

Technical Papers

The Disposition of Intraperitoneally Injected Foreign Matter in Amphibia

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An attempt has been made to compare the behavior of macrophages in various amphibia with similar behavior in mammals, using Thorotrast¹ as an injection material. The author (1) has previously reported that mice injected with Thorotrast dispose of the substance via the urine and bile in the colloidal state, and via the lungs in macrophages. In the latter instance, the macrophages are either cast out of the mouth with mucus and saliva or are swallowed, to leave the body in the feces. Irwin (2) also indicated that in the mammal the lungs function in removal of such macrophages.

Although it has not been possible to make quantitative measurements of movements of ThO₂ in the amphibian body, histological observations have yielded considerable information on the means by which amphibia lose injected foreign matter (Table 1). The number of amphibian forms readily available has imposed a further limit upon this study.

Specimens were killed by decapitation. Pieces of liver, spleen, lung and/or gill, kidney, stomach, upper intestine, muscle with skin, and gonad were fixed in Bouin's fluid, embedded in paraffin, and sectioned at 5 μ . All tissues were stained with Delafield's hematoxylin and eosin.

The specimens used offer a variety of conditions for comparison with the mammal. *Ambystoma* and the adult *Rana* spp. possess lungs; *Desmognathus* have neither lungs nor gills; the larval *R. catesbiana* have both lungs and gills; and the *Eurycea* and *Pseudotriton* larvae have gills only.

Adult Anura: In the frogs, the *R. palustris* killed 3 days post-injection still had large numbers of thorium-filled macrophages in the abdominal cavity. The two *R. sylvatica*, killed at 5 and 10 days, had none. In other respects, except for the skin, the three specimens agree.

In the liver, heavily charged Kupffer cells were numerous, and were widely scattered without regional concentration. Loaded macrophages were common in the lumina of both portal and hepatic veins. There were granules of ThO₂ in liver parenchymal cells, concentrated usually adjacent to the bile canaliculi. There were also free granules of ThO₂ in the bile ducts and gall bladder.

The three spleens contained large quantities of ThO₂. Here nearly all the ThO₂ was located in macrophages packed into or around well-defined sinuses. In

¹ Thorotrast is a colloidal preparation of ThO₂ in aqueous medium approximately 25% ThO₂ by weight, made by Heyden Chemical Corp., New York. The preparation is stabilized by addition of approximately 20% by weight dextrin.

TABLE 1
SPECIMENS, DOSAGES, AND KILLING TIMES

Species	Sex	No.	Dosage (ml) undiluted Thorotrast	Killed (No. days after injection)
<i>Adult forms</i>				
<i>Anura</i>				
<i>Rana palustris</i>	Male	1	0.1	3
<i>R. sylvatica</i>	"	1	.2	5
" "	Female	1	.2	10
<i>Urodela</i>				
<i>Ambystoma maculatum</i>	Male	5	.3	One on each of days 1, 3, 5, 10, and 15
<i>Desmognathus f. fusca</i>	"	1	.2	7
<i>Larval forms</i>				
<i>Anura</i>				
<i>R. catesbiana</i>	?	1	.1	2
<i>Urodela</i>				
<i>Eurycea bistineata</i>	Male	1	.05	5
<i>E. bistineata</i>	Female	1	.05	5
<i>Pseudotriton r. ruber</i>	?	1	0.2	5

the lungs, filled macrophages were located in capillaries and in the tissues outside of capillaries, but adjacent to them. They were also lying free in the air passages or in flakes of mucus on the surface of the lung epithelium. In the kidneys, many glomerular capillaries, the lumina of some of the tubules, and a few capsular spaces contained macrophages with ThO₂. In addition, in the *R. sylvatica* female (10-day specimen), the cells of some of the proximal convoluted segments contained spherical bodies having the appearance of ThO₂, about midway between the base and apex of the individual cell.

The stomach and gut of all three specimens also exhibited macrophages filled with ThO₂, both fixed in the tissues of the walls and free in the lumina. In one (10-day *R. sylvatica*), small macrophages containing ThO₂ appeared to be passing through the epithelium of the gut into the lumen.

Only the *R. palustris* (3-day) had charged macrophages in or immediately beneath the skin. This specimen had a few small macrophages with moderate quantities of ThO₂ passing through the epithelium of the skin glands into their lumina. These macrophages were not numerous and certainly do not indicate that the skin is a major route of ThO₂ removal.

