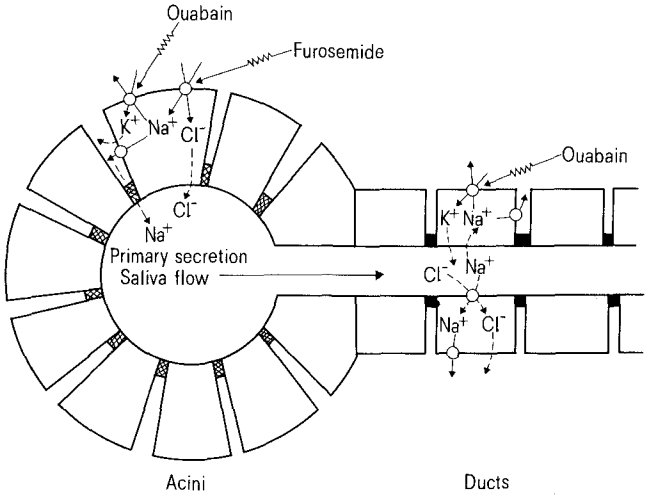

Figure 3. Proposed mechanisms of fluid formation in salivary acinar cells following stimulation with sympathomimetic agents. This mechanism seems to involve ouabain and furosemide-sensitive ion transport systems and the generation of an electrochemical gradient by diffusion of Cl^- across the luminal cell membrane, which favors the passage of Na^+ into the lumen. The presence of Na^+ and Cl^- in the lumen generates an osmotic gradient which causes water movement. An ouabain-sensitive pump is present in duct cells and modulates salivary Na^+ and K^+ concentrations.

Acinar cells are considered as the major source of salivary fluid[5] and effects of the transport inhibitors used in this study on saliva volumes can be ascribed primarily to actions on these cells. Salivary electrolytes, on the other hand, are for the most part the result of the activity of duct cells, which modify the plasma-like primary secretion produced by acinar cells, particularly when the flow is slow[5].

The presence of ouabain in the perfusate modified salivary Na^+ and K^+ concentrations, most likely as a result of inhibition of a Na^+, K^+ pump which is believed to be present in salivary ducts (fig. 3) and to be a major element in the transductal reabsorption[5] of Na^+ and secretion of K^+. The effect of furosemide on salivary electrolytes is consistent with its known action on anion transport[8] and is similar to its effect on the ion composition of saliva secreted in response to acetylcholine[2]. However, studies using the perfused rat submandibular duct[9] indicated that chloride was transported passively and that furosemide had no effect on net transport. The possibility arises, therefore, that furosemide may inhibit chloride secretion in acinar cells, a view that would be consistent with its effect on the NaCl cotransport system presumably present in these cells (fig. 3). Thus, in contrast to ouabain which inhibits the Na^+, K^+ pump in both acinar and duct cells causing, respectively, reduced fluid secretion and altered salivary Na^+ and K^+ concentrations, furosemide probably reduces fluid and salivary Cl^- concentrations by an effect on acinar cells (fig. 3). As expected, the reduced Cl^- concentration observed after furosemide was compensated by an increase in a 'residual anion' concentration calculated as $Na^+ + K^+ + Ca^{++} - Cl^-$. This is mostly HCO_3^- in rat submandibular saliva[5]. The markedly increased salivary Ca^{++} concentrations observed in the presence of furosemide with either isoproterenol or phenylephrine stimulation, do not seem to be the result of decreased volumes of secretion, since a similar effect was not observed with ouabain, at least when phenylephrine was used to stimulate secretion, despite a marked reduction in salivary volume. This effect requires further investigation since the handling of Ca^{++} by salivary glands is a complex process involving several interrelated mechanisms[5].

The presence of similar ionic mechanisms for salivary fluid secretion when cholinergic or adrenergic receptors are stimulated likely represents an integrated physiological mechanism to insure an adequate interaction of the secretory pathways for the 2 major fractions of saliva and the production of an adequate vehicle for the 'wash-out' of macromolecular components of saliva with important digestive and protective functions.

