Tissue culture contamination with swine mycoplasma reemphasizes control problems in maintaining uncontaminated cell lines and poses major questions about sources of these strains in cell cultures.

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Effects of Hypophysectomy and Growth Hormone on Renal Compensatory Hypertrophy in Rats.* (31193)

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For many years there has been disagreement as to whether renal compensatory hypertrophy (RCH) can occur in hypophysectomized rats. McQueen-Williams and Thompson(1) and Cologne(2) reported that in the absence of the anterior hypophysis compensatory renal hypertrophy does not occur. Fontaine and Veil(3), Braun-Menendez and Houssay(4), Rolf and White(5), and Astarabadi(6) concluded that renal compensatory hypertrophy could still occur after hypophysectomy. However, Astarabadi, in more recent investigations(7-9), has found that renal compensatory hypertrophy does not occur in the absence of the pituitary.

In all these experiments the remaining kidney was examined 6-86 days after the nephrectomy, and most commonly 10-25 days after the nephrectomy. Yet Addis(10) has reported that following uniliateral nephrectomy in normal rats the rate of renal compensatory hypertrophy (or restoration of renal tissue as he preferred to call it) is most rapid in the first 48 hours, continuing thereafter at a slower rate. In this study we have observed the response to unilateral nephrectomy at 2, 5 and 10 days after nephrectomy in normal rats, hypophysectomized rats, and hypophysectomized rats receiving hormone replacement.

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Methods. The rats used were male albinos of the Sprague-Dawley strain obtained from the same source, and were divided into 3 groups. Group I consisted of 44 non-hypophysectomized, unilaterally nephrectomized animals maintained on standard laboratory rat chow and tap water. Group II consisted of 35 hypophysectomized, unilaterally nephrectomized rats which were maintained solely on standard laboratory rat chow and 0.3% saline. Group III, 15 animals, duplicated Group II, but were given injections of STH, ACTH and thyroxine.

The nephrectomies were performed under ether anesthesia and alternate kidneys were removed (*i.e.*, the left from the first animal, the right from the second, etc.). The kidney was exposed through a flank incision, the vessels and ureter clamped and tied off, the kidney removed and the incision closed. Upon removal, the kidney was placed immediately into a 10 ml beaker which was then sealed with parafilm and placed in the refrigerator until all the animals in that particular group had been nephrectomized. The kidneys were then taken out in groups of 3, the capsule removed, the kidney split in half, and placed on filter paper. As soon as the third kidney was prepared, the kidneys were weighed in this respective order. One animal each in Groups II and III died following nephrectomy. The data from one animal in Group I was eliminated because of an error in the weighing procedure. The nephrectomies in Groups II and III were performed on the day after the hypophysectomy.

The hypophysectomies were performed *via* the external auditory canal and were judged initially by the appearance of diabetes insipidus. At autopsy the sellae turcicae were examined for tissue remnants. The data from 2 animals of Group II and 2 animals of Group III were eliminated because tissue remnants were found in their sellae.

Bovine growth hormone (NIH) was reconstituted in 0.9% NaCl solution with the addition of a few drops of m/10 NaOH to make it clear. One ml of this solution containing 1 mg of growth hormone (STH) was injected subcutaneously twice a day for 2 days starting on the day of the nephrectomy. Then the dose was reduced to 0.5 ml twice a day until the conclusion of the experiment. Parke, Davis ACTH in dry form was dissolved in water and diluted to 0.04 unit per ml. One-half ml of this solution was injected subcutaneously twice a day starting on the day of the nephrectomy and continuing for the duration of the experiment. Calbiochem L-thyroxine (sodium salt B grade, was dissolved in water and diluted to 20 μg per ml. One-half ml of this solution was injected subcutaneously once a day beginning on the day of the nephrectomy and continuing for the duration of the experiment.

Each group of animals was divided into 3 sub-groups, and on the 2nd, 5th and 10th days following the nephrectomies the remaining kidney was removed and weighed in the manner described. The data from one animal in Group II were eliminated because on gross examination the remaining kidney was found to be hydronephrotic.

The initial kidney weight was related to the initial body weight and body surface area (cm^2) and the remaining kidney weight to the final body weight and body surface area (cm^2). The surface area was calculated from the formula: $S = 12.54 \times W^{0.60}$, where S is the surface area in square centimeters and W is the body weight in grams(11). Statistical evaluation of the significance between value of the means was determined by the T-test using paired variates for the intragroup calculations and unpaired variates for the intergroup calculations.

Results. The results are summarized in Table I and Fig. 1-3. Group I, the non-hypophysectomized controls, demonstrated a very rapid renal compensatory hypertrophy within the first 48 hours. Thereafter, the compensatory hypertrophy continued, but at a slower rate.

