Peterson, J. E., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 163.

4. Wilens, L., McClusky, T., Somoza, C., *ibid.*, 1956, v93, 121.

5. Wilens, L., Gallo, G., *A. M. A. Arch. Path.*, 1957, v64, 570.

6. Hollister, L. E., Kanter, S. L., *Gastroenterol.*, 1955, v29, 1069.

7. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.*, 1952, v195, 357.

8. Erspamer, V., Correale, P., Fioredonata, L., *Arch. Internat. Pharmacodyn.*, 1956, v106, 122.

9. Shore, P. A., Silver, S. L., Brodie, B. B., *Science*, 1955, v122, 284.

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Mitosis Stimulating Factor in Serum of Unilaterally Nephrectomized Rats.* (24347)

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Glinos and Gey found that serum from partially hepatectomized rat has growth-promoting effects on primary liver explants and on strain of fibroblasts cultured *in vitro* with respect to initiation of outgrowth, organization and morphology of colonies, and duration of growth period(1). In addition, Friedrich-Freska and Zaki injected a rat with rat serum obtained 2 days after partial hepatectomy and found that the number of mitoses in liver increased 20 times whereas normal serum caused only a slight increase. The effect of serum was specific for liver and due to non-dialyzable protein (albumin or globulin) fraction(2). Teir and his co-workers investigated a growth-stimulating factor in liver homogenates from newborn or partially hepatectomized rats. When such homogenates are injected intraperitoneally into intact rats, the number of mitoses in liver of normal rat increases(3). They suggested existence of a local growth factor in addition to the general systemic growth regulation. In the present investigation, an attempt was made to demonstrate the presence of a growth-promoting factor in serum of unilaterally nephrectomized rat with respect to mitotic activity.

Methods and materials. Outer medullas of kidneys from Holtzman strain albino male rats, weighing 60 to 80 g, were cut in fragments approximately 1 mm square and explanted in plasma clot on 12 × 50 mm cover glass No. 1. The clot consisted of equal parts of heparinized rooster plasma and embryonic extract from chicken incubated for 8 days. Three explants were placed on each cover glass, and after clot had become firm, 2 such cover glasses were inserted back to back in one roller tube. Two to 3 hours after explantation, 2 ml of Eagle's medium were added to each tube. The cultures were incubated at 37°C, and after 3 days the cultures growing most abundantly were chosen and divided into 2 groups; experimentals and controls. The media of cultures were changed, and experimentals incubated at 37°C with serum (diluted to final concentration of 15 to 20% with Eagle's medium) from the unilaterally nephrectomized rat, obtained on 2nd and 15th postoperative days, and in one case, with serum from sham operated rat, obtained on 2nd postoperative day. Controls were incubated with serum (diluted in the same way as the experimentals) from the normal rat.

After incubation with serum for 3 days, the cultures were fixed and stained according to Jacobson's method for determination of mitotic ratios. The pancreas, anterior pituitary, and bladder of the rat (for organ specificity) and the outer medulla of puppy kidney (for species specificity) were cultured and treated in the same way. Blood was drawn aseptically from the rat by cardiac puncture using

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TABLE I. Mitotic Ratios in Cultures of Outer Medulla of Rat Kidney (in % of Total Counts).

Serum used	Controls R	P	M	A	T	N_t (N_e)	Exp.* R	P	M	A	T	N_t (N_e)
Untreated 1.	97.36	.45	1.05	.35	.79	2002 (6)	94.52	1.79	2.34	.50	.85	2007 (5)
			—2.64—						—5.48—			
2.	97.50	.35	.90	.40	.85	2005 (4)	96.20	.60	1.40	.65	1.15	2004 (6)
			—2.50—						—3.80—			
Sham operation	99.21	.29	.25	.10	.15	2028 (6)	99.31	.15	.34	.15	.05	2043 (7)
			—.79—						—.69—			
Obtained on 15th post-op. day	99.20	.20	.40	.05	.15	2005 (9)	99.65	.05	.20	.00	.10	2008 (10)
			—.80—						—.35—			
Heated at 56°C 1.	99.33	.16	.23	.11	.17	6138 (4)	97.81	.54	.74	.31	.60	6113 (5)
			—.67—						—2.19—			
2.	98.26	.42	.75	.24	.33	6069 (7)	96.69	.78	1.21	.43	.89	5842 (6)
			—1.74—						—3.31—			
Heated at 100°C	97.91	.46	.71	.34	.58	4122 (3)	98.07	.52	.64	.35	.42	4071 (2)
			—2.09—						—1.93—			
Dialyzed against Gey's B.S.S.	99.91	.00	.00	.09	.00	1149 (7)	99.40	.05	.25	.10	.20	2002 (4)
			—.09—						—.60—			

* Serum was obtained on 2nd postoperative day following uninephrectomy except as noted.

