Mitotic Responses of the Compensating Rat Kidney to Injections of Tissue Homogenates*

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SUMMARY

Mitotic activity in the remaining kidneys of unilaterally nephrectomized rats reaches a peak about 48 hours after operation. To test for the possible existence of tissue-specific growth-regulating factors, such rats were given injections of various tissue homogenates *ca.* 30 hours after uninephrectomy. Intraperitoneal administration of homogenates of fresh, cooked, or frozen kidneys, or suspensions of trypsin-dissociated kidneys, all reduced the 48-hour mitotic peak of hyperplastic kidneys by approximately 50 per cent. Saline-injected controls were insignificantly altered. Subcutaneous injections of frozen kidney homogenates did not adversely affect compensatory renal hyperplasia.

Intraperitoneal injections of fresh liver, testis, spleen, and blood homogenates inhibited renal mitosis as effectively as did kidney homogenates. Blood plasma from normal or uninephrectomized rats, however, were not effective. Egg albumin suppressed mitosis, but egg yolk did not.

In view of these failures to demonstrate a tissue-specific effect of homogenates on renal mitosis, together with similar discrepancies in comparable studies of liver, the existence of growth-regulating agents related to organ mass per se, and distinct from other functional demands, must remain problematical.

The principle of replacement therapy has proved its usefulness in many ways, not the least of which is in the experimental investigation of tissue and organ function. Interpreting growth and differentiation as parameters of cell functions, developmental biologists have recognized the rationale of attempting to study compensatory organ growth by replacing lost tissue in altered form. Correlations between the degree of alteration to which the replaced tissues may have been subjected and the effects of such treatments on compensatory growth-responses should logically disclose factors responsible for regulating growth.

Under the impetus of the theoretical considerations advanced by Weiss (35, 36), numerous experiments have been designed to demonstrate the existence of tissue-specific intracellular growth-stimulators (templates) or extracellular growth-inhibitors (antitemplates). According to this view, growth should occur when the balance between these two hypothetical factors is upset in favor of the former, either by reducing the relative concentration of antitemplates or by increasing that of the templates. Such effects can be achieved by appropriate experimental manipulations of the serum or tissues, respectively. Replacement or addition of extra tissue, however, should inhibit or stimulate growth in homologous organs depending on whether it remained viable and capable of further antitemplate production or underwent cellular disruption to release supplementary templates.

Fundamental to this theory is the belief that instances of compensatory growth represent "active maintenance of the total mass of each organ system" (35) irrespective of functional activities, an interpretation consistent with the classical notion of functional overload only if one equates mass with function. The distinction between these two possibilities can best be revealed by studying them separately. Inasmuch as function is insepa-

* This investigation has been supported in part by a research grant (B-923) from the National Institutes of Health, and in part by a fellowship from the Department of Embryology of the Carnegie Institution of Washington.

Received for publication February 28, 1963.

rable from mass, the crucial experiment must involve the administration of tissue mass rendered incapable of performing the normal physiological activities characteristic of its kind. This has been variously attempted by observing organ growth under the influence of grafts, suspensions, homogenates, or extracts of homologous tissues. In some instances—e.g., endocrine glands—replacement of tissue mass is usually tantamount to functional compensation, in which case a categorical conclusion concerning the roles of mass vs. function in growth control cannot easily be reached. In other organs, whose functional units may be at the cellular or histological levels of organization, it is possible to effect functional disruption of the cells by mechanical means without doing violence to molecules presumed to be important in growth regulation. The liver, which lends itself particularly well to experiments along these lines, has been extensively investigated. The present experiments have been undertaken to study comparable phenomena in the rat kidney, which likewise possesses numerous functional units (nephrons) vulnerable to mechanical destruction.