The hypophysectomized Group II which did not receive hormone therapy also demonstrated a rapid renal compensatory hypertrophy in the first 48 hours which paralleled the response of the non-hypophysectomized group. However, instead of the compensatory hypertrophy continuing, there was a regression in kidney size at 5 days, with no subsequent change at 10 days. The body

TABLE I. Renal Compensatory Hypertrophy in Unilaterally Nephrectomized Rats. Summary of data.

		No. animals	% Δ BW	% Δ KW	% Δ K/S
Day 2	I	20	— .3 (8.7)	17.4† (10.0)	17.7† (6.7)
	II	12	—5.2 (3.3)	11.4† (8.5)	15.1† (9.2)
	III	4	6.4 (4.0)	15.4† (3.6)	11.4† (2.8)
Day 5	I	18	14.2 (6.4)	39.6† (15.6)	28.8† (11.4)
	II	11	—6.3 (5.6)	—5.5 (11.0)	—1.7 (12.2)
	III	4	8.7 (2.2)	15.7 (10.6)	9.4 (8.6)
Day 10	I	5	35.2 (11.7)	85.2† (11.4)	54.8† (8.7)
	II	8	—7.3 (7.0)	—4.0 (7.3)	1.4 (7.2)
	III	4	32.7 (8.4)	42.6† (14.8)	20.2* (10.2)

I = non-hypophysectomized, unilaterally nephrectomized group. II = hypophysectomized, unilaterally nephrectomized group. III = hypophysectomized, unilaterally nephrectomized with hormone replacement group.

BW = body wt in g. KW = kidney wt in mg. K/S = kidney wt in mg/surface area in cm^2.

$$\% \Delta = \frac{\text{final value—initial value}}{\text{initial value}} \times 100.$$

Standard deviation in parentheses. * Significant at .05 level. † Significant at .01 level.

Statistical evaluation of significance of the difference between the value of the means for % Δ KW revealed $P < .01$ for I *vs* III, III *vs* II at days 5 and 10. For % Δ K/S a $P < .01$ was found for I *vs* III, at days 5 and 10 and $P < .05$ for III *vs* II at day 5 and $P < .01$ for III *vs* II at day 10.

weight showed a slight, progressive loss at 2, 5 and 10 days.

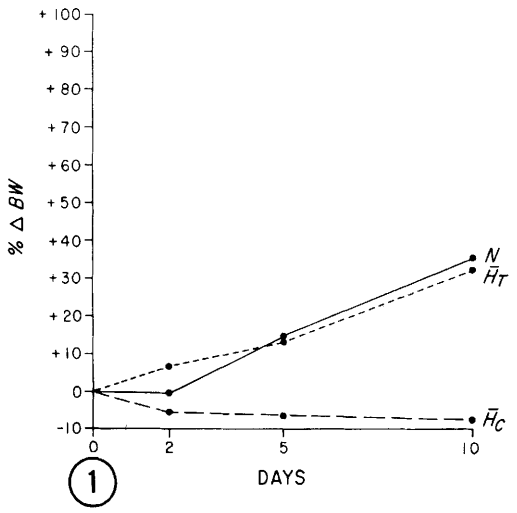
In the hypophysectomized, hormone-treated Group III there was an initial renal compensatory hypertrophy which was comparable to that of Groups I and II. Following this initial phase there was no change at 5 days, followed by further renal enlargement at 10 days which was much less than that of the normal Group I. The body weight increased in each of the 3 periods, and at the 5 and 10 day periods was identical with the average control animal weights.

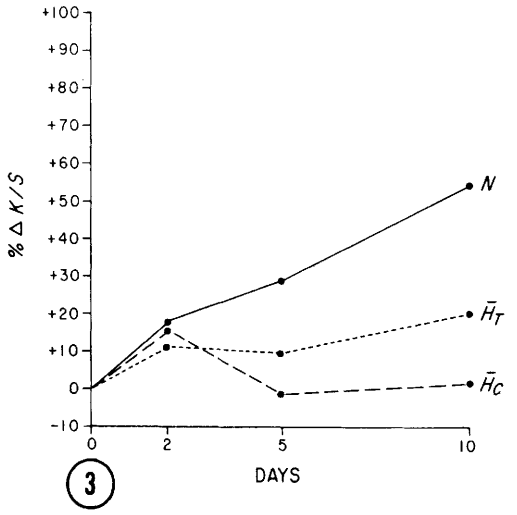
Discussion. Extensive studies by Addis and Lew(10) have characterized the normal growth of kidney tissue in rats and the relative acceleration in single kidney growth after removal of the contralateral kidney. These data indicate that between 30 and 200 days of age the male kidney normally grows at 0.72 times and the female kidney at 0.65 times the rate at which the body increases in weight. Their data further indicate that the maximum rate of compensatory hypertrophy is in the first 2 days after nephrectomy with about 75% being accomplished in 5 days and 85% in 10 days. Addis also demonstrated that in unilaterally nephrectomized animals the change in kidney weight correlates well with the change in kidney protein and may be used instead of the latter in studying RCH(14). Some of the difficulties in interpreting the effect of hypophysectomy on RCH are due to the continued body growth of rats during the experimental period, making the initial kidney weight irrelevant, and the loss of body weight after hypophysectomy, which renders the true kidney to body weight ratio uncertain. To minimize both of these factors it was chosen to perform the nephrectomy immediately after hypophysectomy and to observe kidney weights during the period of maximum response, *i.e.*, 2, 5, and 10 days. As Goss(12) has shown Na^+ retention to be a necessary condition to RCH, our hypophysectomized rats were maintained on 0.3% saline.