R, resting stage; P, prophase; M, metaphase; A, anaphase; T, telophase; N_t, total No. of cells counted; N_e, No. of explants.

a syringe containing 0.2 ml of heparin solution (2×10^{-5} unit/ml) in Gey's balanced salt solution (B.S.S.), centrifuged and the serum used for incubation with tissue cultures. Serum was diluted to final concentration of 15 to 20% with Eagle's medium before incubation. Rate of mitotic division of epithelial-like cells of cultures was estimated by recording % cells in mitotic stages out of total cells counted. Samples were pooled from 1 to 10 explants and in most cases each sample consisted of slightly over 2000 cells. The results are shown in Tables I and II.

Results. The mitotic ratio in epithelial-like cells from the outer medulla of rat kidney, cultured *in vitro* and incubated with serum obtained from unilaterally nephrectomized rats on 2nd postoperative day, was

TABLE II. Influence of Serum from Uninephrectomized Rats upon Mitotic Ratios of Puppy Kidneys and Different Rat Organs in Tissue Culture.

	Controls R	P	M	A	T	N_t (N_e)	Exp.* R	P	M	A	T	N_t (N_e)
Species specificity												
Puppy kidney 1.	99.57	.12	.25	.06	.00	1632 (6)	97.57	.35	1.14	.25	.69	2024 (5)
			—.43—						—2.43—			
2.	98.95	.40	.45	.05	.15	2011 (3)	98.13	.30	1.08	.10	.39	2030 (2)
			—1.05—						—1.87—			
Organ specificity												
Rat bladder 1.	100.00	.00	.00	.00	.00	1234 (2)	100.00	.00	.00	.00	.00	1837 (2)
			—.00—						—.00—			
2.	99.92	.08	.00	.00	.00	1243 (4)	100.00	.00	.00	.00	.00	639 (2)
			—.08—						—.00—			
Rat ant. pituitary	99.28	.29	.43	.00	.00	695 (1)	99.50	.20	.20	.10	.00	2010 (2)
			—.72—						—.50—			
Rat pancreas	99.82	.06	.06	.06	.00	1605 (4)	100.00	.00	.00	.00	.00	479 (2)
			—.18—						—.00—			

* Serum was obtained on 2nd postoperative day following uninephrectomy except as noted.

R, resting stage; P, prophase; M, metaphase; A, anaphase; T, telophase; N_t, total No. of cells counted; N_e, No. of explants.

approximately twice as high as that obtained from controls, incubated with serum from normal rats (experimentals-controls: 5.48%-2.64%; 3.80%-2.50%) (Table I). This was not the case when cultures were incubated with serum from sham operated rats (0.69%-0.79%). Further, there was no increase in mitotic ratio in the cultures incubated with serum obtained from unilaterally nephrectomized rat on 15th postoperative day, as compared with controls (0.35%-0.80%).

The mitotic ratio in epithelial-like cells from rat kidney, cultured and incubated with serum obtained from unilaterally nephrectomized rat on 2nd postoperative day, and further heated for 30 minutes at 56°C was also approximately twice as high as that obtained from controls treated in like manner (2.19%-0.67%; 3.31%-1.74%). In this case, after heating for 30 minutes at 56°C, the serum was centrifuged and the supernatant used.

Serum was also kept for 20 minutes at 100°C in boiling water bath, centrifuged and the supernatant used for incubation with cultured rat kidney. There was no difference in mitotic ratios between experimentals and controls (1.93%-2.09%).

Serum was dialyzed for 48 hours against 3 liters of Gey's solution at 4°C, and used for incubation with cultured rat kidney. The mitotic ratio in the experimentals appeared to be higher than that of the controls (0.60%-0.09%).

When untreated rat serum was incubated with the epithelial-like cells from puppy kidney cultures (Table II), the experimentals showed mitotic ratios approximately 3 times greater than those of controls (2.43%-0.43%; 1.87%-1.05%). When rat serum was incubated with the epithelial-like cells from the bladder, anterior pituitary, and pancreas of the rat, there were no significant differences in mitotic ratios between experimentals and controls (0%-0.08%; 0%-0% in rat bladder; 0.50%-0.72% in rat anterior pituitary; 0%-0.18% in rat pancreas).

Discussion. It follows from our results that a mitosis stimulating factor appears in serum of unilaterally nephrectomized rats within 2 days following uninephrectomy, but disappears within 15 days after operation. The factor seems to be organ specific (acting strictly upon the kidney) but not species specific. Furthermore this factor is heat stable at 56°C, but its activity is destroyed by boiling at 100°C. The mitosis stimulating factor appears to be non-dialyzable.

