TABLE II. Thymus Weights of Young and Adult Pyridoxine-Deficient Pair Weighed and Normal Rats.

Series	Treatment	Weeks on diet	*Ad libitum* normal		Pair weighed normal		Deficient	
			No. of rats	Wt of thymus, mg	No. of rats	Wt of thymus, mg	No. of rats	Wt of thymus, mg
Young	Depletion	5	11	323 ± 26	12	125 ± 10	9	31 ± 3
Adults I	"	9	5	309 ± 22	5	104 ± 12	5	30 ± 3
	"	8	10	190 ± 17	10	95 ± 9	10	38 ± 4
Adults II	Replacement	7*			7	205 ± 13	7	218 ± 12

* 7 weeks of replacement following 8 weeks of depletion.

anition induced lymphopenia of the same degree in young and adult pyridoxine-deficient animals and their respective pair weighed controls. Granulocytosis occurred only in the pyridoxine-deficient young and adult animals. All blood and thymus changes were rapidly restored to normal following realimentation of the deficient and pair weighed adult animals.

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Received September 15, 1953. P.S.E.B.M., 1954, v85.

Effect of Streptococcal Hyaluronic Acid (SHA) in Mice, Rats, Rabbits and Dogs. (20911)

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There are no published data on the toxicological effects of hyaluronic acid prepared from pathogenic bacteria, although pathological changes in tissues of the host have been attributed to the hyaluronic acid produced by invading organisms(1,4).

Materials and methods. The sodium salt of SHA was prepared by Dr. George H. Warren from a virulent strain of *Streptococcus pyogenes*, Group A Type 9 (T9/63/3) obtained from the Hospital of the Rockefeller Institute. It contained 3.67% N, and was free from S, P, and halogen. All solutions of SHA for injection were prepared with physiological salt solution. Autopsies were performed on all the animals listed in Table I, and on a similar number of controls. Sections of heart, lung, liver, kidney, spleen, thyroid, adrenal, pituitary, thymus, bone marrow, pancreas, salivary gland and lymph node from all animals were prepared for microscopic study. In addition, brain and aorta from

TABLE I.
Toxicity of Streptococcal Sodium Hyaluronate.

Species	Dose, mg/kg	Route	No.	Deaths
Rat	150	IV	28	0 in 10 days
	10/day	IP	14	0 after 13 inj.
Mouse	10	IV	10	0 in 10 days
	100			0
	125			0
	160			0
	200			0
	250			0
	320			0
	400			0
	500			0
	630			0
	800			0
	1000	IV	10	0 in 10 days
	10/day	IV	20	0 after 14 inj.
	150/day	IP	20	0 *idem*
Dog	10	IV	4	0 in 10 days
Rabbit	5	IV	14	1 in 14 days
	100/day	IV	7	0 after 14 inj.
	25	IP	16	0 21
	8	IV	9	0 28
	8	IV	9	0 42

some of the animals were also included. The sections were stained with hematoxylin and eosin, trichrome stain and the combined polysaccharide stain of Ritter and Oleson(2). Pituitary was stained with aniline blue and bone marrow with Giemsa stain. The body weight of the dogs was recorded daily and that of the rabbits weekly. Seven rabbits received intravenously 100 mg SHA/kg daily for 14 days and were catheterized at 8 A.M. on days 0, 0′, 3, 7, and 14. The urine specimens were analyzed for glucosamine content(3).

Results. The non-toxicity of SHA is evident from the data contained in Table I. Most of the animals received amounts of SHA far in excess of that which would be released in a massive septicemia with *Streptococcus pyogenes*. The weight of the heart, lungs, liver, kidneys, spleen, thyroid, adrenals, pituitary and thymus were not significantly different from the weights of these organs obtained from the control animals. The veins used for multiple injections showed no evidence of gross inflammatory changes or thrombosis when proper injection technic was employed. Animals that received multiple doses by intraperitoneal injection showed no residue of unabsorbed material, nor were there signs of gross peritoneal inflammation or irritation. On gross examination of the viscera, abnormalities other than those common to laboratory animals were not noted. Thymus weights showed some decrease and adrenal weights an increase in animals that received daily intravenous injections of 100 to 150 mg SHA/kg.