TABLE 1

EFFECTS OF THE ADMINISTRATION OF VARIOUS MATERIALS ON THE MITOTIC RATES OF KIDNEYS IN UNINEPHRECTOMIZED RATS*

Material injected	Hours until sacrifice	No. kidneys	No. mitoses per 1,000 cells ± S.E.
Intact controls		12	0.24 ± 0.06
Not injected	48	24	1.48 ± 0.21
Saline	48	24	1.17 ± 0.19
Fresh kidney	48	12	0.70 ± 0.26
Cooked kidney	48	12	0.52 ± 0.23
Frozen kidney	48	12	0.64 ± 0.18
Frozen kidney	72	12	0.58 ± 0.09
Frozen kidney	144	11	0.61 ± 0.08
Frozen kidney (subcutaneous)	48	12	1.43 ± 0.15
Trypsin-dissociated kidney suspension	48	6	0.74 ± 0.66
Hanks BSS	48	6	1.81 ± 0.77
Centrifuged kidney supernate (0.5 ml. intravenous)	48	12	0.80 ± 0.11
Saline (0.5 ml., intravenous)	48	12	1.16 ± 0.19
Fresh liver	48	12	0.77 ± 0.23
Fresh testis	48	12	0.62 ± 0.13
Fresh spleen	48	12	0.51 ± 0.11
Fresh blood	48	12	0.69 ± 0.09
Fresh plasma	48	12	1.33 ± 0.16
Plasma of nephrectomized rat	48	12	1.31 ± 0.32
Fresh egg albumin	48	12	0.80 ± 0.12
Fresh egg yolk	48	12	1.20 ± 0.15

* Unless otherwise indicated, experimental animals were given injections intraperitoneally approximately 30 hr. after uninephrectomy (18 hr. before sacrifice) of 1–2 gm. of tissue homogenized in saline.

MATERIALS AND METHODS

Groups of twelve male Sprague-Dawley rats weighing between 100 and 150 gm. were subjected to left nephrectomy between 9 and 11 A.M., were administered various test materials between 3 and 5 P.M. the following day, and sacrificed 48 hours after the original operation (18 hours after injection). Operations and sacrifice were carried out under nembutal anesthesia. Kidneys recovered at sacrifice were fixed in Bouin's, sectioned serially (7μ) through the midtransverse region, stained with hematoxylin, and inspected for mitoses in 50 randomly selected cortical fields (450×) throughout ten cross-sections per kidney. In each organ approximately 21,400 cells were scanned, and the proliferative activity was expressed as numbers of mitoses per 1,000 cells.

Preliminary studies were undertaken to determine the mitotic activity in kidneys of intact rats and in those of rats 48 hours after uninephrectomy when the maximum degree of hyperplasia occurs (10). The basic experiment involved injecting uninephrectomized rats intraperitoneally with 1–2 gm. of tissue homogenized in a glass tissue grinder in enough 0.9 per cent NaCl to make up a volume of *ca.* 2 ml. Variations of this experiment included administration of similar doses of homogenates of fresh, frozen, or cooked (100° C. for 3 hr.) kidney injected intraperitoneally or subcutaneously, and sacrifice of recipient rats 48, 72, and 144 hours after uninephrectomy. Other rats each received intraperitoneal injections of viable cells dissociated from single kidneys in 0.25 per cent trypsin and suspended in Hanks BSS. Still another series was given intravenous injections of the supernates of frozen rat kidney ultracentrifuged at 80,000× *g* for 2 hr. The specificity of the effect was determined by similar intraperitoneal injections into uninephrectomized rats of homogenates of other tissues (liver, testis, spleen, blood) as well as of fresh egg albumin and yolk. Controls received equivalent amounts of saline solution. The various experiments are itemized in Table 1.

RESULTS

Confirming previous studies (10), the mitotic activity in the remaining kidney 48 hours after uninephrectomy was approximately 6 times as great as in unoperated controls. Injection of 2 ml. saline intraperitoneally, about 18 hours prior to sacrifice at 48 hours, did not significantly alter the number of mitotic figures in the residual kidney. This series of rats served as controls for the following experiments involving intraperitoneal injections of various materials. Although in many

