

In terrestrial vertebrates, the large intestine is the site of marked bacterial proliferation, whereas the contents of the stomach or small intestine appear to be sterile or sparsely populated with bacteria[1-3]. The mechanical cleansing of the lumen by continuous motor activity, aided by mucus secretion, seems the most important mechanism to keep the small intestine free from debris and bacteria; in contrast, debris accumulate and many microorganisms fluorish in the large intestine, where movements are sluggish and mucus is largely disimbibed or demolished[4,5]. In fish, on the contrary, the hindgut is not enlarged[6,7], the faeces present large quantities of mucus[8] and the intestinal flora appears to be absent or restricted to a very scanty and labile form[1].

The finding that a true indigenous microbial flora is associated with the hindgut of adult amphibia[1-3] and the consideration that the larval state seems to be an old inheritance of the amphibia, handed down from their fish ancestors, prompted us to investigate if stable bacterial populations are established in the intestines of tadpoles.

Larvae from different species (*Rana esculenta, R. temporaria, Bombina variegata, Bufo viridis*), in stages from 24–29 (according to WITSCHI[9]), were examined for the intestinal bacterial content, using techniques previously described[1,2]. Full evidence was obtained that, in the hindgut of tadpoles, a multiform microbial flora is firmly established, that it continues to fluorish even after fasting periods of 1–2 weeks, ultimately being handed down, through the metamorphosis, to the adult hosts.

Are tadpoles provided, then, with an 'enlarged hindgut'? We were not able to find in the literature adequate references on this point. But, from our dissections, it became evident that tadpoles have a very well developed 'large intestine': this (Figure 1), 10–15 mm long and 1–1.8 mm in diameter, has an elongated, pyriform profile;

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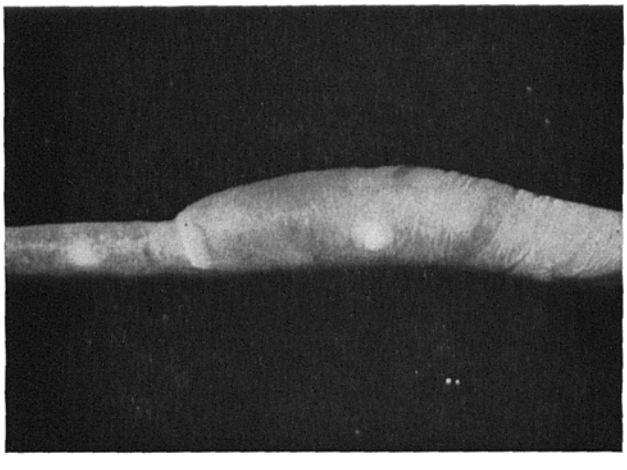

Fig. 1. The 'large intestine' of a tadpole of *Rana esculenta*, stage 25. × 10.

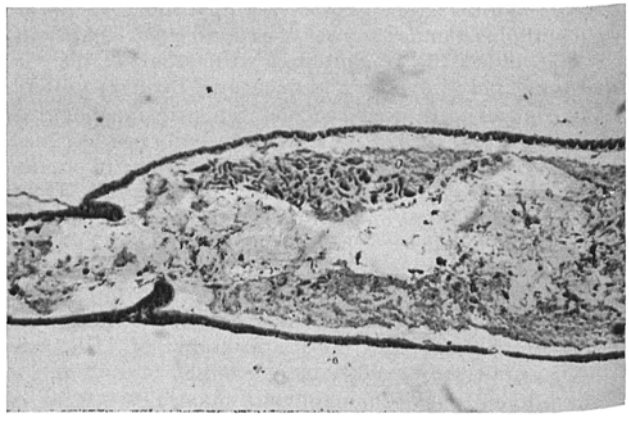

Fig. 2. Same specimen of Figure 1, logitudinal section. From left to right: end of the 'small intestine'; valve; 'large intestine' showing the central mucus flow and the characteristic agglomerate of opalinids. × 25.