MEASUREMENT OF SODIUM-RETAINING SUBSTANCES IN HUMAN URINE

Bertha Singer

Presented to the Faculty of Graduate Studies
and Research in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy.

McGill University Clinic,
Royal Victoria Hospital,
Montreal, Canada.

August 28th, 1952.

ACKNOWLEDGEMENTS

The author is greatly indebted to Dr. J. S. L. Browne who provided the facilities for this investigation and offered valuable criticism throughout the course of the work.

Thanks are also due Dr. E. H. Venning who has given advice whenever necessary and who attended to many of the technical arrangements required by this study.

Dr. C. P. Leblond of the Department of Anatomy and Dr. R. D. H. Heard of the Department of Biochemistry of McGill University have given advice regarding the handling of radioactive isotopes. Dr. J. S. Foster of the Radiation Laboratory at McGill University kindly provided several lots of radioactive material when it was not available at Chalk River.

This project would have been impossible to carry through without the cooperation of a great many doctors, nurses and technicians in several Montreal hospitals. Special thanks are due to Dr. F. McCall of the Children's Memorial Hospital, Dr. R. Laing of the Montreal General Hospital, and Drs. J. Wener, G.A. Simpson, A. Carballeira, H. Elrick, J.A.P. Pare, R.R. Soicher, N. Kalant, L.G. Johnson and W. Leith of the Royal Victoria Hospital.

Mr. H. Rammus prepared many of the urinary extracts and Miss H. Stone performed most of the surgery required in this study. The non-radioactive sodium determinations were made on the flame photometer by Miss H. Thomson of the Department of Experimental Surgery of McGill University.

Special thanks are due to the many friends, and particularly Mrs. A. Heller, who assisted in the editing and preparation of the manuscript which was typed by Miss Freda Jell. The figures were prepared by Miss Shirley Ellis.

TABLE OF CONTENTS

SECTION A - SURVEY OF THE LITERATURE

SECTION B - EXPERIMENTAL WORK

INDEX TO TABLES AND FIGURES

SECTION A - SURVEY OF THE LITERATURE

The Adrenal Cortex and Electrolytes

Recognition of the role of the adrenal cortex in electrolyte metabolism dates back to 1927 (1,2). Since then, a great deal has been learned about the effects of adrenalectomy, and replacement therapy, on electrolyte metabolism. A number of adrenal cortical compounds have been isolated, and their biological activity in adrenalectomized animals, and in patients with Addison's disease, has been ascertained. No attempt will be made, in this section, to cover all of the findings reported on the subject of the adrenal cortex and electrolytes, as several excellent reviews have appeared in recent years (3,4,5). However, some findings, pertinent to the present study, are presented below.

In 1932, Loeb (6) found that there was a fall in plasma sodium in Addisonian crisis, and that the administration of sodium chloride would render the patient symptom-free (7). The fall in plasma sodium following adrenalectomy in the dog was related to the abnormally large quantity of sodium in the urine (8). This urinary loss of sodium was attributed, by Harrison and Darrow (9), to a disturbance in the tubular reabsorption of this ion from the glomerular filtrate.

Anderson and co-workers noted that adrenalectomized rats, unsupported by salt treatment, had increased total sodium excretion, and an increased rate of excretion of administered sodium-24, as compared with normal animals (10,11,12). They found the reverse to be true for potassium. The simple access to a 1% solution of table salt resulted in marked differ-

ences in the handling of sodium and potassium by these animals. It was found that adrenalectomized rats, which had been drinking saline for five to eight days, had a 48-hour urinary sodium output, and rate of sodium-24 excretion, which was almost identical with that noted in normal animals without added salt in their drinking fluid. Since the salt intake of the adrenalectomized animals was higher than it was in the normal animals, and since the urinary output was almost identical in both groups, it would appear that the saline-treated adrenalectomized animals in these studies were storing sodium.

