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THE DISTRIBUTION OF ^{35}S-SULFADIAZINE AND ^{14}C-TRIMETHOPRIM IN RAINBOW TROUT, SALMO GAIRDNERI

By

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BERGSJØ, T., I. NAFSTAD & K. INGEBRIGTSEN: *The distribution of ^{35}S-sulfadiazine and ^{14}C-trimethoprim in rainbow trout, Salmo gairdneri.* Acta vet. scand. 1979, *20*, 25—37. — The distribution of ^{35}S-labelled sulfadiazine and ^{14}C-labelled trimethoprim was studied in rainbow trout by use of whole body autoradiography and liquid scintillation. As compared to mammals, gastrointestinal absorption and elimination were slow. Accumulation in the skin and the uveal tract of the eye was observed for both drugs tested. The results also indicated that the bile was an important route of excretion. Considerable radioactivity was still present in the skin at 144 hr. survival time.

^{35}S-sulfadiazine; ^{14}C-trimethoprim; rainbow trout; Salmo gairdneri; whole body autoradiography.

In the therapy of bacterial infections in hatchery fish the sulphonamides are among the most widely used drugs. Trimethoprim is another drug of interest for the chemotherapy of microbial diseases. Combinations of sulphonamides and trimethoprim will probably become important drugs in the aquaculture.

Some investigation has been done on therapeutic efficacy and residues of sulphonamides in fish. Very little, however, is known about distribution and excretion of these important drugs.

In the present investigation ^{35}S-sulfadiazine and ^{14}C-trimethoprim were administered orally to rainbow trout, and their distribution and excretion were studied by whole body autoradiography and liquid scintillation counting.

MATERIAL AND METHODS

^{35}S-sulfadiazine (2-(^{35}S)-sulphanilamidopyrimidine) and ^{14}C-trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl) (2-^{14}C)pyrimidine) were obtained from the Radiochemical Centre, Amersham, England. The trimethoprim was a gift from the Wellcome Foundation Ltd., England. The specific activity on the date of delivery was 101 μCi/mg and 11.1 μCi/mg for ^{35}S-sulfadiazine and ^{14}C-trimethoprim, respectively. Radiochemical purity was 99 % as determined by thin-layer chromatography on silica gel. The therapeutic dose of sulfadiazine and trimethoprim given to fish was 20 mg and 2 mg per 100 g body weight, respectively. In order to dilute the labelled sulfadiazine, 18 mg of the labelled compound was mixed with 1500 mg of unlabelled sulfadiazine in 6 ml 1 M-NaOH as a solvent (equivalent amounts) and freeze-dried. The specific activity of the resultant mixture was 0.66 μCi/mg on the date of use.

The test animals were healthy rainbow trouts (Salmo gairdneri). The fishes were divided into 3 groups: Groups 1 and 2 were kept in running fresh water at 7 °C and recevied no feed during the experiment. The trouts of Group 1 weighing 140 ± 18 g were given 25 mg/fish of the above-mentioned sulfadiazine-mixture, while the fishes of Group 2 weighing 248 ± 27 g were given 5 mg/fish of ^{14}C-trimethoprim.

The fishes in Group 3 weighing 134 ± 17 g were kept in running fresh water at 15 °C and were fed commercial fish pellets during the experiment. They were given 3 mg/fish of ^{14}C-trimethoprim.

The fishes in all groups were allowed to adapt to the test conditions for 14 days before the experiment started.

Whole body autoradiography

The powdered drugs were weighed and divided into gelatin capsules No. 2 (Parke, Davis & Co., England). The capsules were placed in the stomach of the unanesthetized fish by a pair of anatomical tweezers. After the administration of the capsules the fishes were carefully watched for 15 min. to ensure that the capsules were not regurgitated (*Nakatani* 1962).

