Effect of Reciprocal Allogeneic Renal Transplantation Between Dalmatian and Non-Dalmatian Dogs on Urinary Excretion of Uric Acid.* (30975)

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Most breeds of dog, including mongrels, convert most of the uric acid that is formed from the metabolism of purines to allantoin and excrete far more allantoin than uric acid in the urine. The Dalmatian dog (English coach dog), however, excretes relatively small amounts of allantoin and large amounts of uric acid in its urine(1). The conversion of uric acid to allantoin in dogs occurs in the liver and ceases once the liver has been removed(2,3). The livers of Dalmatian and non-Dalmatian dogs both contain the enzyme, uricase, which catalyzes the oxidation of uric acid to allantoin(4). Friedman and Byers (5) found that the sum of the uric acid and allantoin excreted per unit time in the urine of Dalmatian and of non-Dalmatian dogs was virtually the same. These workers observed also that although the Dalmatian dog converted blood uric acid to allantoin less readily and completely than did the non-Dalmatian, the difference was not sufficiently great to account for the Dalmatian's large urinary output of uric acid and small output of allantoin. Friedman and Byers also found that although the renal clearance of uric acid and of creatinine were approximately the same in the Dalmatian, the clearance of uric acid in the non-Dalmatian dog was much less than the creatinine clearance. This they interpreted to indicate that the Dalmatian, unlike the non-Dalmatian, did not reabsorb uric acid in the renal tubules so that it could be converted to allantoin in the liver and, therefore, excreted larger amounts of uric acid in the urine. Wolfson and associates(6), using a different method for estimating urate, found that the renal clearance of urate in the Dalmatian dog exceeded the glomerular filtration rate and concluded that active tubular secretion of urate must occur in these animals. Evidence from stop-flow analysis of renal function has been added since to support this conclusion(7,8). The mongrel dog normally shows net tubular reabsorption of uric acid although renal tubular excretion of uric acid has been demonstated when sodium urate is given intravenously during osmotic diuresis induced by administration of mannitol(9).

Now that methods are available to reduce the immune response that follows the allogeneic transplantation of a kidney from one dog to another and, therefore, to delay rejection of the transplanted kidney, the concept that the peculiarity of urinary excretion of uric acid by Dalmatian dogs is predominantly renal in origin can be tested directly. The present report describes such tests performed by preparing Dalmatian dogs with non-Dalmatian kidneys and non-Dalmatian dogs with Dalmatian kidneys and by measuring urinary excretion of uric acid subsequently.

Methods. The concentration of uric acid in urine and in serum was measured by the uricase method described by Kalckar(10) and modified by others(11-13). Uric acid, unlike allantoin, absorbs ultraviolet light at a wavelength of 293 mμ. With this method, the absorbancy was measured spectrophotometrically† both before and after the uric acid was converted to allantoin by commercially prepared uricase.‡ The concentration of uric acid in solution is proportional to the difference in absorbancy before and after enzymatic action. Some of the urate in the urine, especially from Dalmatian dogs, was found to be precipitated, and estimations of uric acid from the supernates resulted in values that were falsely low. Therefore, as a

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† Beckman DU, Beckman Instruments, Fullerton, Calif.

‡ Dermatube-U, Worthington Biochemical Corp., Freehold, N. J.

preliminary step, precipitated urates in a 5-ml aliquot of mixed urine were dissolved by adding 40 ml of distilled water and 5 ml of a supersaturated solution of lithium carbonate and placing the mixture in a water bath at 100°C for 10 minutes.

Dogs were kept in metabolic cages in an air conditioned environment at a temperature of 72°F. As a routine, the animals were fed 2 cans of dog food§ daily and were given water *ad libitum*. Twenty-four-hour specimens of urine were collected without the use of a preservative, the volume and specific gravity were measured, and an aliquot was used for measurement of the concentration of uric acid. Catheterization of the urinary bladder was avoided, because it was considered that significant infection might occur in dogs receiving immunosuppressive therapy. Therefore, because the amount of urine remaining in the bladder at the end of any 24-hour period was uncertain, the values for the output of uric acid per 24 hours cannot be considered to be precise, although the mean values of successive daily tests are more nearly so.

In preliminary testing, a group of Dalmatian and a group of non-Dalmatian dogs were compared with respect to the concentrations of uric acid in serum and the daily output of uric acid in urine. The latter parameter characterized Dalmatian and non-Dalmatian dogs into 2 clearly separated groups and was used in subsequent experiments to assess the effect of reciprocal renal transplantation on the urinary excretion of uric acid.

