minished as pregnancy progressed. 17α-Hydroxyprogesterone pellets were ineffective in maintaining pregnancy in castrate mice. When the day of finding the vaginal plug was counted as day 1, duration of pregnancy in the intact untreated CHI mouse was usually 19 or 20 days. In the Brown Belt mouse, it was usually 20 or 21 days.

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Inhibition of Renal Growth Following Unilateral Nephrectomy in the Rat.* (28568)

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Unilateral nephrectomy in the rat stimulates growth of the remaining kidney. The growth response is manifested within 24 hours by a substantial increase in dry weight (1) and within 48 hours by an appreciable increase in mitotic activity in the remaining kidney(2). Unilateral ureteral transection (ureteroperitoneostomy), a procedure thought to be equivalent to unilateral nephrectomy in accomplishing transitory reduction of renal excretory function, does not elicit the growth response(3,4). This observation has been the basis for rejection of the hypothesis that the growth response of the remaining kidney following unilateral nephrectomy is initiated by reduction of renal excretory function. An alternative hypothesis, namely, renal growth response is dependent on reduction of non-excretory (endocrine) renal function has supplanted it. Repetition of the unilateral ureteroperitoneostomy studies in this laboratory revealed that the procedure was uniformly accompanied by a striking inflammatory reaction within the peritoneal cavity. This was not observed following unilateral neprectomy or sham operation. Furthermore, animals with unilateral ureteroperitoneostomy often lost more weight during the experimental period than did control animals.

These observations suggested that failure of growth stimulation following unilateral ureteroperitoneostomy may be related to peritoneal inflammation and/or decreased post-operative food and water intake. The following studies were designed to evaluate this this possibility.

Methods. Non-fasted Holtzman male rats were laparotomized under ether anesthesia between 9:00 A.M. and 11:00 A.M. Either left nephrectomy, sham nephrectomy (manipulation of viscera to expose left kidney)

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TABLE I. Alteration of Body Weight, Kidney Weight and Incorporation of P^{32} into Rat Kidney DNA Following Unilateral Nephrectomy, Sham Nephrectomy or Unilateral Ureteroperitoneostomy.

Exp No.	Treatment	No. of rats	Body wt: Initial (g)	Body wt: % change	Kidney wt, % change§	Relative specific activity of right kidney DNA phosphorous‖
1	a. Left nephrectomy	6	199 ± 6*	− 6.4 ± .4*	+ 7.1 ± 1.5*†	
	b. *Idem* + talc	9	204 ± 3	− 8.3 ± 1.1	− 1.6 ± 1.5	
	c. Left ureteroperitoneostomy	6	205 ± 6	− 9.3 ± .9	+ .4 ± 2.3	
2	a. Left nephrectomy	4	161 ± 3	− 8.1 ± .4	+10.0 ± 1.4‡	499 ± 54*
	b. *Idem* + talc	4	164 ± 5	−13.8 ± 1.1	+ 1.4 ± 1.2	197 ± 15
	c. Sham nephrectomy	4	165 ± 3	− 7.2 ± .6	+ 1.3 ± 1.8	170 ± 31
3	a. Left nephrectomy	8	141 ± 3	− 2.4 ± 1.1	+13.6 ± 1.8†	
	b. *Idem* + starvation	8	141 ± 5	−22.9 ± 1.1	− 2.0 ± .9	
4	a. Left nephrectomy	4	131 ± 3	− 9.2 ± .9	+13.7 ± 2.1‡	251 ± 36
	b. *Idem* + starvation	4	129 ± 2	−21.6 ± 2.4	− .8 ± 2.1	67 ± 8
	c. Left ureteroperitoneostomy	4	127 ± 1	−10.1 ± .7	− 3.4 ± 3.5	115 ± 22

* Mean and standard error. † Based on dry wt. ‡ Based on wet wt.

§ $\frac{\text{Right kidney wt} - \text{left kidney wt} \times 100}{\text{Left kidney wt}}$.