One animal from each of the Groups 1 and 2 was sacrificed after 2, 4, 8, 12, 24, 48 and 72 hrs. In addition, 1 animal in Group 2 was sacrificed after 144 hrs. In Group 3, 1 animal was sacrificed

after 3, 6, 12, 24, 48, 72 and 144 hrs. The fishes were anesthetized in a saturated solution of benzocaine in water at the stated survival time, embedded in a mixture of carboxymethylcellulose and water and frozen in hexane cooled to —78 °C with solid CO_2. Sagital sections (30 µm) were cut on a PVM-microtome and dried at —15 °C according to Ullberg's autoradiographic technique (*Ullberg* 1954). Autoradiograms were made by apposition of the sections against X-ray films (Kodirex, Kodak). Exposure was carried out at —20 °C for 4 weeks. After exposure the films were developed in Kodak D 19.

Liquid scintillation counting

Samples of liver and muscle tissue collected from the frozen blocks were homogenized following addition of water 1:3.

To parallels of 400 µl were added 200 µl ethanol 96 % and 1 ml of tissue solubilizer Soluene-350 (Packard, Switzerland). Following incubation for 24 hrs. at room temperature 400 µl Perdrogen (Riedel-De Haen AG, Seelze-Hannover, Germany) was added to decolorize the samples. After the addition of 4 ml scintillation fluid (Dimilume-30, Packard) and subsequent equilibration at room temperature for 15 min., all samples were counted in a liquid tri-carb scintillation spectrometer (Packard 3310) for 10 min.

Then 100 µl internal standard ^{14}C-Toluene 5.09×10^5 dpm/g (Packard) was added to selected numbers of samples and the counting efficiency calculated accordingly.

The results were expressed as disintegrations per min. per mg tissue (dpm/mg).

RESULTS

Whole body autoradiography

Group 1 (^{35}S-sulfadiazine, 7 °C)

Fig. 1 shows an unstained whole body section of a rainbow trout demonstrating the gross anatomy of the fish.

Two hrs. after administration of the isotope-labelled sulfadiazine only negligible absorption had taken place, as radioactivity was limited almost exclusively to the stomach.

At 4 hrs., a strong activity was still seen in the stomach, however, part of the drug had now passed on to the pyloric caeca and the descending intestine. Furthermore a certain degree of absorption had taken place, as radioactivity was distributed

throughout the body. The highest activity, when excluding the gastrointestinal tract, was observed in the blood, the liver, the kidney and the skin. Lower activities were exhibited in the musculature, the gills and the bile, while the bone, the cartilage and the tendon tissues showed the lowest activities.

At 8 hrs., an accumulation of radioactivity in the skin and the uveal tract of the eye had taken place. The activities in the bile and the gills had increased to approximately the same level as that of the blood (Fig. 2).

The tendency of accumulation in the bile, the skin and the eye increased with the survival time. At 72 hrs. high activity in the stomach was still present. The high activity observed in the entire intestinal lumen could be partly due to bile content. The activity in the bile was considerably higher than in the blood, the liver and the kidney, respectively (Fig. 3).

Group 2 (^{14}C-trimethoprim, 7 °C)

Up to 12 hrs. after administration of the isotope-labelled trimethoprim, activity could be observed only in the stomach.

At 24 hrs., the drug was distributed throughout the body, the highest activity being in the lumen, the mucosa of the stomach and the intestine, the kidney and the uveal tract of the eye. High activity was also present in the bile. The skeletal muscle, the heart, the gills and the muscular layer of the stomach and intestines showed approximately comparable activities, while the blood, the skin and the cartilage of the skull exhibited the lowest activities (Fig. 4).

At 48 hrs., an obvious accumulation in the bile was observed. The highest activity, in exclusion of the bile, was found in the lumen, the mucosa of the entire gastrointestinal tract, the kidney and the uveal tract of the eye. A moderate accumulation was present in the muscular layer of the stomach and intestine and in the skin. At 144 hrs., the activity of the skin had further increased (Fig. 5).