Adult Urodela: The five *A. maculatum* gave a picture similar to, but differing consistently from, that of the frogs. All five had some filled macrophages in

the abdominal cavity. In all, Kupffer cells throughout the liver contained large amounts of ThO_2 . With the exception of the 15-day specimen, there were free loaded macrophages in all vessels of the liver, and some granules of ThO_2 in parenchymal cells. The spleen had, in every case, considerable numbers of ThO_2 -charged macrophages located within distinct sinuses. The lungs contained cells with ThO_2 in capillaries, in the tissues outside the capillaries, free in the air passages, and free in the lumina of larger blood vessels. The kidneys had the same appearance as those of the frogs described above.

The stomach and gut differed from those of the frogs in showing very few loaded macrophages in the lumen or in the wall, and in having no appearance whatever of discharge of ThO_2 through the epithelium. The same was observed in relation to the external skin. There was no sign of passage of ThO_2 into the lumina of the glands, and very little ThO_2 in subcutaneous macrophages. The epithelium of the cloaca, however, although without ThO_2 in or beneath the surface layer, was thickly covered with mucus containing vast numbers of heavily loaded macrophages, as well as fragments of such cells and free granules of ThO_2 . The cloaca of the frogs was not studied.

In the one *Desmognathus* (lacking both lungs and gills), ThO_2 was distributed in exactly the same manner as in the adult *Ambystoma*, except, of course, for the lung. Otherwise, the difference between the two was strictly one of degree. The kidney and cloaca had much heavier and denser amounts of ThO_2 in *Desmognathus*.

Larval forms: These in general agree with the adults in the distribution of ThO_2 observed. The liver could not be distinguished from that of adults on the basis of thorium distribution. The larval spleens contained some large masses of Thorotrast granules free in sinuses, along with larger quantities in macrophages. The gut contained some ThO_2 in macrophages in the walls and lumen, but none of the larvae gave evidence of transfer of Thorotrast through the epithelium.

The kidneys differed slightly from those of adults in showing as a constant feature granules of material that seemed to be ThO_2 within cells of the proximal convoluted segments of the tubules, apparently being discharged. Larval skin had the appearance of adult skin. The *R. catesbiana* larva had both gills and lungs, but the larval urodeles had gills only. The *Rana* lung had not yet begun to function in respiration. In all four larvae, the gills were observed to contain filled macrophages in capillaries, in the tissues outside the capillaries, and on the external surface of the gill epithelium, either sticking directly to the latter or trapped on it in attached flakes of mucus. The lungs of the *R. catesbiana* tadpole contained ThO_2 in macrophages in capillaries and in the tissues outside the capillaries, but none in the lumen of the lung.

In no specimen studied was there any significant amount of ThO_2 in skeletal muscle. Isolated macro-

phages located between muscle fibers contained a few granules only.

The presence of ThO_2 -filled macrophages in both portal and hepatic veins suggests, as more comprehensive data have done for the mammal, that filled macrophages move through the liver, presumably largely from the spleen.

The amphibian lung and gill observations further suggest that whichever of these is active in respiration is also responsible for the release of a part at least of the ThO_2 introduced into the body.

Furthermore, the presence of granules of ThO_2 free and in macrophages in various parts of the kidney—i.e., glomeruli, lumina of tubules, cells of the proximal convoluted segment, and also in the cloaca—suggests that the kidney is at all times active in the removal of circulating Thorotrast. The one *Desmognathus*, lacking both lungs and gills, showed a much higher degree of saturation in the kidney than other specimens and much more ThO_2 in the cloaca. This indicates that in the absence of lungs and gills the kidney is the chief organ of disposal of ThO_2 . This is in contrast to the situation in mammals, in which the kidney removes a very small part of any ThO_2 injected, and the lung, absent in *Desmognathus*, is the chief organ of Thorotrast removal.

That the skin, which serves also for respiration, may serve as an organ for disposal of macrophages carrying ThO_2 is suggested by the finding of such cells in the lumina of skin glands in one of the frogs. However, the rarity of the observation (one frog, no urodeles) indicates that this route is not an important one.

Further investigation of this problem in a more quantitative manner would be highly desirable for comparison with the situation in mammals.

References

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Radioactivity Induced in Yeast by 100 Mev X-Radiation¹

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Mayneord, Martin, and Layne (1) have shown that 24 mev of x-radiation induces a small amount of radioactivity in tissue, ascribed to production of O^{15} and C^{11} by (γ, n) reactions.

In view of the fact that Mayneord *et al.* were working near the thresholds of these reactions, and that maximum yields are expected at much higher voltages

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