quantitative difference between the pyridoxine deficient and pyridoxine control groups existed when relatively large amounts of murine rickettsia were injected. The animals reported in this paper were bled prior to immunization and tested by complement-fixation. All of these sera were found to be negative.

Summary. These studies indicate that nicotinic acid deficiency does not appreciably influence the production of circulating complement-fixing antibody when either relatively small or large amounts of antigenic stimulation were employed. Pyridoxine deficiency, however, influenced unfavorably the antibody response when either small or large amounts of the antigen were injected. The single immunizing injection (0.0073 mg N) did not bring forth circulating antibodies that were demonstrable by complement-fixation.

Received August 13, 1951. P.S.E.B.M., 1951, v78.

Role of Sodium Chloride, Water, Kidneys, and Adrenals in the Production of Congestive Edema.*† (19066)

WILLIAM O. REINHARDT.

From the Division of Anatomy, University of California Medical School, Berkeley.

Evaluation of the respective roles of the adrenals and kidneys in the genesis of edema is dependent on the availability of an animal form and technic whereby an edematous state can be regularly and simply produced. The experiments reported here provide evidence that such an edema can be produced in the rat by ligation of the inferior vena cava, if the animal is allowed free access to 1% NaCl solution postoperatively. This animal preparation has been employed to study the effects of removal of the adrenals and/or kidneys on the development of edema. It is demonstrated that edema does not develop in the nephrectomized rat. However, removal of the adrenals in the nephrectomized rat does result in the development of an edematous state, suggesting that renal retention of sodium and water is not an obligatory factor in congestive edema formation.

Methods. Unfasted male rats of the Long-Evans strain, maintained on this laboratory's Diet XIV, were subjected under brief ether anesthesia to various operative procedures, using clean surgical technics, employing midline ventral abdominal wall incisions. The inferior vena cava was ligated at the caudal margin of the liver, proximal to the right renal vein, with a single silk suture, taking care to include only the vena cava in the ligature. Bilateral nephrectomy was carried out by ligation of the renal pedicles distal to the entrance of the adrenal veins into the renal veins. Bilateral adrenalectomy was carried out by cautery dissection, the glands being removed en bloc with surrounding fat, together with any accessory adrenal cortical nodules visualized. Various sham operations were performed for control purposes. Care was taken to suture doubly all incisions, in order to avoid wound rupture postoperatively as the result of excessive edema formation. After recovery from the brief period of anesthetization, the animals were given access to either tap water or 1% NaCl in tap water for drinking purposes and were allowed free intake of Diet XIV. The animals were weighed postoperatively twice daily. Maximum gains or losses in body weight within the first 24-36 hour period postoperatively were noted and calculated as % change from the onset body weight. The data are presented in Table I.

Results. Ligation of the inferior vena cava

* Assisted by a grant from the National Institutes of Health, U. S. Public Health Service.

† Grateful acknowledgement is made to Dr. H. M. Evans of the Institute of Experimental Biology, and to Dr. Choh Hao Li of the Department of Biochemistry, for various kinds of assistance. Appreciation is expressed to Marian Cleeves, Elizabeth Swirnofsky, Roslynn Kenigsberg and Paul Yparraguirre for their cooperative efforts.

TABLE I. Effect of Adrenalectomy and Nephrectomy, Singly and in Combination with Ligation of the Inferior Vena Cava, on Fluid Retention and Edema Formation in the Rat, as Manifested by Changes in Body Wt.*

Operative procedure	No. of rats	Fluid intake	—% wt change, gain or (loss)— % ± stand. error	Range, %
Controls and sham-operations	13	NaCl	3.9 ± .6	—1 to +8
	5	Water	(6.7 ± .5)	
Ligation of inferior vena cava†	50	NaCl	17.3 ± 2.6	—7 to +72
	16	Water	(.4 ± .7)	
Bilateral nephrectomy	13	NaCl	2 ± 1.3	—4 to +12
Bilateral nephrectomy and ligation of inferior vena cava	12	NaCl	4.6 ± 1.7	—2 to +13
	11	Water	.1 ± .6	—2 to +2
Adrenalectomy and nephrectomy	7	NaCl	(3.2 ± .9)	—7 to +5
Adrenalectomy and ligation of inferior vena cava	13	NaCl	20.2 ± 3.2	+5 to +40
	5	Water	(2 ± 1)	—5 to 0
Bilateral nephrectomy, adrenalectomy and ligation of inferior vena cava	11	NaCl	12.5 ± 4.7	—2 to +46
	5‡	NaCl	27 ± 4.5	+21 to +46
	5	Water	(1 ± 1.4)	—5 to +3

* Operative procedures carried out under ether anaesthetic. Animals were given immediate postoperative access to food and either tap water or a 1% solution of NaCl; edema was measured quantitatively by wt changes which were maximal in 24-36 hr.

† All inferior vena cava ligations were carried out between the caudal edge of the liver and the right renal vein.

