# Defect of uric acid uptake in Dalmatian dog liver

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*Summary.* The uptake rate of uric acid into liver slices of the Dalmatian dog was found to be significantly lower compared with that in the beagle. Since there was no difference in the hepatic uricase activity between the two breeds, the abnormality of uric acid metabolism in the Dalmatian dog is considered to result from a defective hepatic transport system for uric acid.
*Key words.* Dog liver; liver, dog; uric acid uptake; liver slices; uricase, hepatic.

Purine metabolism in the Dalmatian coach hound, unlike that in dogs of other breeds resembles that of man; it is characterized by hyperuricaemia[1,2] and hyperuricosuria[3,4]. Because of these features the Dalmatian dog is especially suitable for the study of the physiological, biochemical and pharmacological actions of uric acid[5], and the purine availability of diets rich in nucleic acids[6,7], in relation to man.

Allogeneic transplantation of the livers between Dalmatian and mongrel dogs[8] revealed that it is the liver which determines the differences in uric acid metabolism. Since the hepatic uricase in Dalmatian dog is quantitatively capable of oxidizing as much uric acid as that in the mongrel dog[9] a defective urate transport system in the Dalmatian hepatocytes has been suspected[3,10,11]. However, experimental data on the rate and mechanism of uric acid uptake by liver tissue in the Dalmatian dog or in any other species have not been published up to now. The present studies were conducted to characterize urate uptake in liver slices of beagle and Dalmatian dogs.

*Material and methods.* Liver samples from nine purebred Dalmatians and six beagles, which had been used in a different research project, were obtained under an anaesthesia especially developed for dogs[12]. The liver samples were quickly removed, placed in oxygenated Krebs-Ringer phosphate solution[13] and kept at 4 °C. The liver slices, obtained with a modified Stadie-Riggs microtome, were similar in weight, averaging approximately 25 mg wet wt per slice. The extracellular volume was determined[14] with $^{14}$C-inulin (inulin-($^{14}$C)carboxylic acid, 185–370 MBq/mmol, Amersham Buchler, Braunschweig). The intracellular volume was calculated from the difference of total water content, measured by drying the tissues to a constant weight at 70 °C, and extracellular volume. The incubation medium was the Krebs-Ringer phosphate solution (pH 7.4) containing 0.1 mmol/l uric acid and (2-$^{14}$C)uric acid (1.85–2.2 GBq/mmol, Amersham Buchler, Braunschweig). The slices were preincubated in oxygenated Krebs-Ringer solution for 15 min at 37 °C and the incubation was started (zero time) by transferring the slices to vials, each with 5 ml incubation medium under an oxygen gas phase. The incubation was carried out at 37 °C with gentle shaking of the vials. At the end of the incubation periods, the slices were removed, washed in physiological NaCl soluiton, blotted, weighed and burned in a sample oxidizer (Tri-Carb, Packard Instrument, Frankfurt/M.). Radioactivity was measured by counting in a liquid scintillation spectrometer (Tri-Carb, Model 3385, Packard Instrument, Frankfurt/M.).

For the determination of uricase activity, liver samples were immediately placed in liquid nitrogen, ground to powder and homogenized (5 g wet wt/50 ml) in the Krebs-Ringer solution for 1 min (Ultra-Turax, Janke & Kunkel, Staufen/Br.). Five ml of the incubation medium with different amounts of uric acid and 2 ml of the liver homogenate were incubated under an oxygen gas phase at 37 °C. At the end of the incubation periods, the uricase reaction was stopped by protein denaturation and the uric acid concentration determined by HPLC[15]. The uricase activity was referred to the DNA content of the liver samples, the determination of which was performed by a method reported elsewhere[16]. $K_M$ and $V_{max}$ were calculated according to Lineweaver and Burk.

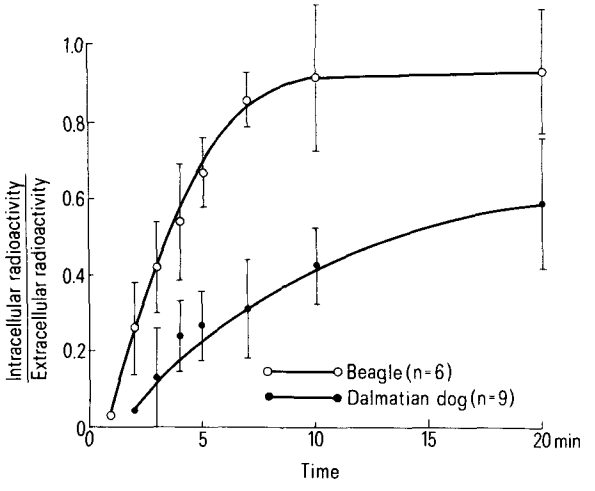
*Results and discussion.* The figure shows the time course of zero-trans influx of (2-$^{14}$C)uric acid in liver slices of beagle and Dalmatian dogs. In beagle liver, nearly complete equilibrium (92%) between intracellular and extracellular radioactivity was attained within 10 min, whereas in Dalmatian hepatocytes intracellular radioactivity has reached only 40% of extracellular radioactivity in the same time. Also, in relative terms in the initial rate of uric acid transport into the hepatocytes appeared to be about three times faster in the beagle than in the Dalmatian.