Group 3 (^{14}C-trimethoprim, 15 °C)

Three hrs. after administration of isotope-labelled trimethoprim at this temperature no absorption had taken place. At 6 hrs., a very slight activity in the stomach wall, the kidney and the liver was encountered, while no activity was observed in the intestine.

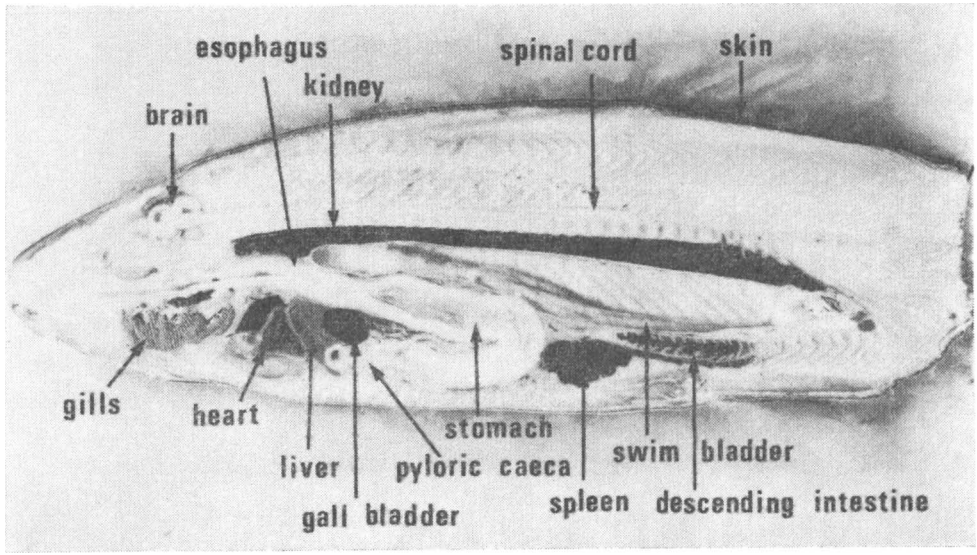


Figure 1. Whole body section of a rainbow trout demonstrating the gross anatomy of the fish. Unstained.

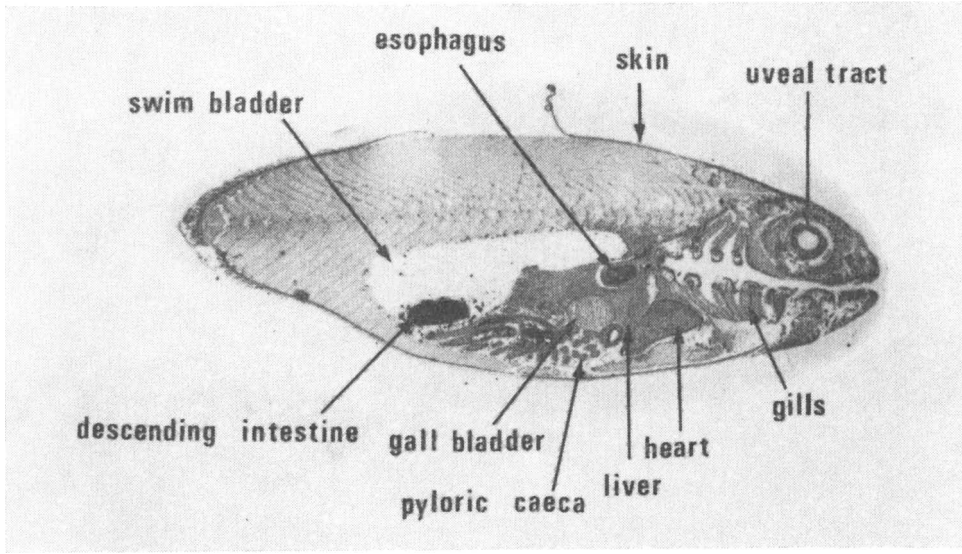


Figure 2. Autoradiogram of a fish kept at 7 °C 8 hrs. after oral administration of ^{35}S-sulfadiazine. Black areas correspond to high concentrations of labelled substance. Accumulations can be seen in the skin and the uveal tract of the eye.