The dynamic aspects of sodium metabolism in adrenal insufficiency in dogs have been studied by Stern and co-workers (13) who reported that more sodium is lost from the extracellular compartment than is accounted for by the negative external balance. This finding supports the suggestion put forward in 1937 by Swingle and his associates (14), that adrenal hormone deficiency results in the storage of sodium within the cells, as well as in renal loss. Early research on the distribution of tissue electrolytes in adrenal insufficiency had revealed an increase in potassium and a decrease in sodium concentration of skeletal muscle and red blood cells (15). Stern and co-workers (13) have re-examined the question of tissue electrolyte distribution following adrenalectomy, in an effort to find the missing sodium. Examination of the liver, spleen, heart, muscle, brain, gut and skin failed to show any indication of its presence in these tissues. Since Harrison, Darrow and Yannet (16) had suggested that one quarter of the total body sodium existed in bone and cartilage, Stern and his associates investigated the sodium concentration in this

tissue as well but found, if anything, a slight decrease in its concentration. However, only the mid-femur was studied, and it was shown that adjacent portions of femur vary considerably in sodium concentration. These investigators also studied the sodium turnover in adrenalectomized animals, using radioactive sodium. Only five adrenalectomized dogs were studied, and the results were not statistically significant. However, the trend was to a more rapid turnover rate in animals in insufficiency. This confirms the aforementioned work with rats of Anderson and co-workers. Studies on bone sodium indicated that only one-third of the bone sodium of normal animals was exchangeable, and that equilibrium was reached within three hours. In adrenal insufficiency, the rate of turnover was so low that equilibrium was not reached during the time interval studied, i.e. six hours. Therefore, the proportion of bone sodium which turns over in adrenal insufficiency is not known.

Effect of Various Hormones on Sodium Metabolism

Desoxycorticosterone (DOC)

In 1937, Steiger and Reichstein (17) completed a partial synthesis of desoxycorticosterone, and showed it to have adrenal cortical activity. A year later, Reichstein and von Euw (18) isolated it in small quantity from an adrenal extract.

This hormone has been shown to correct the disturbances of body sodium and potassium in human and experimental adrenal insufficiency. When given in large doses, it has been reported to cause hypertension in Addisonians, particularly when the salt intake was high (19). Other

clinical symptoms of overtreatment were edema (19) and cardiac insufficiency (20). In animals, treatment with large doses over a long period of time, has resulted in a syndrome of polydipsia and polyuria (21).

The sodium retaining effect of DOC is not confined to the kidney. Administration of this substance results in a fall in sweat (22,23), salivary (24), and stool sodium (25).

There is evidence to indicate that DOC has some influence on the permeability of certain membranes. Seifter et al (26) have reported that DOC increases the enhancing effect of hyaluronidase on osmosis through the bladder membrane, and this has been confirmed, for the skin, by Opsahl (27). Overman and his associates (28) have found an increase in the thiocyanate space in adrenalectomized dogs on desoxycorticosterone-acetate (DCA) therapy, without significant change in the chloride space. They conclude from this that there is a cellular permeability change to the thiocyanate ion on DCA treatment. The same investigators have studied the transport of sodium-24 in adrenalectomized dogs on DCA therapy. Their results suggest that there is an increase in capillary permeability under these circumstances.

For many years, numerous investigators were unable to confirm the presence of DOC in adrenal extract, as demonstrated by Reichstein and von Euw (18). More recent studies, with paper chromatographic separation of steroids, have indicated that this material may be present in the adrenal gland. Hechter, Pincus and associates (29,30) have reported the presence of small quantities of DOC in beef adrenal perfusate. Zaffaroni and Burton (31) have identified DOC in a commercial extract of beef

adrenal gland, using chromatographic separations and spectrophotometric analysis. Burton has also reported the presence of a substance very similar to DOC, by these methods, in a large pool of normal urine treated with β-glucoronidase (32,p.506), although he had previously been unable to detect it in non-enzyme treated normal urine (33).