There is a wide scattering in the mitotic index of controls, which is probably due to differences in the chemical composition (of embryonic extract, plasma, etc.) of different batches of the culture fluid. Therefore, each control should be compared with its corresponding experimental, because in both cases the same batches of culture fluids were used.

Following unilateral nephrectomy, the remaining kidney shows an almost immediate response to uninephrectomy, and *in vivo* it has been shown that mitotic activity in the remaining kidney increases considerably within 2 days following uninephrectomy and continues until approximately the 10th postoperative day(4,5,6). Our results suggest that this increased mitotic activity is due to a humoral factor present in the serum of the unilaterally nephrectomized rat, but not present in the serum of the normal rat. Thus, the mechanism of renal compensatory hypertrophy following uninephrectomy is probably initiated by a humoral factor. The question naturally arises as to the nature of the factor which stimulates mitosis in the remaining kidney after uninephrectomy.

Discussion. Bollman and Mann claimed that accumulation of urinary products was the primary factor in renal compensatory hypertrophy(7), *i.e.,* they found that hypertrophy of the remaining kidney after nephrectomy was greatly increased by accumulation of urinary products in blood following transplantation of the ureter to the duodenum, so that urine was drained into the duodenum; however, no control data were given.

Pure ACTH has no renotropic action(9). The hypophysis is involved in the mechanism of renal compensatory hypertrophy(8,10), but its presence does not seem to be absolutely necessary(11-16). Renin has an inhibitory action on renal compensatory hypertrophy and presumably acts in a self-regulating mechanism in the control of organ growth (25).

Inasmuch as the procedure of uninephrectomy is to be considered as a stress factor for the rat, it is logical to assume that the stress mechanism may be involved in renal compensatory hypertrophy. However, the stress reaction is a more or less generalized phenomenon. In renal compensatory hypertrophy following uninephrectomy, only the remaining kidney undergoes extensive hypertrophy and increases its size to take over the function of the ablated organ. Hormones such as GH(10), thyroxine(17-19) and testosterone propionate(20-23) are involved in the mechanism of the renal compensatory hypertrophy, but these substances act upon various other target organs as well; however, estrogen inhibits renal hypertrophy in the rat(24). It would seem difficult to explain the rather specific phenomenon of renal compensatory hypertrophy through the action of hormones which have more or less generalized effects in the body. These hormones might be involved in stress mechanism following uninephrectomy, but how are these hormones regulated so as to produce an effect solely upon the remaining kidney?

We found that the mitosis stimulating factor had organ specificity, suggesting the possibility of a local growth factor.

As previously mentioned, an organ, but not species specific mitosis stimulating factor has been reported in liver homogenates(3). Furthermore the serum obtained from partially hepatectomized rats on the 2nd postoperative day, has a mitosis stimulating action upon the liver, and this factor is organ specific and proteic in nature, which is in agreement with the results obtained in the present investigation (2).

A mitosis stimulating factor found in the serum of unilaterally nephrectomized rat might represent one or several of the hormones already known, but in view of the organ specificity, this factor should more probably be considered as a substance which had an intimate relation to the local growth regulation of the organ, as in the case of the liver.

The work reported revealed no information as to site of formation of the mitosis stimulating factor.

It might be suggested that renal compensatory hypertrophy following unilateral nephrectomy is a complicated phenomenon of primarily humoral coordination mediated by both the systemic stress mechanism and by a local growth regulatory mechanism.

Summary. A mitosis stimulating factor in serum obtained from unilaterally nephrectomized rat on the 2nd postoperative day was investigated. This mitosis stimulating factor was heat stable at 56°C, non-dialyzable, organ specific, but not species specific. The probable nature of this factor is discussed.

1. Glinos, A. D., Gey, G. O., *Cancer Research,* 1952, v12, 265.

2. Friedrich-Freska, H., Zaki, F. G., *Z. Naturforsch.,* 1954, v96, 239.

3. Blomqvist, K., *Growth stimulation in liver and development following intraperitoneal injections of liver homogenates in the rat,* 1957, Helsinki.