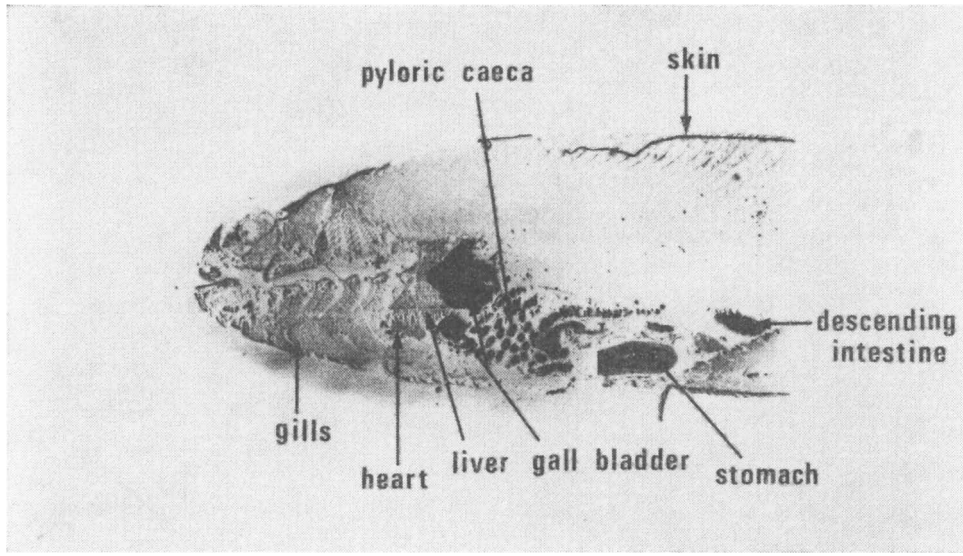


Figure 3. Autoradiogram of a fish kept at 7 °C 72 hrs. after oral administration of ^{35}S-sulfadiazine. Note high activity in the bile. Labelled substance is still present in the stomach.

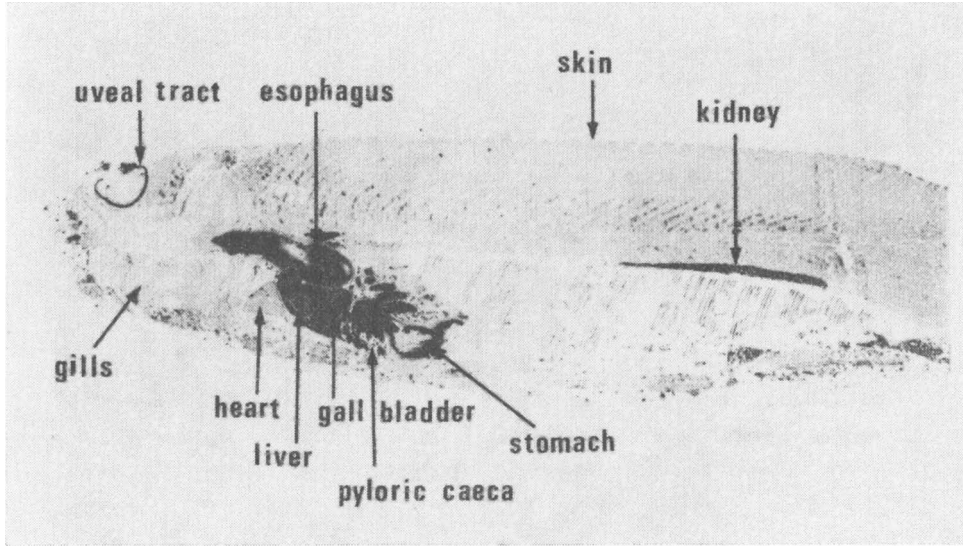


Figure 4. Autoradiogram of a fish kept at 7 °C 24 hrs. after oral administration of ^{14}C-trimethoprim. Labelled substance is distributed all over the body.