"Sodium Factor" of Hartman

In 1940, Hartman and Spoor (34) separated a fraction of adrenal cortical extract which had a specific effect on the retention of sodium. The chemical nature of this substance is still undetermined. It appears to be attached to a protein(35) and has quite different chemical and pharmacological properties from those of DOC (36). The substance isolated by these investigators is more potent than DOC and does not affect potassium metabolism. It is very soluble in dilute alkali, and is in this respect very different from the other active adrenal cortical hormones.

The "Amorphous Fraction"

The term "amorphous fraction" is used to describe that part of the adrenal lipids which is left after the separation of all the crystallizable substances. Wells and Kendall (37) reported that this substance maintained optimum levels of sodium, chloride and potassium, and that abnormal plasma values could not be produced at any dosage level. These properties indicate that the active substance in the "amorphous fraction" is neither DOC nor the "sodium factor" of Hartman.

Grundy et al (39) have recently reported the separation of a highly active mineralocorticoid, from beef adrenal extract, which was

responsible for most of the mineral activity of the preparation. This substance was resolvable from cortisone by paper chromatography only in long runs.

From studies of the reaction with diphenyltetrazolium chloride, and assays of its biological activity, it appears that this substance is twenty times more active than DOC.

The 11-Oxysteroids

Studies in normal (38,40) and adrenalectomized dogs (28,41) have shown that the 11-oxysteroids had a mild sodium-retaining effect, and that 11, 17-oxysteroids had a "sodium-excreting" effect. This "sodium-excreting" effect was only temporary in normal animals (28,42).

Overman and his associates (28,49) have found that cortisone causes a negative balance of sodium, potassium and chloride and an increase in plasma potassium in normal and adrenalectomized dogs, which is opposite in effect to desoxycorticosterone-acetate (DCA). Cortisone and DCA also had opposite effects on the sodium turnover rates, particularly across capillary membranes (49). These findings were more clear-cut in the adrenalectomized animals.

In man, the recent use of cortisone for the treatment of rheumatic and other conditions has resulted in fluctuations in weight and diuresis. Some of the patients developed demonstrable edema, which frequently disappeared without the use of low salt diets or diuretics (43). These findings are difficult to interpret, because the dosages employed were very high, and the effect of cortisone might have been influenced by the patients' adrenal glands.

Fourman and Albright (44) found that 17-Hydroxy-corticosterone (Compound F) had a sodium-retaining effect in a normal man, which was similar to that produced on the first day of ACTH therapy. The authors interpret this as rendering unnecessary the postulate of a DOC-like substance, secreted in response to ACTH. They did not, however, rule out the possibility that ACTH might have been released as a result of their treatment.

In spite of the fact that the presence of cortisone and 17-Hydroxy-corticosterone has been confirmed in the adrenal gland (31,45), in the blood (46,47), and in urine (33), the variability of their effects on sodium has led Kendall to believe that these compounds cannot be responsible for the electrolyte effect of the adrenal cortex (48).

Conn has reported (27, p.193) that corticosterone (Compound B) had a marked sodium-retaining activity, relative to cortisone, both in normal individuals and in patients with Addison's disease. The effects obtained with this substance were similar to those noted with ACTH. This investigator believes that, although Compound B has a lesser effect on electrolyte metabolism than DOC, and a lesser effect on carbohydrate metabolism than cortisone, it has a sufficient effect on both aspects of metabolism to make it a strong contender for the role of "the adrenal cortical hormone". It is present in adrenal cortical tissue in large quantity (31,45).

The Sex Hormones

Thorn and Engel (50) noted mild sodium retention in animals treated with estrone, estradiol, progesterone and testosterone-proprionate.

This has been confirmed more recently by means of a more sensitive assay procedure (51). Although progesterone and estrone have been detected in small quantity in adrenal tissue, their activity is not high enough to account for the electrolyte effect of the adrenal cortex.

17-Hydroxy-11-desoxycorticosterone (Reichstein's Compound S)

Clinton and Thorn (52) have found that Reichstein's Compound S caused sodium retention in normal dogs, but that this effect was not as marked as that of DOC. This substance was highly active in increasing the blood pressure (53,54) and the rate of water turnover (53), in animals.