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Blood and tissue distribution of cyclosporin A after a single oral dose in the rat[1,2]

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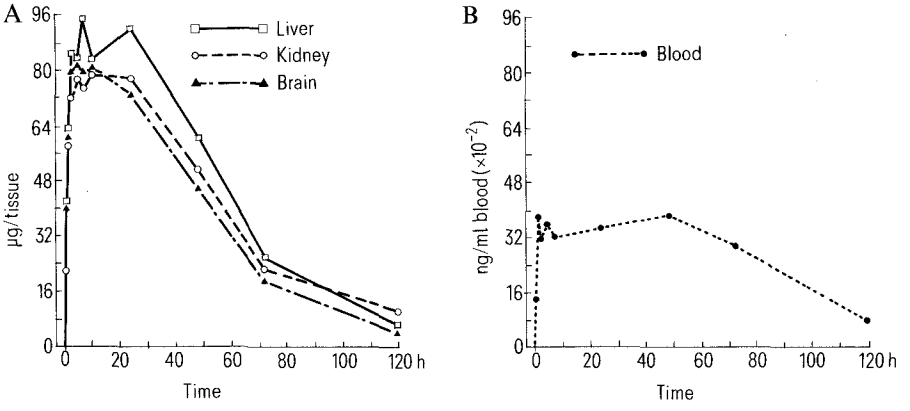
Summary. After a single oral dose of cyclosporin A (82 mg/kg) in rats, tissue (kidneys liver and brain) and blood levels reached maximum values (approximately 80 µg/g and 3.5 µg/ml) between 3 and 7 h after drug administration. Drug elimination continued for at least 5 days. The 24-h urine and bile elimination was 2% for each.

Cyclosporin A (Cy-A), a novel type of immunosuppressive agent[3], is found to be of increasing clinical usefulness in the inhibition of graft rejection in organ and bone marrow transplantations[4-7]. (For a recent review on the in vivo studies with Cy-A, see White[8]). The drug molecule is an undecapeptide of fungal origin[9] with a T-cell associated immunosuppressive activity[10]. The clinical utility of Cy-A is limited mainly by its dose-related nephrotoxicity[11,12].

Although, after short or long term administration of Cy-A, nephrotoxocity as estimated by increased serum creatinine levels has been correlated with cyclosporin concentrations[11,13,14], detailed knowledge of the tissue distribution and elimination



Concentration/time course of Cy-A after a single oral dose (82 mg/kg) in the rat: *A* tissues; *B* blood.

of the drug is lacking. We present here data on the pharmacokinetics of Cy-A in blood and tissue after oral administration to rats. Our experiments were designed in the first place to get insight into the tissue uptake and retention of Cy-A in relation to blood levels and secondly to answer the question whether the observed nephrotoxicity[11,12] could be ascribed to excessive Cy-A levels in the kidneys.

Materials and methods. Experimental procedures: 3-month-old female WAG/Rij rats bred in our own colony received a single oral dose (82 mg/kg b. wt) of Cy-A dissolved in olive oil. The drug was administered via a stomach tube. At different times after drug administration, the animals were bled under light ether anesthesia. At each time-point, the blood samples of 3 animals were pooled and processed for Cy-A determination. The animals were then killed by cervical dislocation and the organs of interest were removed and stored at −20 °C until further processing. Bile cannulation was done via the bile duct. Cy-A determinations: Cy-A was determined by radioimmunoassay[15]. Standard kits for the assay were kindly provided by Sandoz Ltd (Basel, Switzerland). All biological samples (blood, urine, bile and tissues) were tested in duplicate and quantitation was done by use of standard samples prepared in the appropriate biological substance (control blood, urine, bile and liver tissue). Unfortunately, the RIA cannot discriminate between the parent drug and its metabolites. Therefore, when cyclosporin-A levels are mentioned in the text, they mean levels of cyclosporin-A and metabolites.

