irreversibly lethal. The control larvae in unevaporated drop of water remain actively alive.

Discussion. The free-living eggs and larvae of *S. papillosus* are subjected to a great variety of changes in their ecology; in the field they have to face fluctuations of temperature and humidity every day. The present investigation and the observations of the effect of temperature on the free-living stages of this nematode by this author[8] indicate that both temperature and humidity act as concurrent factors. The eggs develop best at 30 °C/100% rel. hum., but below 87% rel. hum. their growth is arrested. The larvae survive for several days at 30 °C/100% rel. hum., but they all die within 24 h at a rel. hum. level of nearly 87% and below. They also die if exposed to desiccation for over 10 min. Prasad[6] observed, in Canada, that the eggs of *S. papillosus* were unable to develop at 92% rel. hum. and below, and that they did not show any preference for 'actual wetness'. The results of the present study, on the contrary, are suggestive of their preference for 'actual wetness'. Prasad's[6] finding about non-preference of the Canadian strain of the *S. papillosus* larvae for actual wetness appears to be significant if it is looked at from the point of view of adaptation of the parasite to escape being embedded in ice for several months of the year. In India and other tropical situations, their preference for actual wetness is a necessary adaptation to escape desiccation and subsequent death due to prolonged periods of higher environmental temperature and dryness. Prasad's[6] statement that longevity of larvae is inversely proportional to temperature increase at a given moisture level lends additional support to the adaptibility of the larvae. It can further be argued that the survival of larvae at different rel. hum. is greatly influenced by the difference of moisture level in the air at different temperatures which bring about changes in the degree of desiccation.

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Labeling of endotoxin lipopolysaccharide with technetium-99m pertechnetate[1]

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Summary. Bacterial endotoxin treated with technetium-99m can be visualized in living animals by gamma camera imaging.

Many attempts to determine mechanisms of endotoxin action have involved following the distribution and fate of toxin injected into experimental animals. Hexavalent ^{51}Cr is most commonly used for labeling endotoxin for subsequent identification in host tissues[4-7], but other isotopes such as tritium[8] and ^{32}P phosphate[9] as well as immunological tagging methods[10] have also been used.

Scintigraphic equipment is now available which permits localization of materials labeled with certain gamma-emitting isotopes. It is possible that such a system could be used with endotoxin to obtain instant dynamic localization of the toxin at any time following injection into an animal. Technetium-99m is an ideal isotope for such studies as it emits a 140 keV gamma ray which is optimal for the detection systems now in use. In addition, this isotope is easily obtainable and, due to its short halflife (6 h), it can be used in dual-isotope studies. Therefore, we have attempted to develop a method for labeling endotoxin with technetium-99m so that its in vivo kinetics in animal models can be studied using gamma camera imaging system analysis.

Materials and methods. *Salmonella typhosa* lipopolysaccharide (Westphal extraction, Difco) was suspended (10 mg/ml) in saline and labeled with either $[^{51}CrO_4]Na_2$ (New England Nuclear) as described by Zlydasyk and Moon[11] or ^{99m}Tc. For daily use, 10–15 mCi of ^{99m}Tc pertechnetate ($^{99m}TcO_4^-$) in saline was eluted from a Tc-99m/Mo-99 generator (Mallinckrodt).

$^{99m}TcO_4^-$ was reduced in the presence of endotoxin. 1 ml of the endotoxin suspension (10 mg) was added to 1 ml of a 1.0 mM $SnCl_2$ solution. 10 mCi of $^{99m}TcO_4^-$ in 0.5 ml saline was added and the entire mixture adjusted to a pH of 4–5 with NaOH. After incubation at room temperature for 10 min, the mixture was passed through 5 ml of sephadex G-25 gel (Pharmacia) in a glass pipette. Endotoxin with ^{99m}Tc was eluted in the 3–5 ml fractions, whereas free isotope remained on the column.

In vivo distribution of ^{99m}Tc-endotoxin was determined in 250 g Sprague-Dawley rats which were anesthetized with halothane (Ayerst Laboratories) and inoculated via the femoral vein with 0.2 ml (approximately 0.5 mg endotoxin)

Table 1. Sephadex G-25 gel chromatography of several endotoxin lipopolysaccharide solutions

	Series 1 Cr-51-endotoxin (n=3)	Series 2* Tc+Sn+endotoxin (n=8)	Series 3* Tc+Sn (n=8)	Series 4* Tc+Sn+gelatin (n=3)
	–	–	–	–
1–2	0.5%	0.2%	0.1%	0.06%
2–3	30.6%	5.0%	0.1%	14.5%
3–4	51.6%	26.3%	0.2%	34.0%
4–5	11.0%	21.4%	0.1%	27.6%
5–6	2.7%	1.8%	0.1%	2.6%
6–7	1.4%	0.4%	0.1%	2.6%
7–8	0.5%	0.2%	0.1%	2.5%
8–9	–	0.1%	0.1%	2.1%
9–10	–	–	–	1.8%
Gel	1.6%	44.7%	98%	10.9%

Results are given in mean percent of activity of isotope administered to the column.