The effect of Compound S in man has not been thoroughly investigated. Fagans, Louis and Conn (55) have recently studied the activity of this material in two normal men, but were unable to demonstrate any metabolic changes after its administration.

Although Reichstein has isolated Compound S from adrenal tissue, many investigators have been unable to confirm its presence in the adrenal gland. Hechter and his associates (29) have not detected this substance in adrenal gland perfusate, and found that it was converted to 17-Hydroxy-corticosterone if it was added to the perfusion fluid. However, a minimum of 1 gram of Compound S has been isolated by Haines (29, p.241) from 5,000 lbs. of hog adrenal tissue which had been "quick-frozen" immediately after killing. He has suggested that this freezing procedure may prevent the conversion of Compound S to 17-Hydroxy-corticosterone before extraction.

Thyroxin

Thyroxin has been reported to be diuretic (62), although it

may, at the same time, produce a reduction of chloride excretion (63). At other times, such as in myxedema, this substance may increase salt excretion (60, p.30).

Epinephrine

Adrenal medullary extract may produce a marked diuresis associated with increased salt excretion (64), or, on the other hand, it may reduce urine volume (65,66). These contradictory results would be more understandable if it were proven that epinephrine causes the release of ACTH, as postulated by McDermott, Fry, Brobeck and Long (67).

Insulin

In diabetes mellitus, there is a severe loss of electrolyte and water. This is probably due to the glycosuria and acidosis of the diabetes. The diabetic animal has, in fact, a polyuria and polydipsia. This increased water exchange and the process of inactivation of ketone bodies increases the amount of salt leaving the body.

Relation of the Anterior and Posterior Pituitary and Adrenal Cortex.

The work of Gaunt, Birnie and collaborators has in the past few years focussed attention on the role of the posterior pituitary gland in conditions of disturbed salt and water balance (56,57,58,59,60). They found that the activity of the anterior and posterior pituitary and the adrenal cortex were inter-related in salt and water metabolism. Their findings may be summarized as follows: a) an intact anterior pituitary is necessary for the induction of diabetes insipidus, by the destruction of the posterior pituitary; b) the posterior pituitary promotes water retention and salt excretion; c) adrenal cortical hormones have opposite effects on

salt and water. They are essential for normal inactivation of antidiuretic hormone (ADH) in hepatic and, perhaps, other tissues.

Clinically, Lloyd and Lobotsky (61) have shown that there is a relation between the antidiuretic activity of the blood and the urinary corticoids, in a wide variety of conditions. Generally, they found that a diuresis was associated with a high ratio of corticoid to antidiuretic substance, and vice versa.

A number of electrolyte and water disturbances which have been associated with increased adrenal cortical activity, have also been attributed to the posterior pituitary. This appears paradoxical in view of the postulated opposite effects of these glands on salt and water metabolism. However, Gaunt and Birnie have pointed out that DOC overdosage may produce either polyuria or edema (60, p.24). Thus, the diuretic effect of DOC may, under certain circumstances, be masked by its sodium-retaining effect.

Various Conditions in Which Disturbances in "Salt-Retaining" Hormone Have Been Postulated.

Addison's Disease

Addison's disease has for many years been associated with adrenal insufficiency. Biochemical changes which occur in this condition include marked depletion of salt, with accompanying loss of body water (6,7). Disturbances in glomerular filtration rate and renal plasma flow, as well as in tubular function, have been noted (68).

Urinary corticoid levels are generally below normal in this condition. Lloyd (69, p.474) has recently noted a relation between the

urinary corticoids and the level of serum antidiuretic substances (ADS) in Addison's disease. Generally, untreated patients had reduced urine volumes and urinary corticoid levels, associated with increased serum ADS. During replacement therapy with DOC, urinary volumes and corticoid levels increased, and serum ADS levels decreased. The origin and nature of the serum ADS detected in this study, and its importance in the pathogenesis of Addison's disease is not known.