4. Rollanson, H. D., *Anat. Rec.,* 1949, v104, 263.

5. Sulkin, N. M., *ibid.,* 1949, v105, 95.

6. Ogawa, K., Sinclair, J. G., *Tex. Rep. Biol. and Med.,* 1958, v16, 215.

7. Bollman, J. L., Mann, F. C., *Arch. Path.,* 1935, v19, 28.

8. Selye, H., Hollett, C., *J. Urol.,* 1945, v53, 498.

9. Simpson, M. E., Li, C. H., Evans, H. M., *Endocrinol.,* 1946, v39, 78.

10. Cater, D. B., Holmes, B. E., Mee, L. K., *Biochem. J.,* 1957, v66, 482.

11. McQueen-Williams, M., Thompson, K. W., *Yale J. Biol. and Med.,* 1940, v12, 531.

12. Fontaine, T., *Compt. rend. Soc. biol.,* 1947, v141, 569.

13. Astarabadi, T. M., Essex, H. E., *Am. J. Physiol.,* 1953, v173, 526.

14. Raaf, D., White, H. L., *Endocrinol.,* 1953, v53, 436.

15. Braun-Menendez, E., Houssay, H. E. J., *Rev. Soc. Argentina Biol.,* 1945, v25, 55.

16. Astarabadi, T. M., Essex, H. E., Grindlag, J. H., *Endocrinol.,* 1953, v52, 653.

17. Valori, P., *Arch. Fisiol.,* 1949, v48, 196.

18. Zeckwer, I. T., *Am. J. Physiol.,* 1946, v145, 681.

19. Kleinsorg, H., Loester, L., *Arch. Intern. Pharm. Therap.,* 1949, v79, 83.

20. Lattinei, J. K., *J. Urol.,* 1942, v48, 778.

21. Mackay, E. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v45, 216

22. Ludden, J. B., Kruger, E., Wright, I. S., *Endocrinol.,* 1949, v28, 619.

23. Kochakian, C. D., *ibid.,* 1944, v35, 224.

24. Schaffenburg, C. A., McCullagh, E. P., *J. Clin. Endocrinol. and Metab.*, 1953, v13, 837.

25. Schaffenburg, C. A., Masson, G. M. C., Corcoran, A. C., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 469.

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Coenzyme Studies in Primaquine-Sensitive Erythrocytes.*† (24348)

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A severe, acute, genetically determined hemolytic anemia may be induced by primaquine, many other drugs of therapeutic importance, and fava beans, in certain otherwise healthy individuals(1-5). Because the biochemical and enzymic abnormalities which characterize erythrocytes of these drug-sensitive individuals were first investigated using primaquine, the syndrome precipitated by this drug as well as by toxicologically related agents has been designated "primaquine-sensitive hemolytic anemia"(5). Except for certain biochemical abnormalities in glutathione and carbohydrate metabolism, erythrocytes of primaquine-sensitive individuals appear to be normal(6). Reduced glutathione content of these erythrocytes is generally lower than normal; however, there is considerable overlapping of values (2). The reduced glutathione content falls still further *in vivo* during drug-induced hemolysis(7). Furthermore, reduced glutathione content is usually unstable when erythrocytes are incubated *in vitro* with acetylphenylhydrazine(2,8). The glutathione reductase activity of primaquine-sensitive erythrocytes is increased (Reaction 2)(9). Two enzymic abnormalities in carbohydrate metabolism of erythrocytes from primaquine-sensitive males have been reported by this laboratory. 1) Activity of glucose-6-phosphate dehydrogenase, the enzyme catalyzing initial oxidative reaction of the hexosemonophosphate shunt, is markedly diminished (Reaction 1)(10). In some sensitive females deficiency may be considerably less than in males(2). 2) The activity of aldolase is increased in erythrocytes of primaquine-sensitive males(11). Aldolase catalyzes the reversible splitting of FDP, supplying substrate for the oxidative reaction of the Embden-Meyerhof pathway (Reactions 3 and 4).

Enzymic abnormalities in primaquine-sensitive erythrocytes focus attention on the pivo-

Reaction 1:

$$\text{‡G-6-P} + \text{TPN}^+ \xrightarrow[\text{Dehydrogenase}]{\text{G-6-P}} \text{6-PG} + \text{TPNH} + \text{H}^+$$

Reaction 2:

$$\text{GSSG} + \text{TPNH} + \text{H}^+ \xrightarrow[\text{Reductase}]{\text{GSSG}} 2\,\text{GSH} + \text{TPN}^+$$

Reaction 3:

$$\text{FDP} \underset{\longleftarrow}{\xrightarrow{\text{Aldolase}}} \text{GA-3-P} + \text{DHAP}$$

Reaction 4:

$$\text{GA-3-P} + \text{Pi} + \text{DPN}^+ \underset{\longleftarrow}{\xrightarrow{\text{GAPD}}} \text{1,3-DPG} + \text{DPNH} + \text{H}^+$$

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‡ DPN+, DPNH, oxidized and reduced diphosphopyridine nucleotide, respectively; TPN+, TPNH, oxidized and reduced triphosphopyridine nucleotide, respectively; GSH, GSSG, reduced and oxidized glutathione, respectively; G-6-P, glucose-6-phosphate; 6-PG, 6-phosphogluconate; FDP, fructose diphosphate; DHAP, dihydroxyacetone phosphate; GA-3-P, glyceraldehyde-3-phosphate; 1,3-DPG, 1,3 diphosphoglycerate; Pi, inorganic phosphate; GAPD, glyceraldehyde phosphate dehydrogenase.