In all groups there was a relative increase in kidney weight at 2 days. Available evidence in humans indicates that the action of STH does not persist as long as 3 days(13). Therefore, this immediate RCH is probably independent of STH, since it was present in the hypophysectomized animals. Subsequently the kidney weight falls in the hypophysectomized animals and at 10 days parallels the slight weight loss. The animals which were hypophysectomized and treated with STH, thyroxine and ACTH showed a normal weight gain, but an increase in kidney weight which

was only half that of the control Group I. When the kidney weight is compared to the increase in surface area, as done by Astarabadi(9), there appears to be a significant renal hypertrophy, but when compared to body weight, this apparent hypertrophy disappears. Since Addis and Lew have demonstrated that normal kidney growth is somewhat less than total body growth, the former treatment is probably the more valid(10) and indicates one of the problems in the evaluation of the data in this and similar experiments (Table II).

As Addis(15) has shown, the protein content of the diet has a marked effect on RCH. The difference in protein intake of the hypophysectomized and non-hypophysectomized rats and the importance of this difference cannot be accurately assessed. However, the achievement of a normal increase in body weight in the group receiving replacement therapy indicates an adequate protein intake





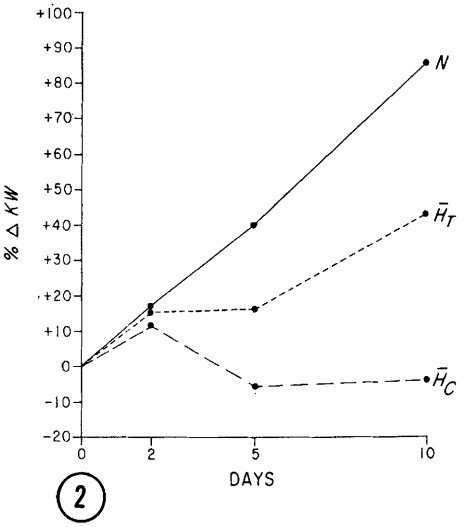


FIG. 1. Percent change in body weight.

$$\% \Delta BW = \frac{BW_2 - BW_1}{BW_1} \times 100.$$

BW_1, BW_2 = Initial and final body weights in g.
N = Non-hypophysectomized, unilaterally nephrectomized, Group I.
$\bar{H}_C$ = Hypophysectomized, unilaterally nephrectomized, Group II.
$\bar{H}_T$ = Hypophysectomized, unilaterally nephrectomized, replacement hormone treated, Group III.

FIG. 2. Percent change in kidney weight.

$$\% \Delta KW = \frac{KW_2 - KW_1}{KW_1} \times 100.$$

KW_1, KW_2 = Initial and final kidney weights in mg.
Other symbols as for Fig. 1.

FIG. 3. Percent change in ratio of kidney weight to surface area.

$$\% \Delta K/S = \frac{KW_2/S_2 - KW_1/S_1}{KW_1/S_1} \times 100:$$

K_1/S_1, K_2/S_2 = Initial and final ratios of kidney weight in mg to surface area in square centimeters.
Other symbols as for Fig. 1.

TABLE II. Renal Compensatory Hypertrophy in Unilaterally Nephrectomized Rats 10 Days After Nephrectomy. Comparison of results obtained using kidney weight (mg)/100 g body weight and kidney weight (mg)/surface area (cm^2).*

	% Δ KW/BW	% Δ KW/S
I. Astarabadi(9)		
Controls	+10%	+39%
Hypophysectomized	+11%	+ 9%
Hypophysectomized with STH	+ 4.5%	+20%
II. Fogelman and Goldman		
Controls	+38%	+55%
Hypophysectomized	+ 3%	+ 1%
Hypophysectomized STH, ACTH & thyroxine	+ 7%	+20%

* Calculations were made using mean values in the formulas:

1. $\frac{KW_2/BW_2 - KW_1/BW_1}{KW_1/BW_1} \times 100.$

2. $\frac{KW_2/S_2 - KW_1/S_1}{KW_1/S_1} \times 100.$

in this group. The attainment of RCH at day 2 and the subsequent fall in kidney size out of proportion to the small loss in body weight suggests that the failure of RCH in the hypophysectomized non-treated group cannot be ascribed solely to a decreased protein intake.

