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Renal and Adrenal Relationships in Compensatory Hyperplasia.* (29837)

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Growth in many organs can be stimulated either by partial ablation or by functional overload. In most cases, the two methods are known to be equivalent(1), but in physiologically complex organs such as the liver and kidney, it has never been discovered how the effects of partial ablation are translated into subsequent cellular proliferation in the residual tissues. Although functional overload has long been recognized as an obvious possibility, certain recent explanations(2) have favored the postulation of tissue-specific growth-regulating factors related more to maintenance of predetermined organ mass than to re-establishment of physiological proficiency. However, in view of vain attempts to demonstrate the existence of such factors in the case of the kidney, either in the serum or in renal tissue itself(3,4), the following experiments were designed to test the importance of strictly functional alterations on compensatory renal hyperplasia (CRH).

Methods. All experiments were carried out on male Sprague-Dawley rats weighing 100-120 g. Animals were kept in a temperature-controlled room at 76-78°F and illuminated from 0830 to 2230 daily. Operations and sacrifices were performed between 0900 and 1100. Nephrectomies and adrenalectomies were done under ether anesthesia *via* the dorso-lateral approach.

As previously described(4), results were assessed in terms of the mean per cent of renal cortical cells in mitosis ± S.E. Mitotic counts were made on the cortical areas of 7 μ kidney sections after fixation in Bouin's and staining with hematoxylin. Fifty randomly selected fields from 10 sections per kidney (5 fields per section) were scanned for mitotic figures at 430× magnification. The resulting counts divided by the total number of cells examined per kidney (approximately 21,400 cells) yielded a mitotic index expressed on a percentage basis. To reduce the effects of individual variations, rats were usually sacrificed in groups of 12, and their mitotic indices averaged. In experiments not involving nephrectomy, groups of 6 rats were used and their 12 kidneys were counted separately.

Role of salt and adrenals in compensatory renal hyperplasia. After subtotal nephrectomy, the incidence of cell division in the remaining kidney rises to a maximum of about 5 times normal during the second postoperative day(5-7). Williams(7) has shown that most of the mitotic figures at this time are in the cells of the proximal tubules. Recent investigations of the influence of the adrenal cortex on this process have yielded divergent results. Goss and Rankin(6) have claimed that bilateral adrenalectomy abolishes the CRH that normally takes place after unilateral nephrectomy. Williams(8), however, showed that nearly normal CRH occurred in adrenalectomized uninephrectomized rats. Subsequently, Astarabadi(9) observed compensatory increases in the relative weights of remaining kidneys during the 2 weeks following total adrenalectomy. Since the last 2 investigators maintained their rats on saline drinking water, while the first did not, it seemed plausible that this might account for the lack of agreement.

Accordingly, parallel series of rats were subjected to uninephrectomy and bilateral

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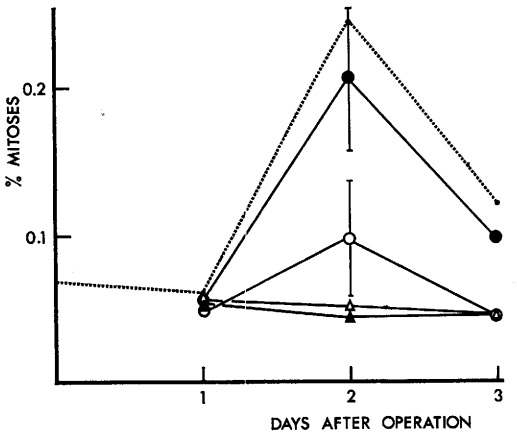

FIG. 1. Role of adrenal glands in compensatory renal hyperplasia (% mitoses ± S.E.). CRH in adrenalectomized rats drinking 0.9% NaCl (●) is nearly equivalent to that in control rats (broken line) after unilateral nephrectomy. In uninephrectomized adrenalectomized rats maintained on fresh water (○), CRH is considerably depressed. When both kidneys are intact, adrenalectomy either with (▲) or without (△) supplemental NaCl has no effect on normal levels of renal proliferation.