In order to exclude the possibility that the difference in the rate of uric acid uptake reflects differences in hepatic uricase activity, the kinetic parameters of this enzyme in liver homogenates of both breeds have been determined. The data were referred to the DNA content in liver, which was similar in the two breeds (Dalmatian dog: 2.4 ± 0.5 mg DNA/g wet wt; beagle: 2.2 ± 0.2 mg DNA/g wet wt). Confirming earlier studies[9,17] no differences in hepatic uricase activity between the beagle and the Dalmatian dog were found (table).

Kinetic parameters of hepatic uricase in beagle and dalmatian dogs

| | $K_M$ (μmol/l) | $V_{max}$ (nmol/g DNA × min) |
|---|---|---|
| Dalmatian dog n = 9 | 522 ± 261 | 388 ± 167 |
| Beagle n = 6 | 575 ± 271 | 445 ± 174 |

Liver homogenate was added at zero time to the incubation medium containing uric acid. Loss of urate was determined by HPLC. $K_M$ and $V_{max}$ were calculated according to Lineweaver and Burk. The results are the means ± SD.




($^{14}$C)uric acid uptake in liver slices from beagle and Dalmatian dogs. Liver slices were transferred at zero time to the incubation medium containing uric acid (0.1 mmol/l) and ($^{14}$C)uric acid. The uptake was determined by measuring radioactivity. Values shown are means of similar experiments. Vertical bars represent ± SD.

The figure shows the urate uptake in the liver slices, and the difference in the uptake rates between beagle and Dalmatian dog must originate from a defective transport system. From the finding that the hepatic 'clearance' of uric acid in the non-Dalmatian dog was greater than in the Dalmatian dog[3,10] it has been suggested that the hepatic cellular membrane prevents in some way the oxidation of uric acid in the Dalmatian liver. The data in this paper demonstrate that the uptake rate into Dalmatian hepatocytes is lower by a factor of about 3 as compared to that in the beagle. This result appears to prove that the genetic abnormality of uric acid metabolism in the Dalmatian coach hound is due to a change in urate transport through the membranes of hepatocytes.

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## Genetic analysis of permethrin resistance in the house fly, *Musca domestica* L.

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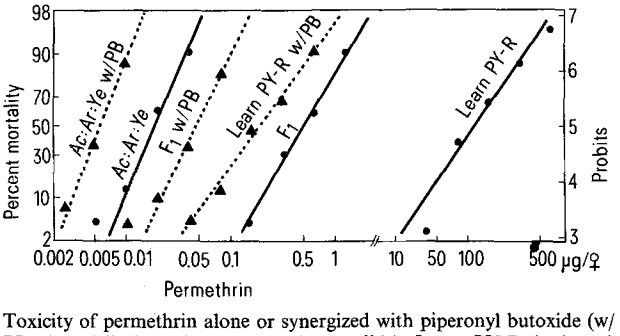
*Summary.* The genetic control of mechanisms conferring resistance to permethrin was investigated in *Musca domestica* L. Factors conferring resistance were found on autosomes 1, 2 and 3, with their relative dominant effects being 3 > 1 > 2. Three combinations of chromosomes (i.e. 2+5, 3+5 and 1+3+5) were found to interact, but in a less than additive manner. Data obtained with synergists suggest that the mixed function oxidase system is responsible for part of the resistance. Pyrethroid resistance on chromosome 1 has not been previously reported.
*Key words.* Fly, house; *Musca domestica;* permethrin resistance; resistance, genetic control.

Pyrethroid insecticides have emerged into prominence largely as a result of the successful modification of the structure of the natural pyrethrins to increase stability and insecticidal activity[2]. Although these new field-stable compounds have been used for only a few years, cases of control failures due to the development of resistance have been reported[3]. Earlier studies of the genetics of resistance to the natural pyrethrins have shown that four factors, one each on chromosomes 2 and 5 and two on chromosome 3, confer the resistance[4]. Due to changes in the structure of pyrethroids, reports that the newer pyrethroids may possess a different type of action [5-7] and the great economic importance of these insecticides, we conducted a chromosomal analysis of resistance in the house fly using the widely used pyrethroid, permethrin, in an attempt to identify the chromosomes involved and to quantify their respective contribution to resistance.

*Materials and methods.* House flies, *Musca domestica* L., were collected from a dairy (Learn) near Ithaca, New York, following three years of limited spraying with permethrin, and were subsequently selected with this insecticide in the laboratory for 18 generations to achieve a circa 6000-fold level of resistance (Georghiou et al., unpublished data). This strain (Learn PY-R) is cross-resistant to all pyrethroids tested to date and has a low to moderate resistance to several organophosphates and carbamates (Scott and Georghiou, unpublished data). Three susceptible strains were also used; *ac;ar;ye* (bearing the morphological markers *ali-curve, aristapedia* and *yellow eyes* on chromosomes 1, 2 and 4 respectively), *ac;ar;bwb;ocra* (bearing the morphological markers *ali-curve, aristapedia, brown body* and *ocra eyes* on chromosomes 1, 2, 3 and 5, respectively) and NAIDM (non-marked strain). Bioassays involved application of 1 µl acetone (controls) or permethrin in acetone to the thoracic notum of 3-day-old female flies under $CO_2$ anesthesia.




Toxicity of permethrin alone or synergized with piperonyl butoxide (w/PB, dotted line) to the *ac;ar;ye* (susceptible), Learn PY-R (resistant) and $F_1$ (*ac;ar;ye* ♀ × Learn PY-R♂) strains of house fly.