For allogeneic renal transplantation, the dogs were operated on in three groups of three, composed of one Dalmatian and 2 non-Dalmatians, in each of which several measurements of daily urinary excretion of uric acid had been made preoperatively. Each of the non-Dalmatian dogs received a kidney from the Dalmatian and had its own kidneys removed. A kidney from one of the non-Dalmatian dogs was transplanted to the Dalmatian. At surgery it was discovered that one Dalmatian dog had a kidney that was unsuitable for transplantation because it had 2 small renal arteries instead of a single artery and, therefore, the final group of dogs with allogeneic renal transplants consisted of 3 Dalmatians and 5 non-Dalmatians. The kidneys were transplanted intraperitoneally into the pelvic regions of recipients using, in each case, an end-to-end anastomosis between the renal artery and the central end of the divided common iliac artery, an end-to-side anastomosis between the renal vein and the common iliac vein, and an anastomosis between the ureter and the urinary bladder. Thus, finally, each Dalmatian had only a single kidney from a non-Dalmatian dog and each non-Dalmatian had only a single kidney from a Dalmatian dog. Each animal was given 1,000,000 units of aqueous penicillin intraperitoneally at the conclusion of the operation and 400,000 units of procaine penicillin with 500 mg of streptomycin intramuscularly daily for 5 days thereafter.

Azathioprine (Imuran‖) was the principal immunosuppressive agent used in these experiments and was given orally daily in single doses. The initial doses, given for 2 days before operation, were 8 to 10 mg per kilogram whereas subsequent daily doses varied from 1 to 6 mg per kilogram. The dosage of azathioprine was adjusted in response to daily estimations of blood urea nitrogen concentration (BUN) and the leukocyte and platelet counts, with the goal of giving enough of the drug to keep the BUN as low as possible without producing dangerously low concentrations of leukocytes and platelets. Blood hemoglobin concentrations and hematocrit values were estimated weekly.

In 5 of the 8 dogs operated on, actinomycin C (Sanamycin¶) was used periodically in doses of 10 μg per kilogram intravenously in addition to azathioprine. Three of these animals also underwent X-irradiation to the region of the transplanted kidneys on the day of surgery and the 2nd, 5th, 7th, and 9th days thereafter in doses of 150 rad from a 6-mv linear accelerator.

Results. Serum uric acid concentrations in 5 Dalmatian dogs that had not been fed for

§ Hill's Dog Food, Hill Packing Co., Topeka, Kan.

‖ Burroughs Wellcome and Co., Tuckahoe, N. Y.

¶ FBA Pharmaceuticals, New York.

TABLE I. Serum Uric Acid Concentrations and Daily Urinary Excretion of Uric Acid in Intact Dalmatian and Non-Dalmatian Dogs.

Type of dog	n*	Serum uric acid (mg/100 ml)	n*	Urinary uric acid (mg/24 hr)
Dalmatian	5	1.3 ± .22†	7	461 ± 28
Non-dalmatian	4	.5 ± .13	10	58 ± 6.8

* No. of dogs.
† Mean ± standard errors of mean.

24 hours averaged 1.3 mg per 100 ml (standard error 0.22). The comparable mean value for 4 non-Dalmatian dogs was 0.5 mg per 100 ml (standard error 0.13). The differences in daily urinary excretion of uric acid between the two classes of dogs were much greater, the mean values being 461 ± 28 mg per 24 hours for 7 Dalmatians and only 58 ± 6.8 mg per 24 hours for 10 non-Dalmatians (Table I). Clearly, the urinary excretion of uric acid was a more valid parameter for differentiating the two groups of dogs and for studying the effects on uric acid metabolism of transplantation of kidneys between them.