adrenalectomy. One group of animals was maintained on 0.9% NaCl drinking water, the other on fresh water. Twelve rats per series were sacrificed on each of the following 3 days Accessory series of animals were either unilaterally nephrectomized or bilaterally adrenalectomized, the latter group receiving either saline or fresh drinking water. The mitotic activity curves in Fig. 1 reveal that adrenalectomy in the absence of supplemental salt depresses CRH (though not always to normal levels). But if extra salt is provided, adrenalectomy has no significant effect on CRH. Clearly, a major role played by the adrenal cortex in affecting renal growth is to promote the retention of sodium. The absence of significant CRH in rats depleted of sodium as a result of adrenalectomy, and its restoration by NaCl replacement therapy, suggests that this compound is intimately involved in regulating kidney growth. This does not mean that all renal proliferation depends upon NaCl, for adrenalectomy does not alter the normal low level of mitotic activity characteristic of unoperated kidneys regardless of whether or not salt is given in the drinking water (Fig. 1).

Effect of adrenal cortical hormones on compensatory renal hyperplasia. In view of the demonstrated importance of the adrenal glands in CRH, the next logical step was to test the effects of cortical hormones. Cortisone could be eliminated as a necessary factor in CRH, since previous investigations (6) showed that it not only suppressed CRH when administered to uninephrectomized rats, but abolished most renal proliferation altogether. Desoxycorticosterone (DCA), however, was found to be of importance, as indicated by the following experiments.

Three groups of 12 rats each were uninephrectomized. The experimental group was adrenalectomized and given twice daily subcutaneous injections of 20 mg DCA in 0.1 ml sesame oil. The other groups, one of which was adrenalectomized, were both injected with sesame oil as controls. All animals were given fresh water to drink and were maintained under the conditions described above. As Fig. 2 illustrates, the 48-hour mitotic peak in the DCA-treated group was in a range comparable to that of the controls, while adrenalectomy without supplemental DCA inhibited CRH, as expected. Indeed, similar injections of DCA into unoperated rats for periods up to 4 days were found to induce a transient but significant ($P<.05$) renal hyperplasia on the third day

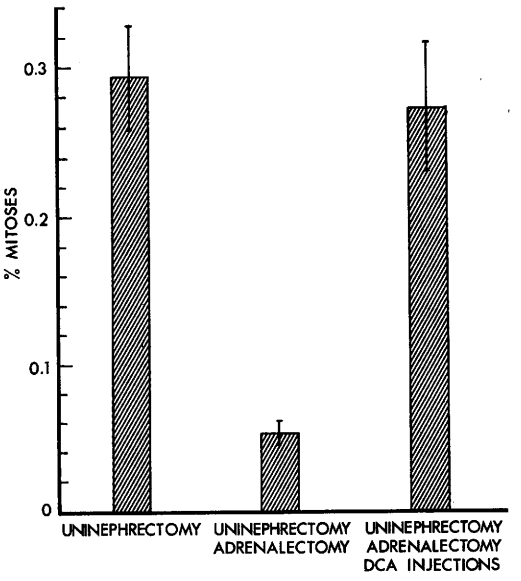

FIG. 2. Influence of adrenalectomy and DCA injections on CRH 48 hours after unilateral nephrectomy.

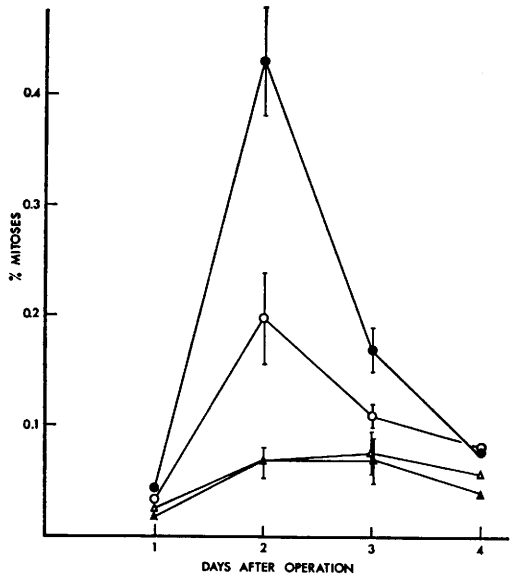

FIG. 3. Effect of unilateral ligation of ureter on % renal mitosis ± S.E. In ipsilateral hydronephrotic kidneys (●) there is an extreme outburst of cell divisions. Contralateral unoperated kidneys (○) exhibit more moderate hyperplasia. In sham-operated kidneys (▲) and opposite intact ones (△) no significant hyperplasia occurred.

of treatment. These experimental results suggest that the adrenal glands may be essential to CRH because of the mineralocorticoids they secrete.

