gine. This variation in nutritional requirement has been retained in JA-1 and JA-2 for 23 and 16 subculture generations *in vitro* and in 3 and 18 *in vivo* transplant generations, respectively. Thus, this change was stable and heritable under the conditions described. Some growth and histological characteristics of these variants were described.

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Plasma Ribonuclease Uptake by Rat Kidney Cortex.* (24806)

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Marked increases in serum ribonuclease (RNase) activity follow bilateral nephrectomy, hydronephrosis(1,2) or temporary ligation of the renal pedicles(3); and similar increases are also found in human renal disease (4,5). Further work(6) suggested that serum RNase is inactivated by kidneys, for only minor variations in serum enzyme activity were detected in dogs on which the ureter-venous anastomosis was performed bilaterally. A mechanism of inactivation agreeing with histological and physiological information obtained mainly with administered proteins (*cf.* 7,10), and involving reabsorption of filtered RNase followed by inactivation by the tubular cells was therefore suggested(6,11). It will now be shown that, fitting with above hypothesis, a marked correlation is found between blood serum and kidney cortex RNase activities; further, that enzyme activity increases in the remaining kidney soon after unilateral nephrectomy.

Material and methods. Male Wistar rats, weighing 150-300 g were used. Blood samples of approximately .5 ml were taken from tail tips after warming animals at 37°C for 10 min. Unilateral nephrectomy was performed under ether anesthesia through a lumbar approach. An equal number of left and right kidneys were removed. Sham operated animals were included in several experiments. After removal of kidneys, the capsule was stripped off, and slices less than 1 mm thick were made with razor blade. Half percent homogenates in .85% sodium chloride containing 4% (v/v) n-butanol were prepared with glass homogenizer and Teflon pestle and stored at 2°C. Preliminary experiments showed that the activities obtained with this medium(12) were higher than those of water homogenates. Part of the activity of homogenates, however, was still particle-bound, for it could be sedimented by centrifugation at 5000 g for 15 min. Triplicate samples of 5 μl serum or 50 μl of homogenates were assayed for RNase activity at pH 7.4 as previously described(1,3). Results are expressed in optical density units after subtraction of appropriate blanks. In some experiments "alkaline" phosphatase activity was estimated in 5 μl samples of serum or 20 μl samples of kidney homogenates with disodium phenylphosphate as substrate(13).

Results. Correlation between blood serum and kidney cortex RNase activities. Fig. 1 shows results of 2 experiments in which 31 rats were used. No significant difference

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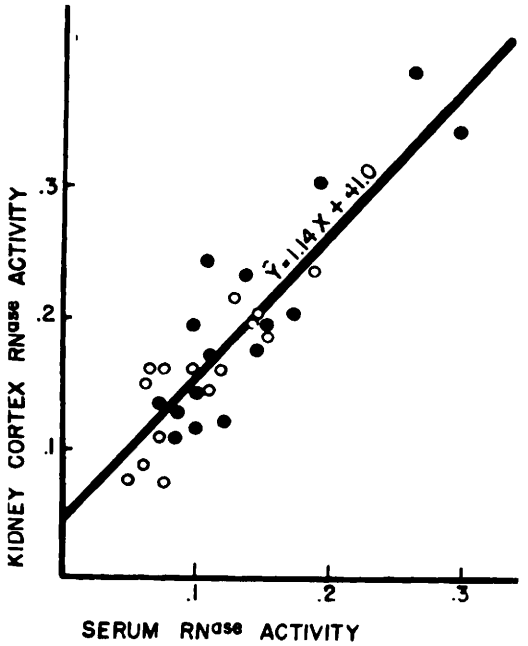

FIG. 1. Kidney cortex RNase activity against serum enzyme activity (optical density units). Each point stands for individual rat. Open and closed circles represent 2 different experiments.

was found between results of the 2 experiments when separately analyzed by standard regression technics(14). A regression line for the whole data was therefore calculated. The 95% limits for the regression coefficient were .91 and 1.37. The calculated correlation coefficient was + .88. Fig. 1 also shows marked individual variability between intact animals, as previously noted(3,11). When "alkaline" phosphatase was assayed in similar material, no significant correlation was found between blood serum kidney cortex enzymatic activities.