The results of these studies and of those reported by Astarabadi indicate that the pituitary is essential for RCH. The work of Lowenstein and Stern(16) and of Ogawa and Nowinski(17) suggests the presence of a "renotropic" factor in rat serum 48 hours after unilateral nephrectomy. There are a number of possibilities for such a factor: (1) "Renotropin" is STH. This seems unlikely since STH replacement produced a normal weight gain, but failed to produce normal RCH either in the study reported by Astarabadi or in the present experiment. (2) "Renotropin" is a non-pituitary agent which requires STH for effectiveness. This is also unlikely in view of the failure to achieve normal RCH with replacement therapy. (3) "Renotropin" is a pituitary hormone separate from STH. This would be more compatible with Astarabadi's findings that crude extracts of the anterior pituitary caused a degree of hypertrophy which exceeded the normal(6), but that purified STH could not produce normal RCH in unilaterally nephrectomized, hypophysectomized rats(8) (Table II). (4) "Renotropin" is a non-pituitary agent which requires an intact pituitary, and possibly an unidentified specific pituitary component, in order to be effective. An increase in the circulating levels of "renotropin" would be expected after the removal of one kidney if a humoral mechanism produces RCH. The observation of RCH 72 hours after hypophysectomy and 48 hours after nephrectomy implies that "renotropin" is being produced. There can be no increase in "pituitary renotropin" after hypophysectomy. The later failure of continued hypertrophy is apparently due to the absence of pituitary hormones. For these reasons the last alternative seems most probable.

Summary. The effect of hypophysectomy on renal compensatory hypertrophy (RCH) was observed in unilaterally nephrectomized rats receiving no therapy and those receiving replacement therapy and was compared to the RCH attained by non-hypophysectomized unilaterally nephrectomized rats at 2, 5, and 10 days. In the latter group there was an initial rapid RCH seen at day 2 which continued, but at a slower rate, through day 10. In the hypophysectomized group without replacement therapy there was also an initial RCH in the first 48 hours. However, instead of the RCH continuing there was a regression in kidney size at 5 days and with no subsequent change at 10 days. In the hypophysectomized group receiving hormone replacement there was an initial RCH comparable to the other two groups, but the further renal enlargement at 10 days was much less than that of the non-hypophysectomized animals. The results of this experiment and of other reported studies imply the existence of a renotropic factor which is not produced in the pituitary, but which requires an intact pituitary for full effectiveness.

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Production and Inhibition of Gas in Various Regions in the Intestine of the Dog.* (31194)

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The production and composition of gases in the lumen of the intestine have been ascribed to a fermentation process of various types of foods consumed(1,2). Although it has been accepted that this process takes place in the colon, there has been little attempt to investigate the relationship between the colon and various levels of the small intestine as gas-producing areas following injections of a gas-producing food into these areas(3,4,5).

To study this relationship, experiments were so designed that known amounts of gas-producing and non-gas-producing foods could be introduced into surgically prepared intestinal segments of normal and antibiotically pretreated dogs. The assumption was that the fermentation, or gas-producing process, is associated with the intestinal flora which are susceptible to various types and concentrations of antibiotics.

Material and methods. Female mongrel dogs having an average weight of 12.8 kg were dewormed with Tenarids‡ worm tablets one week prior to their use. The animals were fasted 24 hours before anesthetization with nembutal, after which time a 10 cm midline incision was made on the abdomen. Isolated intestinal segments 50 cm in length were prepared in the duodenum, upper jejunum, and lower ileum by placing ligatures about the intestine. The colonic segment measured only 40 cm in length due to the shortness of this region in the dog. Cannulae 3 inches in length, made from one-half inch diameter flexible plastic tubing, were placed at each end of the 4 segments. Plastic adapters were also fitted to each cannula so that a segment could be closed off, or coupled to a gas collection apparatus when desired.

To record the degree of gas production and the inhibition that occurred due to antibiotics in various segments of the intestine and colon, 3 different series of experiments were performed using the following diets: (1) a non-gas-producing control-methyl cellulose homogenate, (2) a gas-producing navy bean homogenate, and (3) a navy bean homogenate after pretreating the animals with various antibiotics.

The methyl cellulose homogenate was prepared by homogenizing 50 g of methyl cellulose in enough distilled water to make a 200 ml volume; 50 ml of this homogenate were injected into each of the 4 segments. The material was inserted through the cannula in

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