In this series of renal allografts, one Dalmatian dog lived 43 days with a non-Dalmatian kidney and one non-Dalmatian dog lived 36 days with a Dalmatian kidney. Each animal had renal function that was sufficient to maintain the BUN at concentrations less than 35 mg per 100 ml (normal, 10 to 15 mg per 100 ml) for several days during which time the animals appeared healthy and ate normally. During these periods, the mean urinary excretion of uric acid was 555 mg per 24 hours for the Dalmatian dog and 51 mg per 24 hours for the non-Dalmatian, values not significantly different from those measured before renal transplantation in the same dogs (Table II). Additional evidence that the urinary excretion of uric acid remained characteristic of the host, despite the presence of only a foreign kidney, is supplied by data from another Dalmatian in which the average daily output of uric acid in urine, which was 484 mg per 24 hours before renal transplantation, remained as high as 205 mg per 24 hours after operation despite impairment of renal function so that the BUN ranged from 70 to 80 mg per 100 ml. In addition, a non-Dalmatian dog with a Dalmatian kidney, maintaining BUN values ranging from 50 to 70 mg per 100 ml, excreted an average of only 32 mg of uric acid daily (Table II).

The 4 remaining dogs lived for too short a time to provide data.

Discussion. Without treatment, dogs with only allogeneically transplanted kidneys usually live for from 1 to 2 weeks, with renal function gradually becoming worse and finally ceasing as the kidney undergoes progressive rejection. With immunosuppressive therapy, such transplanted kidneys may function longer, although variation from almost no prolongation of function to months of life-supporting function are encountered. Apart from failures caused by technical problems, these variations are attributed currently to differences in histocompatibility between donors and recipients that are controlled genetically and dictated by chance. Ordinarily many experiments must be done to find a combination of animals in which small and therefore tolerable doses of immunosuppressive drugs impair the immune response sufficiently to permit prolonged survival with good renal function.

TABLE II. Effect of Replacing Kidney of Dalmatian Dogs with Kidney from Non-Dalmatian Dogs and *vice versa* on Urinary Excretion of Uric Acid.

	Mean urinary excretion of uric acid					
Host	n*	With own kidney (mg/24 hr)	n*	BUN (mg/100 ml)	After renal transplantation (mg/24 hr)	Difference (mg/24 hr)
Dalmatian	3	607 ± 67†	5	<35	555 ± 36	— 52
Non-dalmatian	4	39 ± 6.0	8	<35	51 ± 5.8	+ 12
Dalmatian	9	484 ± 39	5	70–80	205 ± 31	—279‡
Non-dalmatian	7	46 ± 6.8	6	50–70	32 ± 5.6	— 14

* No. of observations.
† Mean ± standard error.
‡ Significantly different ($P < .001$).

In this small series the results indicated that Dalmatian dogs with allogeneic transplants of non-Dalmatian kidneys continued to excrete the large amounts of uric acid in urine daily that are typical of Dalmatians and that non-Dalmatian dogs with Dalmatian kidneys excreted uric acid in the small amounts typical of non-Dalmatian dogs. Renal function was impaired to a greater or lesser degree in all the dogs postoperatively, but because severe impairment of renal function curtails excretion of uric acid sharply, the persistently high values obtained in the Dalmatian dogs, and their differences from the normal values obtained in the non-Dalmatians, seem to us to be significant. Although antibiotics and various immunosuppressive agents including a purine analogue, azathioprine (1-methyl-4-nitro-5-imidazolyl-6-thiopurine) were necessary complications, these agents were given to Dalmatian and non-Dalmatian dogs alike and cannot be considered to have been determining factors in the results that were obtained.

To account for these findings, while retaining the concept that differences in the urinary excretion of uric acid by Dalmatians and non-Dalmatians result principally from differences in the kidneys of the two kinds of dogs, it would have to be assumed that, immediately after allogeneic transplantation, the kidney of one kind of dog adopted the functional characteristics of the kidneys of the other. We know of no data to support this assumption. There seems to be no doubt that in Dalmatian and non-Dalmatian dogs the kidneys handle uric acid differently, but probably it will prove profitable to accept the doubt cast by these experiments on the concept that the kidney itself is responsible for the contrasting patterns of urinary excretion of uric acid and to reevaluate the problem of transport of uric acid to the renal cells in the two kinds of animals.

Summary and conclusions. When a kidney from a non-Dalmatian dog was substituted for the kidneys of a Dalmatian and *vice versa,* with rejection delayed by immunosuppressive therapy, the Dalmatian recipient continued to excrete the large amounts of uric acid in the urine that are characteristic of the species whereas the non-Dalmatian continued to excrete small amounts of uric acid, characteristic of non-Dalmatians. These data cast doubt on the concept currently held that the kidneys are predominantly responsible for the differences in urinary excretion of uric acid, characteristic of Dalmatian and non-Dalmatian dogs.

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