Cushing's Syndrome

Cushing's syndrome is generally associated with a disturbance in protein and carbohydrate metabolism (70), but a number of reports indicate that there may be a disturbance of electrolytes as well (71,72). The patients presenting these symptoms generally have a severe alkalosis associated with a reduced concentration of chloride and potassium.

Conn (22) has found a lowered sweat-sodium concentration in a patient with Cushing's syndrome associated with adrenal cortical carcinoma.

Congestive Heart Failure

A number of factors have been brought forward to explain the accumulation of edema fluid in chronic congestive heart failure. Some explanations of this phenomenon are based on hemodynamic changes due to the cardiac disturbance itself, while others include disturbances in endocrine activity.

a) "Forward" or "Backward" Failure. A widely held view of the sequence of events in congestive heart failure is a modified theory of "forward failure". According to this theory, congestive failure develops

in the following manner: reduced cardiac output, reduced glomerular filtration rate, retention of salt and water, increased blood volume, venous congestion, edema. There is a considerable body of evidence in support of this theory.

Warren and Stead (74) observed that in some cardiac patients the body weight and plasma volume increased after the administration of salt, before any significant rise in venous pressure was detected. Additional supporting evidence for this view is found in the work of Merrill (75) who noted a diminished renal blood flow and glomerular filtration rate in all of the patients whom he investigated.

Studies of spontaneous nocturnal diuresis in uncompensated congestive failure, by Brod and Fejfar (78), indicated that the diuresis was always secondary to increases in renal plasma flow. They believe that the changes noted were due to a redistribution of blood flow, rather than changes in cardiac output. Davies (81) has found that there was a reduction of glomerular filtration rate and renal plasma flow in almost all patients, regardless of whether they had "low" or "high" output failure.

In a review of his findings, as well as those of others, Leiter (76) has concluded that the initial determining factor in the retention of salt and water is the reduction in glomerular filtration. As a result very little salt is presented for reabsorption by the distal tubules. The renal tubules behave normally, and respond in the usual manner to increases or decreases in the amount of filtrate and its content of salt.

The mechanism of the renal ischemia of the "forward failure"

concept is not known. Merrill and his associates have suggested that renin may play a role in this phenomenon (85). They found significant quantities of renin in renal venous blood in eight of eleven patients. Mokotoff and Shorr (73, p. 628) have found large amounts of "vaso excitor material" (VEM) in venous blood in ten of twelve patients in congestive failure. Increases in VEM were accompanied by increases in vaso depressor material (VDM). This substance is antidiuretic, acting, it is believed, via the posterior pituitary gland.

In the "backward failure" concept, the ventricle fails to pump adequately. The blood returning to it accumulates proximally in the atrium and veins, and venous pressure increases. The increase in hydrostatic pressure results in a greater loss of fluid and electrolytes into the tissue spaces, resulting in the formation of edema.

Strong evidence against the "backward failure" concept has been presented by the Bradleys (77), who found that it takes a pressure of 14-22 mmHg. in the renal vein (three times the normal level) to reduce the renal plasma flow 25%. In spite of this great pressure, none of the reductions approached the values of from 50% to 80% noted in almost all patients with congestive failure. Although the "backward failure" theory was once widely accepted, studies of this kind, as well as the fact that many patients with venous hypertension have little edema, have resulted in reducing the number of its supporters.