Table 2. Tissue distribution of a technetium-99m-stannous ion-endotoxin mixture

Organ	5 min (n=4)	15 min (n=4)	30 min (n=4)	45 min (n=4)
Whole blood	2.4 ± 0.2%/ml	1.4 ± 0.2%/ml	1.3 ± 0.1%/ml	1.1 ± 0.1%/ml
Kidney	1.0 ± 0.2%/g	1.7 ± 0.5%/g	2.2 ± 0.2%/g	3.1 ± 0.2%/g
Whole kidney	1.4 ± 0.3%	2.1 ± 0.4%	2.8 ± 0.2%	3.8 ± 0.4%
Liver	2.5 ± 0.4%/g	2.9 ± 0.1%/g	3.6 ± 0.2%/g	3.6 ± 0.4%/g
Whole liver	29.0 ± 0.9%	34.2 ± 1.1%	42.6 ± 2.2%	42.5 ± 2.0%
Spleen	2.4 ± 0.2%/g	5.3 ± 0.8%/g	4.9 ± 0.2%/g	5.2 ± 0.7%/g
Whole spleen	2.2 ± 0.1%	5.1 ± 1.1%	5.2 ± 0.4%	5.8 ± 1.2%
Lung	1.8 ± 0.3%/g	1.6 ± 0.2%/g	1.3 ± 0.1%/g	0.8 ± 0.1%/g
Whole lung	1.0 ± 0.3%	0.9 ± 0.1%	0.8 ± 0.1%	0.5 ± 0.1%
Testicle				
Whole testicle	0.04 ± 0.01%	0.04 ± 0.01%	0.03 ± 0.01%	0.02 ± 0.01%

Data are expressed percent of the injected dose.

of the test preparation. Uptake of the radioactive material in the spleen, liver, lung, kidney, testicle and muscle as well as the clearance rate from the blood were determined at 5, 15, 30 and 45 min following injection. Specific activity in these samples was determined on a gamma well counter.

Results and discussion. With sephadex gel analysis we found that the ^{51}Cr-labeled endotoxin, the mixture of ($^{99m}TcO_4^-$ + $SnCl_2$ + endotoxin) and the mixture of ($^{99m}TcO_4^-$ + $SnCl_2$ + gelatin) all eluted greater than 50% of their activity in the 3–5 ml fraction (table 1). The mixture of ($^{99m}TcO_4^-$ + $SnCl_2$) eluted only 0.4% of the administered activity in the 3–5 ml fraction.

In vivo experiments performed with the endotoxin-containing mixture displayed a well-defined time-organ uptake relationship (table 2). Blood clearance consisted of 2 components: a 15-min, short T 1/2 component and a long T 1/2 component lasting several hours. Renal activity increased from 5 to 45 min. Isotope activity in the liver increased to 42% of the injected dose by 30 min post-injection. Lung and testicle activity decreased at the same rate as blood pool activity.

By using the methodology described in this report, we have produced an endotoxin-^{99m}Tc mixture which is distributed in vivo in a manner similar to that reported for other endotoxin-isotope preparations[4-9]. Therefore ^{99m}Tc may be a useful label for scintigraphic studies of endotoxin in vivo. Unfortunately it is still not possible to be certain that the isotope is actually bound to the toxin. Ordinarily this problem is resolved by comparing the distribution of free label to that of the labeled product. The chemistry of ^{99m}Tc, however, is very complex and not well documented with regard to labeling an organic complex such as endotoxin[12]. This isotope can hydrolyze in aqueous solution to form a colloidal complex which can localize predominantly in the liver.

We found that the elution profile of ^{51}Cr-labeled endotoxin on sephadex G-25 can be mimicked with the addition of gelatin or endotoxin to a mixture of $^{99m}TcO_4^-$ and $SnCl_2$. This could mean that the endotoxin or the gelatin act as hydrolyzed technetium colloid stabilizers. If a protein or a lipopolysaccharide happens to have a significant colloid-stabilizing effect, then there is no assurance that a stabilized technetium colloid will not pass the sephadex column and appear in the final preparation[12].

It is hoped that future studies of the biologic and biochemical properties of endotoxin-^{99m}Tc mixtures will further establish the reliability of this procedure for labeling endotoxin with this isotope.

Gamma camera image of a rabbit injected 15 min previously with the ^{99m}Tc-endotoxin mixture described in this report. This 400,000 count image was obtained with a nuclear Chicago Pho-gamma HP camera equipped with a high resolution parallel-hole collimator.

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