Kidney cortex RNase in remaining kidney after unilateral nephrectomy. Table I shows that as early as 2 hours after nephrectomy, kidney cortex RNase activity increases significantly in the remaining kidney and remains elevated to 72 hours, the maximum time period studied. Differences between paired values were analyzed by the *t* test. Data in Table I were gathered in several experiments on different occasions, so that no stress should be put on differences between values for various time-periods of nephrectomy. Possibly due to high individual variability and relatively small number of animals studied, no temporal trend is discernible in the results. No significant difference in serum RNase activity before and after unilateral nephrectomy was found when at least 5 animals of each of the 50′, 2 hr, 4 hr, and 8 hours groups were sampled. No variations in "alkaline" phosphatase activity of the homogenates were found 4 hours after nephrectomy.

Discussion. The above correlation between kidney cortex and serum RNase activity suggests a relationship between the two leading to at least 3 hypotheses: a) plasma RNase originates from the kidneys; b) kidney RNase is taken up from plasma, and c) plasma RNase comes from one or a number of tissue sources, whereas the kidney produces its own enzyme; there would be a parallelism between ability of tissues to produce serum RNase and that of the kidney cortex in manufacturing its enzyme.

Hypothesis a) does not seem tenable in

TABLE I. Effect of Unilateral Nephrectomy on Kidney Cortex RNase Activity.

Treatment	Time after nephrectomy	No. of animals	RNase activity* Control kidney	RNase activity* Remaining kidney	Difference ± stand. error of differences	P
Sham-operated†		27	164 ± 65.7‡	159 ± 64.9§	5.0 ± 3.2	.05
Nephrectomy	50 min.	6	128 ± 23.0	146 ± 35.1	18.0 ± 8.9	"
	2 hr	6	150 ± 40.5	191 ± 35.3	41.0 ± 10.0	.02
	4	11	139 ± 74.4	208 ± 66.7	69.0 ± 13.5	.001
	8	5	227 ± 58.0	282 ± 32.0	57.0 ± 13.1	.05
	22	9	191 ± 40.0	306 ± 49.7	114.7 ± 14.5	.001
	72	8	156 ± 82.2	230 ± 73.1	74.0 ± 13.5	"

* Mean ± stand. dev.
† Including animals studied after several time periods.
‡ Right kidney.
§ Left kidney.

view of serum enzyme increases induced by bilateral nephrectomy(1); hypothesis b) is supported by data referred to in the introduction; it could imply a biochemical similarity between kidney cortex and serum enzymes. The few data available permitting such a comparison(15,16) suggest that they share common properties. A high RNase activity in kidney "droplets" has been reported by Strauss(17,18); however, determinations were performed at pH 5.0, so that it cannot be assumed that the same enzyme is being assayed. It seems that the amount of reabsorbed RNase is proportional to blood enzyme levels —a reasonable assumption—and that a constant fraction of reabsorbed enzyme is inactivated. In that way serum-kidney cortex correlation would arise. Hypothesis c) gains no support from the literature and involves assumptions as yet unproved; it does not conflict necessarily with hypothesis b).

Should the second hypothesis be confirmed we would have a clear cut example of a tissue enzyme taken up from blood plasma. Whether a similar situation occurs in other organs or other kidney enzymes having a similar origin, should be tested. The "alkaline" phosphatase determinations suggest a different behavior from that of RNase. However, we found a marked lability in serum "alkaline" phosphatase in sham-operated rats (unpublished), possibly influenced by hormonal mechanisms (19). This, together with the probably smaller glomerular clearance of the enzyme, which would delay equilibration between kidney cortex and plasma, may account for the difference.

Increased kidney cortex RNase activity after unilateral nephrectomy may be ascribed to compensatory hyperfunction. As no significant changes were observed in blood serum RNase after that procedure, the increases in kidney enzyme could be the result of a higher load of filtered enzyme due to increased GFR. Alternatively, rate of athrocytosis could be increased in absence of other functional changes.

Study of kidney cortex RNase under other experimental conditions is being undertaken. It is suggested that this enzyme may serve as a useful endogenous biochemical indicator for study of "athrocytosis" by the kidney (*cf.* 20).

Summary. A marked correlation was found between kidney cortex and serum "alkaline" RNase activity in normal rats. The cortex enzyme increased in the remaining kidney after unilateral nephrectomy. It is suggested that the kidney cortex enzyme is taken up from blood plasma through reabsorption from the glomerular filtrate.

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