Some recent publications of H.L. White and associates (82,83) have questioned the role of the diminished glomerular filtration rate in the production of edema in congestive heart failure. These investigators

reported that hypophysectomized dogs, with chronically low glomerular filtration rates, remain in salt balance when untreated, or when supported with sufficient DOC to replace any possible deficit. Raising the filtration rate in these animals to normal or above normal, or of normal dogs to supra normal levels, by growth hormone administration, caused no increase in sodium excretion. They concluded that sodium imbalance cannot be produced by changes in glomerular filtration rate so long as tubular reabsorption is normal. Thus, although they admit that glomerular filtration rate and sodium excretion are reduced in congestive heart failure, they do not think that the reduced filtration rate is actually responsible for the sodium retention. A recent paper in support of these conclusions has been published by Heller (84) who found that patients recovering from congestive failure may become edema-free without showing a significant increase in glomerular filtration rate.

b) Endocrine Disturbances Fuchter and Schroeder (86), Warren and Stead (74), Davies (81), and many others have suggested that changes in endocrine activity may play a role in the retention of sodium in congestive failure.

Although many investigators have suggested that increased adrenal cortical activity may be a factor in this condition, very few publications have appeared giving concrete evidence of such activity. One report, published by Deming and Luetscher (51), on the urinary sodium-retaining substances in this condition, is available. These investigators, using an assay based on the total urinary sodium excretion of adrenalectomized animals, were able to show that five of six patients with uncompensated

congestive failure had significant quantities of sodium-retaining materials in their urine extracts.

Reduced levels of salivary sodium and sweat sodium (87) have been interpreted as indication of increased circulating salt-retaining corticoids (24,22,87). White et al (88) have found that salivary sodium levels were low in 27 patients with congestive failure. Reynolds (89), however, was unable to find the reduction in sweat sodium which might be expected in this condition.

Studies on the shifts of electrolytes and water during recovery from congestive heart failure, by Iseri and his associates (90), suggest that there is a cellular uptake of sodium during this period. Although this would not be expected if a regression from hyperactivity of the adrenal to normal were occurring, the authors believed that further studies of the adrenal gland in congestive failure and during recovery were indicated.

Parrish (91) has studied the urine of 10 patients in congestive heart failure, by biological assays. He found that four of ten patients had increased urinary adrenal cortical activity when measured by the "survival time" assay which measures, primarily, "salt-active" hormones (92). Increased "glucocorticoid" excretion was noted in all ten patients by the liver glycogen assay.

The urinary corticoid excretion in patients with congestive heart failure has recently been studied by Lasche and co-workers (93). In the majority of 23 patients with congestive cardiac failure they noted low urinary levels of formaldehydogenic steroids and 17-ketosteroids which

were raised by the administration of mercurial diuretics. ACTH, without mercurial diuretics, did not cause an increase in the urinary corticoids, although a fall in eosinophils did occur. The patients had "hypoglycemic unresponsiveness" following a test dose of insulin, and the fall in eosinophils caused by this treatment was below normal. These findings suggest that although the depressed corticoid excretion in congestive heart failure may be due, in part, to renal retention, there does appear to be a reduced ability of the adrenals to secrete "glucocorticoids" in response to stress. These findings are in disagreement with the aforementioned results of Parrish. Recent unconfirmed results of Bornstein and Trewhella (94) seem to indicate that circulating ACTH is elevated in severe congestive heart failure. This might serve to support the work of Lasche et al, since an excess of ACTH would be released to counteract an unresponsive adrenal.

Studies of the diurnal variations of water and electrolyte excretion have been made by Goldman (79), who found a decrease in water and electrolyte excretion during the night in normal individuals. This cycle was reversed in patients with congestive heart failure, and was not always related to increases in creatinine excretion. Similar findings were noted in patients with cirrhosis and ascites. The author has suggested that this phenomenon may be due to changes in a humoral agent produced by the liver when the patient is lying down. Lowered adrenal cortical function during sleep, as reported by Pincus et al (80), may have some bearing on the abovementioned reversed diurnal variations in water and electrolyte excretion.

Another endocrine gland which may play a role in the formation of edema in this condition is the posterior pituitary. An antidiuretic substance has been detected in the urine of patients with congestive heart failure, by means of the intravenous administration test in dogs (95). Dochios and Deifus (96) have detected an antidiuretic substance in the urine of twelve patients in congestive failure, using the rat test, and have correlated values with the degree of clinical edema.