‖ $\frac{\text{DNA phosphous spec. act.} \times 10^4}{\text{Acid sol. inorganic phosphous spec. act.}}$.

or unilateral ureteroperitoneostomy (transection of left ureter) was performed. Forty-eight hours later the animals were re-anesthetized and exsanguinated. Kidneys obtained at initial or final operation were rinsed in isotonic saline, freed of perirenal fat, blotted on filter paper, and promptly weighed. Dry weights were obtained by heating minced tissue to constant weight at 100°C in a vacuum oven. Alterations in wet weight (Exp. 2 and 4) or dry weight (Exp. 1 and 3) are expressed as per cent change of weight of right kidney compared to left kidney [(right kidney weight)-(left kidney weight) (100)/(left kidney weight)].

Phosphorus^{32} (P^{32}) incorporation into kidney deoxyribonucleic acid (DNA) was used as an index of cellular proliferation (Exp. 2 and 4). Thirty-six hours after initial operation animals were re-anesthetized with ether and P^{32} equal to 3 microcuries per gram of body weight was injected into a saphenous vein. Special caging of the animals required after isotope administration necessitated withdrawal of food and water for the remainder of the study. Twelve hours later the animals were sacrificed and the remaining kidneys were cleaned, weighed, and homogenized in cold 5% trichloroacetic acid. Specific activity of trichloroacetic acid soluble inorganic phosphorus was determined by the method of Lindberg and Ernster(5). DNA was isolated from the trichloroacetic acid insoluble residue by the method of Barnum *et al.*(6), its phosphorus content determined by a modification of the Fisk, Subbarow method (7), and its radioactivity determined by counting a plated aliquot with a thin window Geiger detector. The results of the isotope studies are presented as the ratio of the specific activity of DNA phosphorus to the specific activity of acid soluble inorganic phosphorus.

Statistical significance of the difference between mean values was assessed by the standard "t" test. P values less than .05 were considered significant.

Results. Induction of peritonitis by intraperitoneal instillation of 2 ml of a 5% suspension of U.S.P talc in isotonic saline at the time of unilateral nephrectomy abolished the growth response usually observed 48 hours after operation (Exp. 1 and 2, Table I). Neither kidney weight gain nor acceleration of P^{32} incorporation into DNA of remaining kidney was observed if nephrectomy was accompanied by talc induced peritonitis. It is to be noted that in Groups 1c and 2c left and right kidneys were both removed 48 hours after the initial procedure (either uni-

lateral ureteroperitoneostomy or sham nephrectomy). The per cent difference between left and right kidney weights observed in these groups indicates the usual close agreement in weight between kidney pairs. Body weight loss in animals with unilateral ureteroperitoneostomy (1c) significantly exceeded that seen in unilaterally nephrectomized animals (1a).

Withdrawal of food and water during the entire 48-hour experimental period (Exp. 3 and 4) inhibited renal growth response. P^{32} incorporation into kidney DNA of starved, unilaterally nephrectomized animals was depressed and kidney weight gain was prevented. P^{32} incorporation into renal DNA of animals with unilateral ureteroperitoneostomy was also significantly less than that observed in fed, unilaterally nephrectomized animals, a finding which confirms the work of Simpson(3). The difference between P^{32} incorporation into renal DNA of starved, unilaterally nephrectomized animals (4b) and of animals with unilateral ureteroperitoneostomy (4c) is not significant.

The differences in P^{32} incorporation into renal DNA between Exp. 2 and 4 (cf. 2a with 4a) are unexplained at present. A possible cause is day to day variation in basal levels of renal mitotic activity. Regardless of the origin of this variation, the differences induced by unilateral nephrectomy and sham nephrectomy in Exp. 2 and unilateral nephrectomy and unilateral ureteroperitoneostomy in Exp. 4 on P^{32} incorporation into renal DNA are comparable to those obtained using other chemical isolation technics(3) or other indices of cellular proliferation(4).