instances there occurred a substantial reduction in mitotic activity, the differences were seldom statistically significant ($P < 0.1$–0.2) owing to the considerable individual variations characteristically encountered in mitotic counts. Thus, intraperitoneal injection of fresh kidney homogenized in saline decreased mitotic activity in the remaining kidney *ca.* 40 per cent, an amount not significantly different ($P < 0.2$) from the counts in saline-injected control rats. This general effect was not appreciably different when the kidney was previously cooked ($P < 0.05$) or frozen ($P < 0.1$). The depression of mitotic activity by the latter preparations was apparently not attributable to a delay in response, since after 72 and 144 hours the proliferative activity was not altered from that noted at 48 hours. In view of the relatively brief but high rise in mitotic activity previously reported in kidneys undergoing normal compensatory hyperplasia (10, 38), it is possible that the chief effect of kidney homogenate injections is to reduce the amplitude but extend the duration of the compensatory response. No such effect was obtained, however, when similar homogenates of frozen kidney were injected subcutaneously instead of intraperitoneally. Under these conditions, a slight, but not significant, increase in the 48-hour mitotic response occurred, indicating that the rate at which the components of the homogenate are absorbed is probably an important factor in determining the host reaction. Intravenous injections of such concentrated kidney homogenates have lethal effects; however, ultracentrifugation at 80,000 × *g* for 2 hr. yielded a relatively clear supernate which, when injected intravenously in quantities of 0.5 ml., caused a decrease ($P < 0.2$) in mitosis in compensating kidneys almost as great as that following the intraperitoneal injection of homogenates. Intravenous injections of saline (0.5 ml.) did not differ from intraperitoneal injections.

To determine whether the factors responsible for reducing the number of compensatory renal mitoses are tissue-specific, homogenates of other tissues were tested according to the same criteria described above. In all cases (liver, testis, spleen, blood) the effects were comparable to those previously obtained with kidney homogenates ($P < 0.02$–0.2). It is evident, therefore, that this is not a tissue-specific effect. Administration of blood plasma, however, was without influence, indicating that the cellular content, or some derivative thereof, is responsible for the inhibitory effect on renal mitosis. Similar injections of blood plasma from rats that had been uninephrectomized for 48 hr. were likewise ineffectual. On the theory that the amount of protein present in the injected material might be the causative factor, 2 ml. of egg albumin was injected intraperitoneally into uninephrectomized rats and was found to be capable of depressing compensatory hyperplasia ($P < 0.2$). In contrast, egg yolk was ineffective.

Although the above results are reasonably consistent among themselves, they serve to illustrate the difficulties of demonstrating tissue-specific growth-regulating factors. Clearly, the observed effects reflect a nonspecific inhibitory influence on compensatory renal mitosis which appears attributable to the protein content of the various materials injected. Such results, therefore, argue against the existence of tissue-specific growth-regulating molecules in kidney homogenates.

DISCUSSION

The general subject of compensatory hypertrophy and organ growth regulation has been recently reviewed by such authors as Abercrombie (1), Bullough (6), Paschkis (17), Rose (19), Swann (25), and Wright (40). It is pertinent to consider here the data which have accumulated from numerous experiments designed to investigate the effects of tissue injections on organ growth, for in a sense one might expect results comparable to those reported above with reference to the kidney. In view of the extensive literature on this subject as it relates to the liver, our knowledge of this organ's growth responses will be reviewed to the exclusion of other organs, such as the spleen (16) and orbital glands (26), that have not as yet been investigated in such detail. Notwithstanding the inevitable technical differences inherent in comparisons between the results of different investigators, it is not possible to detect consistent effects of injections of normal, adult liver preparations on mitosis in the intact livers of rats and mice. In experiments of this kind, some investigators have reported no effects on liver mitosis (4, 13, 23), others have found increases (18, 21, 28) or decreases (20) in proliferative activity, and some have noted decreases followed by increases (39). Blomqvist (4) detected no effect of adult liver homogenates on normal liver mitosis but noted increased proliferation following injections of newborn liver or regenerating adult liver. Christensen and Jacobsen (7), however, failed to induce liver mitosis by the intraperitoneal administration of minced adult regenerating liver. Wilson and Leduc (39) found decreased mitosis in mouse livers up to 3 days followed by an increase on the 5th day, irrespective of whether the injected homogenates were prepared from guinea pig liver, mouse kidney,

boiled egg yolk, or normal, boiled, or autolyzed mouse livers. Other investigators have reported no changes in liver mitosis following injections of kidney (13, 20), parotid gland (23), thymus (29), or embryo homogenates (13). Although Teir and Ravanti (28) found slight increases in mitotic rates of rat livers after intraperitoneal injections of orbital gland homogenates, Teir *et al.* (27) noted no influences of such injections on hepatic ribonucleic acid or deoxyribonucleic acid. Grafts of tumors (3), including hepatomas (30), are also known to increase liver mitotic activity, and Malmgren (13) has reported significant rises in hepatic mitotic rates in mice bearing grafts of mammary carcinomas or receiving injections of breis or saline extracts of such tissues.