At 12 hrs. the drug was distributed throughout the body. The highest radioactivity, when excluding the gastrointestinal content, was found in the mucosa of the stomach and the intestine and in the uveal tract of the eye. A relative high activity was present in the kidney, the liver and the bile. The gills and the spleen showed somewhat lower activities, as did the muscular layer of the gastrointestinal tract.

At 24 hrs. an accumulation had taken place in the bile. The activity in the liver had decreased, as compared to that of the 12 hr. sections. In the skin there was an accumulation of radioactivity. The activity in the skeletal muscle, the heart and the muscular layer of the stomach and intestine had increased as compared to the blood (Fig. 6).

No difference was observed between sections obtained at 24 and 48 hrs., respectively.

Seventy-two hrs. after administration, the activity was considerably reduced in most tissues, except from the skin where activity was still accumulating and the uveal tract of the eye, the bile and the mucosa of the intestines, which showed approximately the same activities as before. Activity had now disappeared from the gills, the body and heart muscle, the blood, the CNS and the cartilage (Fig. 7).

At 144 hrs. after the administration of the drug, radioactivity had nearly left all structures but the uvea and the skin. Some activity was also still present in the bile, the gastrointestinal tract and the kidney. The last-mentioned organs are not demonstrated in the section in Fig. 8, which shows an autoradiogram of a fish after 144 hrs.

Liquid scintillation counting of ^{14}C*-trimethoprim*

The results of the liquid scintillation counting of muscle and liver tissue are shown in Table 1. The results seem to be well in accordance with what was seen on the autoradiograms. The liver values were about 6—7 times that of the muscle values. In cold water fishes, the concentrations reached a maximum after 48 hrs., while in warm water fishes, the peak was found at 24 hrs. However, the cold water fishes had higher values than those of the warm water all through the experiment.

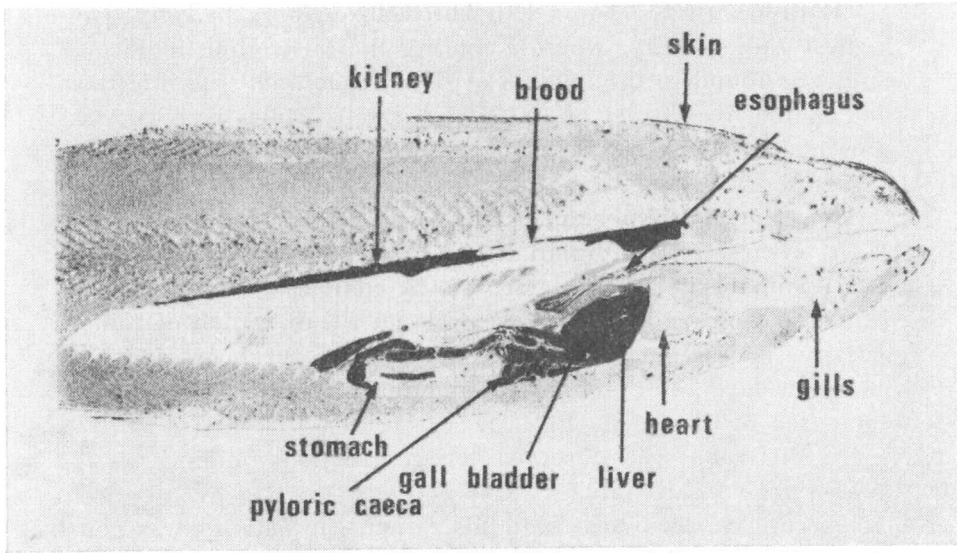


Figure 5. Autoradiogram of a fish kept at 7 °C 144 hrs. after oral administration of ^{14}C-trimethoprim. Labelled substance is still present in most tissues. Note accumulation in the skin.