Influence of adrenals on hydronephrotic hyperplasia. Still another phenomenon affected by adrenalectomy is the renal reaction induced by ligation of one ureter. This causes ipsilateral hydronephrosis accompanied by extreme hyperplasia to about twice the levels typical for normal CRH(6,10-12). Although Benitez and Shaka(12) have recently reported that mitotic activity in the contralateral kidney remains normal under these conditions, others(6,10,11) have detected a moderate rise in proliferative rate.

In the present investigation, the left ureters were ligated in 4 groups of 12 rats each, one group being sacrificed daily for 4 days. Sham operations were performed on equal numbers of control rats. In the latter group mitotic activity remained normal throughout the 4-day observation period (Fig. 3). The hydronephrotic kidneys of the operated rats, however, exhibited a remarkable rise in mitotic activity on the second day, which returned to normal by the fourth day. The opposite intact kidneys also underwent proliferation (Fig. 3). Although their degree of hyperplasia was not as great as that in the ligated kidneys, it was significantly above ($P<.02$) the normal mitotic levels in the sham-operated controls.

When this experiment was performed on adrenalectomized rats (without supplemental salt), the vicarious hyperplasia in the nonligated kidneys was reduced to normal levels (Fig. 4). Clearly, the hyperplastic response of the intact kidney to ligation of the opposite ureter depends on the presence of sufficient salt, as controlled by the adrenal cortex. In this respect, it resembles CRH following uninephrectomy.

In the hydronephrotic kidneys of adrenalectomized rats, mitotic activity was reduced by about the same amount as in the contralateral organs ($P<.01$), thus leaving it still well above normal (Fig. 4). Hence, the exaggerated mitotic response of the hydronephrotic kidney represents the summation of several effects. In part, it is attributed to NaCl and the adrenal cortex, as shown in the present account. Another fraction of the hydronephrotic hyperplasia may owe its origin to the local traumatic effects of tubular

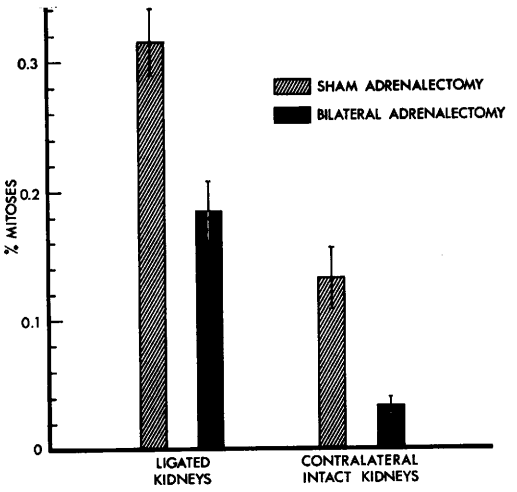

FIG. 4. Effect of adrenalectomy on renal hyperplasia occurring 48 hours after ureteral occlusion. In absence of adrenals, incidence of cell division in hydronephrotic kidneys (left) was significantly reduced. In opposite unoperated kidneys (right), adrenalectomy inhibited vicarious hyperplasia, but did not affect normal rate of background proliferation.

distentions. Represented by the residual hyperplasia seen in the hydronephrotic kidneys of adrenalectomized rats deprived of supplemental salt, this response is probably akin to local wound healing, and may be mediated *via* pathways other than those involved in CRH caused by functional overload. In addition to the above effects, still another portion of renal proliferation is represented by normal background mitosis. This is independent of adrenal cortical influences as Fig. 1 indicates, but what causes it remains to be determined.

Discussion. Proliferative responses in the kidney derive from a number of different causes which produce additive effects on rate of cell division. The experiments described above demonstrate the role of NaCl in stimulating renal mitosis, an influence possibly related to juxtaglomerular physiology and hemodynamic factors. DCA produces similar effects, probably by promoting the retention of sodium. Other electrolytes may eventually be found to cause comparable reactions in the kidneys. Whether or not they all affect the same physiological pathway can be determined only by observing renal mitotic responses to treatments with various salts in combination.