Results and discussion. In our study we used an oral Cy-A dose of 82 mg/kg. The figure shows the tissue and blood concentrations vs time after a single oral dose (82 mg/kg) in the rat. For all 3 organs studied (liver, kidney and brain), the maximum concentration is about the same (of the order of 80 µg/g) and is reached at between 3 and 7 h after administration. Thereafter, there is a rather slow elimination of Cy-A from the organs; even 5 days after drug administration significant amounts of Cy-A and/or its metabolites were detected (in the order of 10 µg/g). No differences in drug uptake were found between the brain and other tissue, e.g., kidney. Apparently, owing to its lipophilic nature, Cy-A can pass the blood-brain barrier.

Blood levels of Cy-A increased rapidly after a single oral dose and remained constant for about 2 days. We chose whole blood instead of plasma or serum determinations because of the observed temperature-dependent fluctuations in plasma or serum concentrations as compared with whole blood[16]. The 24-h excretion of Cy-A in urine and bile was 2% of the total administered dose for each. To what extent these figures were due to excretion of Cy-A metabolites is unknown, because the RIA used for the determination of Cy-A in biological materials cannot discriminate between the parent drug and its metabolites as mentioned before.

Cy-A is a powerful immunosupressant which will probably play a crucial role in future immunosuppressive regimens for organ and bone marrow transplantation. Clinical studies[17,18] suggest that the observed nephrotoxicity may be reduced by dose-adjustment based upon routine monitoring of Cy-A levels. Our findings suggest that from the slow elimination rate from the tissues examined a high degree of cumulation of Cy-A and/or its metabolites can be anticipated upon chronic administration of Cy-A. In one post mortem study in man[17] in which tissue Cy-A levels were determined after repeated drug administration, it was shown that kidney tissue did not show elevated drug levels as compared with other tissues such as spleen, heart, liver and brain. Further studies will be needed to solve the problem whether the nephrotoxicity of Cy-A is due to 'excessive' drug uptake and retention by the kidneys or to a higher intrinsic sensitivity for Cy-A toxicity than other organs. Another difficulty in interpreting the blood and tissue levels in relation to nephrotoxic effects may be the fact that the ratio parent drug/metabolites is unknown at the moment. This will be of especial interest if a low bioavailability of the drug is the result of an extensive first pass metabolism by the liver[17].

We have shown here that a single oral dose of Cy-A in the rat leads to significant tissue drug levels which last for a number of days. With regard to the supposed working mechanism of Cy-A, namely, an inhibition of the generation of the helper T-cell subpopulation[19], the observed high tissue levels suggest an overdose of the drug. This could especially be the case with patients who are treated chronically (and most patients are) with Cy-A. Thus, our future efforts will be directed towards the elucidation of the pharmacokinetic behavior of Cy-A following repeated administration.

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Passage of hemolysins through the midgut epithelium of female *Ixodes ricinus* L. fed on rabbits infested or reinfested with ticks[1]

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Summary. Antibodies considered in this study are hemolysins synthesized by rabbits against sheep red blood cells. Ingested with the blood meal, they cross the tick midgut epithelium and retain their immunological properties in the hemolymph. During a reinfestation of rabbits, more ticks present these antibodies, and titres are generally higher than during a first infestation. Hemolysins are only found in ticks weighing 180 mg or more.

Rabbits infested with 10 female *Ixodes ricinus* L. acquire a resistance which disturbs the Ixodid biology[2,3]. During reinfestations, the ectoparasites often ingest less blood and lay fewer eggs. Tissue at tick fixation sites is infiltrated with mononuclear and polymorphonuclear cells, in which eosinophils and degranulating basophils are recognized, especially during reinfestation[4]. Some degranulating mast cells are also present. Animals develop immediate and delayed hypersensitivity reactions against tick saliva[3,5]. Specific antibodies against ♀ *I. ricinus* salivary glands have been detected in infested rabbits. They undoubtedly take part in establishment of immunity, since passive transfer of immune serum produce partial resistance against ticks[6,7].

Our work demonstrates that hemolysins, detected with a sensitive hemolysis reaction, cross the midgut epithelium of ♀ *I. ricinus*. They were identified in ticks fed on rabbits immunized against sheep red blood cells. The influence of anti-tick immunity on this phenomenon was assessed.