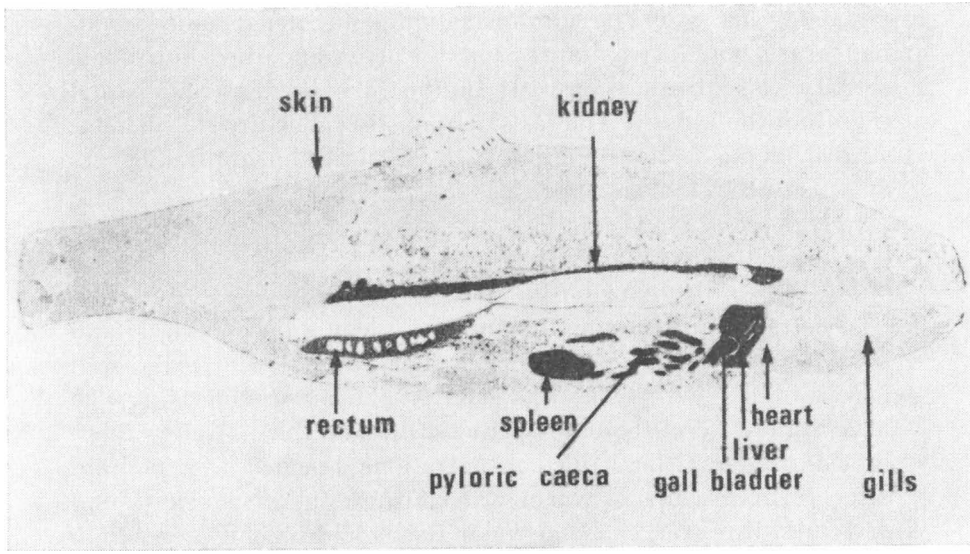


Figure 6. Autoradiogram of a fish kept at 15 °C 24 hrs. after oral administration of ^{14}C-trimethoprim. Note accumulations in the bile and the skin.

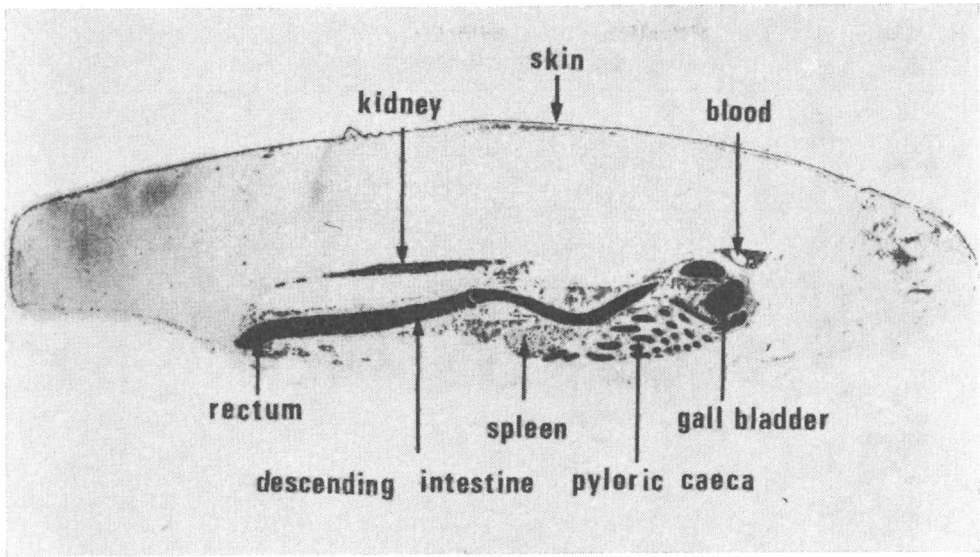


Figure 7. Autoradiogram of a fish kept at 15 °C 72 hrs. after oral administration of ^{14}C-trimethoprim. Radioactivity has decreased in most tissues, except from the bile, the skin, the uveal tract of the eye, the kidney and the intestinal tract.