Equal confusion has characterized attempts to study influences of hepatic preparations on mitosis in regenerating livers. The intraperitoneal administration of minced normal adult liver caused no change in the normal hyperplasia of regenerating livers, according to Christensen and Jacobsen (7). Increased mitotic activity, however, has been noted in regenerating livers following intravenous injections of liver chromatin extracts (14) or intraperitoneal administration of liver homogenates (12, 15). Saetren (20) and Stich and Florian (24) detected decreased proliferation in regenerating livers after injections of normal liver homogenates. The same authors (20, 24), however, found no effect of kidney or brain homogenates on regenerating liver mitosis, nor did homogenates of regenerating livers exert an influence on hepatic hyperplasia (24). Grafts of hepatomas were likewise without effect on proliferative activity in regenerating livers (30).

Though the kidney has not been a popular object of investigation along these lines, the experimental results are almost as inconsistent as those in the liver. Following intraperitoneal injections of normal adult kidney preparations, mitotic activity in recipient kidneys has been found to increase (5, 21) or decrease (20). Injections of liver either diminish renal mitosis (23) or have no effect (20, 21), whereas proliferation in kidneys is unchanged (21) or reduced (23) by administration of pancreatic or parotid extracts, respectively.

When injected into rats undergoing compensatory renal hyperplasia, homogenates of kidney were found to stimulate proliferation after 2 weeks (5), but at 48 hours an inhibition of mitotic activity in compensating kidneys occurs (20, 22). The latter results are consistent with those reported in the present investigation. Following administration of liver homogenates, however, Saetren (20) reported no effect on compensating renal hyperplasia, although Steuart (22) found an inhibitory influence.

Saetren's (20) results are in agreement with those herein reported in one respect—namely, the inhibitory effect of intraperitoneal kidney homogenates on the 48-hour mitotic peak characteristic of compensatory renal hyperplasia. It has not been possible, however, to confirm most of his other results, including the efficacy of subcutaneous injections of kidney homogenates, the heat-labile quality of his inhibitory principle, nor the restriction of this effect to the kidney as opposed to the liver. It is conceivable that we have been studying separate factors—one tissue-specific and heat-labile, the other nonspecific and heat-stable; how such a discrepancy might have arisen is not known.

Judging from the foregoing data, it is not possible to demonstrate unequivocally the existence of tissue-specific growth-promoting factors by administration of homogenates or extracts of liver or kidney. Until the futility of such an experimental approach has been overcome, perhaps by refining the procedures by which tissue preparations are made or by studies of dose-response relationships, the presence of growth-stimulants that are distinct from the functional activities of adult organs must remain only a theoretical possibility. Perhaps it is significant, however, that in the embryo, whose organs are not functionally mature, the application of grafts and extracts of tissues appears to have more consistent effects on homologous host organs than in adult organisms. Grafts or extracts of liver, for example, stimulate hepatic growth in young chick embryos (2, 8, 32, 33, 37), an effect which seems to diminish with increasing age of the host (32). According to Andres (2), similar effects are caused by suspensions of mesonephros. Mitotic activity in the embryonic mesonephros is also stimulated by injection of mesonephric suspensions, but not by liver suspensions (2). The possible specificity of such reactions may find its explanation in the preferential uptake of S^{35} by kidney (9) and liver (34) from labeled adult homologous organs administered to chick embryos. Comparable experiments on 6-week-old mice, however, did not result in the selective uptake of label by the same homologous organs (11).

Thus, the evidence in favor of the existence of tissue-specific growth-stimulators is more convincing in embryonic systems than in postnatal ones. It is possible, therefore, that growth regulation in embryonic organs, whose only function is to grow, is mediated by factors sensitive to relative tissue mass. Once such organs are functionally competent their size may be influenced primarily by physiological demands.

ACKNOWLEDGMENTS

Part of this research was conducted under a fellowship from the Department of Embryology, Carnegie Institution of Washington, for which the author is especially grateful. The technical assistance of Mrs. Sally Hoedemaker is sincerely appreciated.

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