Materials and methods. Infestation of rabbits. 13 Himalayan male rabbits, weighing approximately 2 kg, were used in the experiment. Each rabbit was exposed to 14 ♀ *I. ricinus* fed under capsules on their backs.

Immunization of rabbits. Rabbits were immunized with i.v. injections of 1 ml of 10% sheep erythrocyte suspension per kg of rabbit weight[8]. 2 injections and infestation schemes were used. In 1 scheme, 5 rabbits were given injections on days 1, 2, 3, 4, 8, 10, 13 and 17 of the experiment and were infested with ticks on day 9. In another scheme, 5 other rabbits were first infested with ticks. The immunization process with sheep blood cells began on the day when the last tick dropped from the rabbit and then followed the same procedure as the first scheme. Reinfestation of the second group of rabbits took place on the 13th day of the immunization with sheep red blood cells. 3 control rabbits were not injected with erythrocytes suspensions but were infested and reinfested with ticks.

Collection of hemolymph. The process for collecting hemolymph was similar to that described by Burgdorfer[9]. Generally 2–6 µl (extreme values: 1–30 µl) of hemolymph were collected in capillary tubes. Hemolymph was stored at −20 °C until used.

Titration of hemolysins. Hemolysin titres of rabbit serum or of tick hemolymph were measured[10]. Briefly, 0.5 ml of a serum or hemolymph dilution, prepared in veronal buffer pH 7.4, was mixed with 0.3 ml guinea-pig complement (1/20) and sheep red blood cells 2%. To each tube, 1.7 ml veronal buffer was added. The mixture was incubated for 1 h at 37 °C under gentle stirring. After centrifugation (3000 t/min during 5 min), hemolysin titres in the supernatant were defined spectrophotometrically (524 nm). Specificity threshold of the reaction was fixed at 1/10 for tick hemolymph. Rabbits sera were tested from 1/100.

Results. Hemolysins cross the midgut epithelium of ticks. At the end of the blood-meal during the first infestation (day 0, first scheme), 16.0% of ♀ *I. ricinus* (4/25) show the presence of hemolysins in their hemolymph (table 1). 7 days after feeding, this percentage increases to 50.0% (8/16). During reinfestation

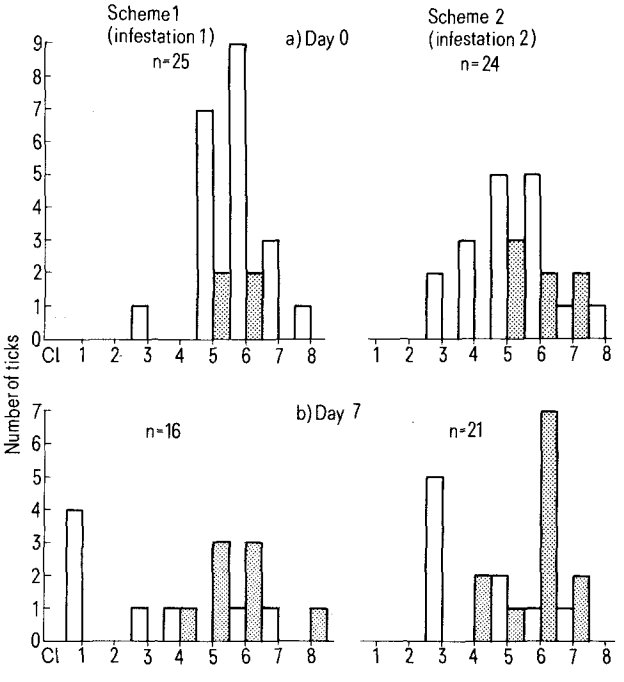

Figure 1. Relation between tick weight and hemolysins crossing. For some ticks, hemolymph was not prepared; these cases are not represented in the figure. □, ticks without hemolysin; ▨, ticks with hemolysins; Cl. 1–7: weight class of 60 mg; Cl. 8: ⩾ 420 mg.