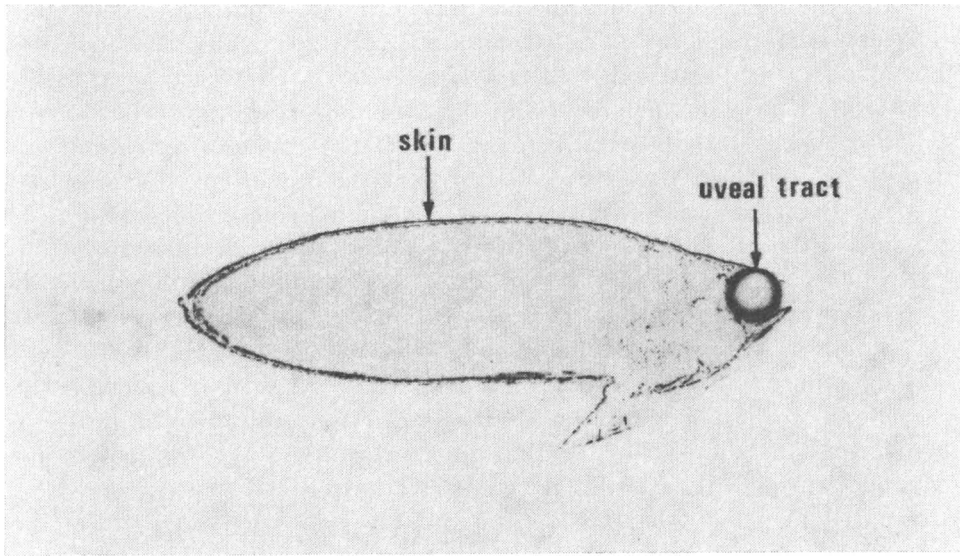


Figure 8. Autoradiogram of a fish kept at 15 °C 144 hrs. after oral administration of ^{14}C-trimethoprim. The highest radioactivity is present in the skin and the uveal tract of the eye. Internal organs are not demonstrated in this section.

Table 1. Radioactivity in liver and muscle at different intervals after oral administration of ^{14}C-trimethoprim. The values are given in disintegrations per min. per mg tissue.

Hrs. after administration	Dpm/mg tissue			
	liver tissue		muscle tissue	
	7 °C	15 °C	7 °C	15 °C
3	—	—	—	4.2
6	—	14.1	—	6.8
8	2.9	—	4.6	—
12	—	408.7	4.4	44.7
24	324.9	—	61.3	55.5
48	803.1	—	129.1	55.7
72	266.6	—	37.2	6.4
144	442.0	46.3	43.9	4.7

DISCUSSION

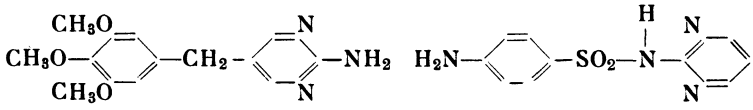
The stomach emptying as observed in the present study was found to be extremely delayed, as compared to mammals. However, the observation is in good agreement with earlier investigations performed on fish. *Windell et al.* (1976) reported that the time for stomach evacuation in rainbow trout on a commercial feeding varied between 16.4 hrs. at 20 °C and 72.4 hrs. at 5 °C.

Both of the 2 drugs administered in the present experiment are expected to be readily absorbed after oral administration (*Goodman & Gilman* 1975). In our experiment, sulfadiazine was partly absorbed after 4 hrs. at 7 °C. Trimethoprim, however, was not distributed in the body until 12 hrs. after administration when fishes were kept at 7 °C. At 15 °C some absorption had taken place after 6 hrs. This absorption was probably from the stomach, since no labelled material could be detected in the intestine at that time.