‡ Represents 5 animals of the preceding group of 11 rats to show extent of fluid retention at its maximum.

at the caudal edge of the liver did not cause edema in animals given access to water postoperatively. If, however, these animals were given free and voluntary access to 1% NaCl solution for drinking purposes, such treatment resulted in retention in the tissue spaces of a major part of the ingested fluid, resulting in an average body weight gain of 17% within a period of 24-36 hours. The retained fluid manifested itself in the form of a peripheral edema, commencing in the caudal half of the body, and gradually involving the entire body (subcutaneous and loose retroperitoneal and mediastinal tissues, with evidence, in some instances, of slight pleural effusion and/or ascites). Such animals became almost globular in shape, and spent their time in constant drinking, exhibiting a marked polydipsia for the proffered saline solution. Within a period of 3-6 days, however, the animals spontaneously lost their edema fluid.

The availability of this method for the production of an acute and massive edema permitted a study of the effects of renal insufficiency and hypoadrenalism. It was found that ligation of the inferior vena cava in the nephrectomized animal was not an adequate stimulus to cause edema formation, nor did nephrectomy without caval ligation cause the development of this condition. Noteworthy, however, was the development of edema in the adrenalectomized animal with ligated vena cava (average body weight increase of 20%). In a similar manner, removal of the adrenals in the nephrectomized rat with ligated cava caused edema formation (average body weight increase of 27% in the 5 most marked examples). It is to be emphasized that intake of unsalted water did not initiate any of the phenomena noted, nor did sham operations eventuate in edema formation.

Discussion. The edema produced in these experiments by ligation of the inferior vena cava is entirely dependent on the availability and intake of an extra source of NaCl and water. Animals with ligation of the inferior vena cava did not develop edema when given access to water alone. It is well established that an adequate salt intake is necessary before edema formation can occur(1). As noted, the rat with ligated vena cava, when given free access to 1% NaCl, exhibits a marked increase in fluid intake which is coupled with a demonstrated relative failure

1. Peters, J. P., *New Engl. J. Med.*, 1948, v239, 353.

to excrete imposed water loads(2). The combination of increased intake and decreased output result in the formation of a temporary acute massive edema which is lost spontaneously upon recovery of renal excretory function. It is presumed that this is coincident with the development of adequate venous collateral circulatory channels and restoration of more nearly normal hemodynamic and hormonal relationships. It is of interest that removal of the kidneys did not permit the development of edema in the rat with ligated vena cava given free access to saline solution, since the fluid intake in these animals remained at a low level. The suggestion must be made that the kidneys may act, either directly or indirectly, as regulators of thirst and appetite for NaCl. The observation that edema does develop in the nephrectomized animal, if the adrenals are simultaneously removed, suggests a reciprocal relationship between these organs in the regulation of thirst and appetite for NaCl, in the presence of venous congestion. Adrenalectomy, *per se,* is known to cause an increased intake of NaCl (3). The adrenalectomized animal with ligated vena cava, but with intact kidneys, given access to saline develops edema to the same degree as does the non-adrenalectomized rat in the present experiments. It is noteworthy that removal of the adrenals in the nephrectomized rat causes a marked thirst and appetite for NaCl sufficient to overcome the effect of nephrectomy and allow the development of massive edema.

To summarize, if venous congestion is produced in an animal which is allowed free access to saline, edema will develop in the presence or absence of the adrenals, or in the adrenalectomized-nephrectomized animal, but not in the nephrectomized animal. The edema appears to be dependent on the development of thirst and appetite for NaCl solution. The kidney thus appears to play an inhibitory role in this phenomenon which is overweighted by adrenal insufficiency.

The observation that adrenal insufficiency is instrumental in allowing edema formation in the nephrectomized animal assumes significance in interpreting the role of the adrenals and kidneys in various clinical states, such as cardiac congestive failure and the nephrotic syndrome. In this respect, Burch and Ray (4), in a recent report on congestive heart failure, have critically reviewed the nature of the etiological factors operative in edema formation, and have stressed the inferential role played by hormonal factors. They point out that the present hiatus in our understanding of the mechanisms of hormonal activity in congestive failure is, to a large extent, consequent on the lack of suitable experimental methods whereby hormonal relationships can be varied under controlled conditions, with resulting certainty that a condition resembling congestive edema will eventuate. It is felt that the present experimental animal remedies the situation by providing an object in which such hormonal alterations can be evaluated. The edematous state demonstrated in these experiments exhibits the pattern and characteristics of the peripheral edema associated with cardiac congestive failure.