It is well known that high protein diets also stimulate renal hypertrophy(13). Inasmuch as this cannot be attributed to the excretion of excess urea, and since the liver also enlarges in animals on protein-rich diets, it seems plausible that the necessity to deaminate amino acids in the liver and kidney might bring about enlargement of these organs. The enhancement of CRH in uninephrectomized rats subjected to partial hepatectomy(14) suggests that these two organs might share a common function upon which their growths depend. Deamination, then, may constitute a growth-promoting pathway distinct from that concerned with salts and DCA as outlined above. If so, heightened intakes of salt and protein would be expected to stimulate more renal proliferation than either one alone.

From the above evidence pertaining to the kidney, it would appear that organs may be stimulated to grow by however many functions they may be called upon to perform. In specific physiological deficiencies, the proliferative response is less than when all functions are affected, as in cases of partial organ resection. Functions mediated by different physiological pathways should therefore give rise to additive growth responses. The more functions an organ has, the more different pathways would be activated by partial ablation, and the greater should be the additive growth reaction. Perhaps this explains why the regenerating liver, with numerous functions, exhibits so much more hyperplasia than do other less versatile organs. This hypothesis calls for greater emphasis on correlating specific functional demands with compensatory growth reactions in a wide variety of organs.

Summary. The compensatory renal hyperplasia that normally follows unilateral nephrectomy is abolished by bilateral adrenalectomy if rats are maintained on fresh drinking water, but is restored when saline drinking water is provided or if such animals are injected with desoxycorticosterone. The adrenals are also important for the heightened proliferation caused by unilateral hydronephrosis. These experiments suggest that the role of the adrenal cortex in renal hyperplasia may be to secrete mineralocorticoids, which promote the retention of sodium and thereby stimulate cell division in the kidneys.

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Histo-Immunological Detection of Collagen Fractions. (29838)

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Demonstration of the antigenicity of collagen(1,2) and application of the fluorescent antibody technique(3) have made possible the detection of collagen antigens in reticulin, as well as in collagen fibers of many organs (4,5,6). Since these studies were performed with crude and heterogeneous preparations of connective tissue, we then used more highly purifed collagen preparations to obtain more specific antibodies, and preliminary results were reported(7). In the present study, by means of a fluorescent labeling technique, an attempt is made to demonstrate more precisely the localization of individual collagen "fractions" within connective tissue structures.

Materials and methods. An acetic acid extract of collagen(2) and more highly purified neutral salt, citrate-soluble and insoluble fractions prepared from leg tendons of adult chickens by the procedures of Jackson(8), were used as antigens. Schedule of heterologous sensitization of adult rabbits and guinea pigs, and a description of the serological tests applied to check the production and specificity of corresponding antibodies, have been published(7). Globulin, or the gamma globulin fraction, was separated from anti-collagen antisera by a salt fractionation procedure, and dissolved to a concentration of 20 mg of protein per ml; it was then conjugated with fluorescein isothiocyanate(9). Unconjugated fluorescein was removed by dialysis against phosphate-buffered saline (pH 7.1) at 4°C. Nonspecific fluorescence was removed by prior absorption twice with a powder prepared from rat or guinea pig liver, followed by centrifugation, or by passage through a column of Sephadex G 25 (10); the material was then lyophilyzed. Samples of unconjugated globulin antibody, also adjusted to a concentration of 20 mg of protein per ml, were lyophilyzed and used for control blocking reactions.

Blocks from several organs containing different types of connective tissue (leg tendon, larynx cartilage, tibial bone, skin, cornea, sclera, stomach, intestine, comb, kidney, liver, testis and aorta) were obtained from adult Leghorn chickens about 2 to 4 months old. A portion of each block was fixed in 4% formol and submitted to silver impregnation, and other small pieces were frozen rapidly and cut at 4 to 8 μ, in a cryostat at —20°C.

Direct or indirect Coons' techniques(3) were applied to the unfixed sections or to sections fixed previously with 90% alcohol or acetone. For these purposes the conjugated globulin fraction or the whole immune serum was used, followed, in the latter case, by the corresponding sheep globulin anti-rabbit globulin fraction (obtained from Microbiological Assoc.). Control reactions were performed in both cases: 1) with serum or globulin fraction, both from normal adult rabbits or guinea pigs; 2) with other immune sera such as rabbit sera anti-chicken testis; 3) by a blocking test with unconjugated anti-collagen sera or its globulin fraction; 4) with immune sera treated previously with 80 to

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