In warm-blooded animals sulfadiazine is expected to be uniformly distributed throughout the total body water, while trimethoprim will reach higher concentration in most tissues compared to blood (*Godman & Gilman*). The same general pattern of distribution was observed in the rainbow trout in our experiment. Two organs should receive special attention because of their capacity of accumulating both of the drugs tested, namely the skin and the uveal tract of the eye. A common feature of

those 2 organs is their content of melanin. Several authors have reported an affinity of certain polycyclic compounds to melanin (*Potts* 1964a,b, *Ullberg et al.* 1970, *Lindquist & Ullberg* 1972, *Dencker et al.* 1975). Among these compounds, chloroquine and chlorpromazine have been most thoroughly investigated. The accumulation of these compounds in the uveal tract can still be found 1 year after administration (*Lindquist & Ullberg*).

Even though sulfadiazine and trimethoprim have not been tested for melanin affinity, their chemical structures make them likely to bear the same property. Thus the accumulation of these substances in the skin and the uveal tract may be considered as a result of melanin affinity associated with the electron donor properties of the compounds (*Potts* 1964b).



Trimethoprim Sulfadiazine

The most important finding concerning excretion was the increasing content of both substances in the bile, which demonstrates that the bile excretion is the major excretorial pathway of these drugs in rainbow trout. This observation is in contrast to the knowledge of the excretorial pattern of sulfadiazine in mammals, in which the kidneys are the major excretorial organ (*Goodman & Gilman*). The route of excretion of trimethoprim in mammals varies between animal species. In man, about 4 % of the administered dose was recovered in feces (*Schwartz & Ziegler* 1969), while in calf the corresponding amount was 50 % (*Wellcome Laboratories,* personal communication).

There is paucity of information concerning the excretorial pattern of drugs in fish. The role of biliar accumulation, however, has come to attention for some drugs (*Adamson* 1967).

Except from the bile, excretion in fish may take place through the gills and the kidneys. Only very lipid soluble compounds are expected to pass the gill membranes by diffusion (*Maren et al.* 1968). In the present experiment, the concentration of sulfadiazine in the gills never exceeded that of the blood. For trimethoprim, however, the radioactivity in the gills was high until 48 hrs., but the concentration of the gills fell approximately concomitant to that of organs other than liver and kidney. These

findings indicate that excretion through the gills is of minor importance for the 2 drugs tested.

As for the urinary excretion, we do not possess at present sufficient data to make clear statements. However, the persisting high radioactivity in the kidneys after the declining of activity in other organs indicates that renal excretion takes place for both substances.

For poicilotermic animals like the fish, the metabolic rate is determined by water temperature. The results of the liquid scintillation counting showed that maximum concentration of the labelled drug in fishes kept at 15 °C was established in half the period of time of what was needed at 7 °C (Table 1). The higher metabolism rate seems to comprise the excretion rate as well, since the warm water fishes never reached the concentration level of the cold water fishes. In the cold water fishes the concentrations were still considerable at 144 hrs., while most tissues of the warm water fishes showed no radioactivity at that time. Compared to mammals, the elimination of the drugs was delayed at both temperature levels.

From a practical point of view, the accumulation of the drugs in the skin should be noted. The phenomenon has 2 important aspects. Firstly, this fact should attract attention when drug treatment for skin infections in fish is concerned. Secondly, the observation will have to be considered when withdrawal times should be stated. Previous limits have been based on the analysis of muscle tissue.

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SAMMENDRAG

Fordelingen av ^{35}S-sulfadiazin og ^{14}C-trimethoprim hos regnbueørret, Salmo gairdneri.

Fordelingen av ^{35}S-merket sulfadiazin og ^{14}C-merket trimethoprim hos regnbueørret ble undersøkt ved hjelp av helkropps autoradiografi og scintillasjonstelling. Man fant generelt en langsom gastrointestinal absorpsjon og eliminasjon sammenlignet med pattedyr. Akkumulering i hud og øye ble observert for begge de undersøkte farmaka. Resultatene indikerte også at gallen var en viktig ekskresjonsvei. Det var fremdeles en betydelig radioaktivitet i huden 144 timer etter administrering.

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