Only the animals that received daily intravenous injections of 100 to 150 mg SHA/kg showed significant microscopic changes; these consisted of lymphoid atrophy in the thymus and depression of spermatogenesis characteristic of the alarm reaction. In none of the animals was there evidence of injury to the parenchyma of the liver, kidney or other organs, or to the ground substance, fibres or cells of the mesenchyme. There was no significant storage of injected polysaccharide by the macrophages of the liver, spleen or lymph nodes, or in the free phagocytic cells. Granules in these cells were stained by the iron-ferrocyanide reaction in some of the animals that received 150 mg SHA/kg but the incidence and intensity was no greater than that seen in the untreated controls.

In the rabbits that received daily intravenous injections of SHA, the glucosamine content of the 8 A.M. urine specimen obtained by catheterization on 2 consecutive days rose from a mean pretreatment level of 18 mg % $\pm$ 5.3% (S.D.) to 72 to 94 mg % $\pm$ 7.9% (S.D.) on the 3rd, 7th and 14th day of treatment; this indicated that a considerable portion of the injected SHA was excreted as free glucosamine or as unchanged polymer.

Summary. Sodium hyaluronate prepared from a virulent strain of *Streptococcus pyogenes* was injected intravenously and intraperitoneally into mice, rats, rabbits and dogs. Single injections up to 1 g/kg body weight and repeated daily injections up to 100 mg/kg for periods of 10 to 42 consecutive days failed to produce pathological changes in the tissues. There was no evidence that this material was stored and it appeared to be either metabolized or excreted in the urine.

Addendum. A paper by Di F. Tinacci and G. Benassi (*Arch. Sci. biol.*, 1953, v37, 114) has recently become available. These authors studied the fate of injected hyaluronic acid

and chondroitin sulfate in a small series of rats and rabbits. They used purified hyaluronate prepared from human umbilical cord but did not give any analysis for the degree of purity. They administered only single doses which were much smaller than the large doses which we used. Their findings were essentially the same as ours with the exception that there may have been an antigenic response in their animals. In our study there were no signs of antigenic response in the tissues nor could we detect antibodies to hyaluronate in the blood of our animals.

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Received February 15, 1954. P.S.E.B.M., 1954, v85.

Creatinine Excretion in Renal Failure.* (20912)

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Early studies on the metabolism of creatinine revealed that the daily urinary excretion was remarkably constant for a given individual(1). It was hoped that if this were the case at varying levels of function, establishment of this constant value for urinary creatinine would allow for its subsequent replacement by k in the conventional clearance equation, $C = UV/P$.† Rewriting the equation $C = k\ P$ would then permit an approximate estimation of C, adequate for clinical purposes, by the single determination of P. The studies to be reported revealed that UV decreased gradually as P increased in renal failure, and therefore UV could not be replaced by a constant. Attempts to determine the mechanism of this decrease are described.

Methods and materials. The Brod and Sirota(2) modification of the Bonsnes and Taussky(3) method was used for the determination of serum and urine creatinine. A modification of this method was used to determine the urine creatine. Fecal creatinine was determined by the method of Williams and Dick(4). All patients were males. Twenty-four hour urine collections were made on patients with chronic renal disease and on normal controls. Patients with acute renal disease may have rapidly changing serum and urine creatinine values, and were excluded. It was believed that the creatinine recovered from these patients would be affected by rapid changes in renal function, and would not always represent the actual 24-hour excretory requirement.

Observations. Table I summarizes the relationship of the serum creatinine values to the amount of creatinine excreted into the urine. Calculation of the relationship of the individual urine creatinine values (y) to the related serum creatinine values (x) by the method of least squares revealed a regression with the equation $y = 1.35 - 0.042x$. Application of Student's distribution(5) indicated that the p value for zero slope was less than 0.01. It therefore is apparent that there is a significant decrease in urinary creatinine as the serum creatinine increases. To our knowledge the only previous reference to this observation was made by Steinitz and Türkand(6), who reported that the product of

* This work was supported in part by a grant-in-aid from the U. S. Public Health Service; and the Riker Laboratories, Inc., Los Angeles, Calif.

† Where C is clearance in ml/unit time, U is urinary concentration of creatinine in mg/ml, V is urine volume/unit time, and P is plasma concentration of creatinine in mg/ml.