The demonstration that hypoadrenalism facilitates the development of edema in the nephrectomized rat subjected to venous obstruction is likewise of interest, because of the recent reports suggestive of improvement in the congestive failure of cardiac origin (5-8), and in the nephrotic syndrome(9-13), after treatment with adrenocorticotrophic

2. Reinhardt, W. O., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 809.

3. Richter, C., *Am. J. Physiol.,* 1936, v115, 155.

4. Burch, G. E., and Ray, C. T., *Am. Heart J.,* 1951, v41, 918.

5. Massell, B. F., and Warren, J. E., *J. Am. Med. Assn.,* 1950, v144, 1335.

6. Sprague, R. G., Power, M. H., and Mason, H. L., *J. Am. Med. Assn.,* 1950, v144, 1341.

7. Mazar, S. A., and Bogdasarian, R. M., *J. Am. Med. Assn.,* 1950, v144, 1369.

8. Dorfman, A., Bergenstal, D. M., Smull, K., Howard, R., Senz, E., Oleson, D., and Muller, R., *Proc. 2nd Conf., Clinical ACTH,* vII, *Therapeutics,* 645, edited by Mote, J. R., Blakiston, N. Y., 1951.

9. Metcoff, J., Kelsen, W., Rance, C. P., Janeway, C. A., *Proc. 2nd Conf., Clinical ACTH,* vI, *Research,* 148, edited by Mote, J. R., Blakiston, N. Y., 1951.

10. Leutscher, J., Jr., *Proc. 2nd Conf., Clinical ACTH,* vI, *Research,* 155, edited by Mote, J. R., Blakiston, N. Y., 1951.

hormone (ACTH) or cortisone. Such observations suggest that a relative or absolute hypo- or dysfunction of the secretory activity of the adrenal cortex, or of the responsiveness of peripheral tissues, may exist in these conditions.

The increased intake of NaCl produced by ligation of the inferior vena cava in the rat is reminiscent of the similar chain of events in animals treated with desoxycorticosterone (DOC)(14). Insofar as it is possible, on the basis of the gross observations reported here, to hazard an explanation of the role of the adrenal cortex in the edema of congestive failure, it may be remarked that in this condition, as the result of changes in venous pressure, in electrolyte balance, and in protein and volume regulation, changes which in themselves may not seem grossly exaggerated, that a relative disproportion may occur in the secretion and/or peripheral effects and/or excretory rates of adrenal salt retaining steroids (DOC-like) as contrasted with the 11-17 oxycorticosteroids [see reviews by Burch and Ray(10), Stead(15), and Smith (16).] In this connection, comment must be made on a possible relationship between the diurnal rhythm of congestive failure (nocturnal dyspnea, etc.) and a similar rhythmic cycle in the secretory activity of the adrenal cortex.

Summary. The edema produced in the rat by ligation of the inferior vena cava and voluntary intake of 1% NaCl solution is studied as altered by adrenalectomy and nephrectomy. (1) The obligatory role of NaCl in the formation of edema fluid is reemphasized. (2) The venous congestion produced by ligation of the inferior vena cava is not a sufficient stimulus to cause edema formation in the nephrectomized rat, but does cause edema if the animal is nephrectomized and adrenalectomized. (3) Under these conditions, it is demonstrated that the kidney does not play an obligatory role in the formation of the edematous state in the adrenalectomized rat. (4) The evidence supplied by these experiments is discussed in relationship to the role of the adrenal cortex in conditions exhibiting generalized edema (congestive heart failure, nephrotic syndrome).

11. Farnsworth, N. B., Dupee, C. F., *Proc. 2nd Conf., Clinical ACTH,* vII, *Therapeutics,* 149, edited by Mote, J. R., Blakiston, N. Y., 1951.

12. Farr, L. E., *Treatment of the Nephrotic Syndrome,* Thomas, Springfield, 1951.

13. Barnett, H. L., Forman, C. W., McNamara, H., and Crery, W. W., *J. Clin. Invest.,* 1951, v30, 227.

14. Rice, K. K., and Richter, C. P., *Endocrinology,* 1943, v33, 106.

15. Stead, E. A., *Circulation,* 1951, v3(2).

16. Smith, H., *The Kidney,* Oxford Press, N. Y., 1951, p691.

Received September 25, 1951. P.S.E.B.M., 1951, v78.

Effect of ACTH Upon Gastric Secretion.* (19067)

SEYMOUR J. GRAY, JOHN A. BENSON, JR., AND ROBERT W. REIFENSTEIN.†

From the Medical Clinic, Peter Bent Brigham Hospital, the Department of Medicine and the Biophysical Laboratory, Harvard Medical School, Boston.

The two principal physiological pathways by which the stomach may be stimulated to secrete hydrochloric acid and pepsin are mediated (a) by way of the vagus nerve and (b) by the release of a hormone, gastrin, produced by the gastric mucosa. Evidence will be presented indicating that the adrenal gland and the adrenal corticoids constitute a third mechanism by which gastric secretion may be stimulated. The vagal phase of secretion is activated by sight, smell, and ingestion of food, insulin induced hypoglycemia, and

* This work was supported in part by the William F. Milton Fund, Wyeth, Inc., and the Atomic Energy Commission.

† This work was done during the tenure of a Postdoctorate Research Fellowship from the Public Health Service.