Discussion. The sequence of events initiating growth of remaining kidney following unilateral nephrectomy is unknown. Although neural mediation of the phenomenon has not been satisfactorily ruled out, 2 humoral theories, both predicated on the existence of a hypothetical blood borne renotrophic agent or agents, have dominated experimental work in this area. The first proposes that transitory reduction of renal excretory function results in increased blood levels of renotrophic material. The second proposes a reciprocal relationship between blood levels of renotrophic material and blood levels of a hypothetical kidney hormone; in this scheme reduction of renal mass, but not reduction of renal excretory function, results in increased renotrophin blood levels. The first hypothesis is not supported by ureteroperitoneostomy experiments which appear to demonstrate that reduction of net renal excretory function is not a sufficient condition for eliciting the growth response. The second hypothesis is supported both by failure of unilateral ureteroperitoneostomy to induce renal growth and by inhibition of renal growth response after injection of crude extracts of renin, an enzyme which may be considered a renal endocrine product(1).

The present studies suggest alternate interpretations for these findings. In the case of the ureteroperitoneostomy studies, it is possible that concomitants of the procedure, namely, peritonitis and reduced oral intake, are responsible for growth response inhibition. The significant loss in body weight following injection of crude extracts of renin (1) suggests that inhibition of renal growth response in those studies may have had as its basis reduction of oral intake. Thus, the possibility that reduced renal excretory function is indeed the initial event in eliciting growth of remaining kidney following unilateral nephrectomy is reopened.

Evidence for mediation of the renal growth phenomenon by a specific renotrophic material is lacking. Aside from the work of Ogawa and Nowinski(8) and Lowenstein(9) which appears to demonstrate renotrophic activity of serum from unilaterally nephrectomized rats its existence is based on conjecture. The present studies neither support nor refute the renotrophin hypothesis but emphasize the sensitivity of the renal growth phenomenon to inhibition by extraneous factors and indicate that studies designed to demonstrate either renotrophic or antirenotrophic effects of various operative procedures or injected materials must be carefully designed and cautiously interpreted.

Summary. Both talc induced peritonitis and food and water restriction inhibit the prompt growth response of remaining rat kidney following unilateral nephrectomy.

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Influence of Calcium-Deficit on Morphine Inhibition of QO_2 of Rat Cerebral Cortex Slices.* (28569)

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There is general agreement that morphine has little effect on the metabolism of brain tissue respiring in the usual Ringer-type solutions. Concentrations as high as 0.01 M had no effect on the QO_2 of rat brain cortex slices respiring in glucose-Ringer's(1), and 0.0056 M was found not to affect glycolysis by rat brain homogenates(2). However, with special media and with certain substrates, inhibition of oxygen uptake by brain tissue preparations has been reported. Quastel and Wheatley(3) worked with chopped whole guinea pig brain suspended in phosphate buffer pH 7.4 containing 0.6% saline. They found that 0.12% (0.0016 M) morphine inhibited the increment in oxygen uptake caused by the addition after 3 hours of glucose, lactate, pyruvate and glutamate, but not succinate or p-phenylenediamine. Seevers and Shideman(4) reported that 0.12% morphine inhibited by 10-30% the QO_2 of rat brain slices in Ringer M/60 phosphate buffer pH 7.35 when the substrate was lactate, pyruvate, or α-ketoglutarate, but not when the substrate was glucose, succinate, fumarate, or malate.

Recently Bell(5) found that the stimulative response of rat cerebral cortex in Krebs-Ringer phosphate medium to applied electrical pulses was partially inhibited by 0.0001 M morphine and practically abolished by 0.001 M morphine. Takemori(6), using a Ringer's solution low in calcium (0.0005 M) reported that 0.001 M morphine inhibited the oxygen uptake of potassium stimulated cerebral cortex slices from control rats, but not from rats tolerant to daily doses of 90/mg/kg of morphine. He later reported(7) the same results with a medium containing the usual amount of calcium (0.0013 M). He also stated that nalorphine reversed the apparent adaptation of brain slices to morphine and claimed(8) complete cross-cellular adaptation with methadone in cortical slices adapted to the depressive effect of morphine.

During a study of the effects of cold exposure on some actions of morphine we attempted to duplicate Takemori's basic observation that morphine could inhibit potassium stimulated oxygen uptake of rat cerebral cortex slices. We were unable to show any inhibitory effect of 0.001 M or 0.002 M morphine on brain slices from control rats and from rats exposed for one month to an ambient temperature of 4°C. In an effort to account for this discrepancy, we noted that Takemori(6) had originally used a low-calcium Ringer's solution and therefore repeated the experiments using calcium-free

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