Nephrosis

The cause of edema in lipoid nephrosis is still open to discussion. One of the most widely held views is that a decrease in plasma albumin, consequent to proteinuria, allows water and other solutes to move in to the interstitial spaces, leading to a reduction in blood volume. This, in turn, is believed to result in decreases in renal blood flow and glomerular filtration rate.

Eder and his group (97,98) showed that reductions in plasma volume were associated with the accumulation of edema. This was generally accompanied by reductions in renal plasma flow and glomerular filtration rate, and in the excretion of salt and water. If human serum albumin caused a diuresis, it also resulted in an increase in both of these renal functions. Onset of diuresis was generally noted in conjunction with an increased plasma volume, as well as glomerular filtration rate, by Barnett et al (99) who believe that these changes are always associated with the mechanism of diuresis, regardless of the method used for inducing it. Luetscher and his co-workers (100) found an increase in the renal clearance of "creatinine" as well as an increase in plasma sodium concentration,

accompanying the elimination of edema. This increase in plasma sodium with loss of edema has also been noted by Orloff and his associates (101).

Other findings on the subject of glomerular filtration rate and plasma volume have not always been in agreement with those noted above. Orloff et al (101) have eliminated edema fluid, with serum albumin, without causing a consistent variation in the glomerular filtration rate. The subject is further complicated by the finding that glomerular filtration rate and tubular functions may actually be above normal in some edematous nephrotics (103,104,105). These findings led Orloff and his associates (101) to conclude that a further increase in the filtration rate could not be expected to result in an increase in the excretion of salt, in these patients. The possibility that the plasma volume may be reduced in nephrosis has been studied by Galan (102), who actually found it to be either normal or increased.

A specific tubular disturbance, with respect to sodium, has been suggested by numerous observers of nephrosis (106,107,108,109,110,101). Although there is no method, at present, for the evaluation of renal function regarding sodium in man, Burnett and his associates (109) have attempted to evaluate this aspect of renal activity in nephrotics by acute sodium loading. They noted that patients with normal, or only moderately reduced filtration rates responded with poor sodium excretion, and concluded that the tubules were acting abnormally.

A number of features of this syndrome, such as apparent specific retention of sodium, beneficial effects of ACTH and cortisone, and spontaneous remissions associated with infections, have pointed to the possibility

of disturbed adrenal cortical function as a factor in the etiology of this condition.

Studies of tissue changes in the nephrotic syndrome in six children at post mortem have been made by Fox and Slobody (110). They found losses of one quarter to two thirds of the potassium per unit dry weight in skeletal muscle. The cells had gained large amounts of water and sodium chloride in concentrations approximating extracellular fluid. These values compared with those obtained with DOC therapy.

Luetscher and Deming (51,106,100) detected elevated quantities of sodium-retaining activity in lipid extracts of nephrotic urine. They noted a fall in the level of these substances when a diuresis was produced with cortisone, ACTH or serum albumin, and suggested that the sodium-retaining substance may be of adrenal cortical origin.

It has been shown that a remission may be initiated by infections such as measles (111) and malaria (112,113). This finding, plus the fact that ACTH or cortisone will also cause remissions, has led Byrne (112) to suggest that nephrosis is one of Selye's "diseases of adaptation".

Luetscher et al (106) had noted that the diuresis obtained with cortisone usually occurred when treatment was terminated, and suggested that improvement occurred during the period of reduced adrenal cortical function following the abrupt cessation of therapy. This suggestion is not supported by the findings of the same group (100), and those of others, that a diuresis often begins while the patient is still on ACTH therapy.

There is considerable evidence for the view that glomerulonephritis may be the result of an antigen-antibody reaction (114). Lange

et al (116) have shown that antibodies to human kidney were present in a high percentage of cases during the different stages of glomerulonephritis, and that this antigen-antibody reaction was complement-binding. They found that the serum complement levels were reduced in acute glomerulonephritis and the nephrotic syndrome, and that they returned to normal after subsidence of symptoms of acute glomerulonephritis or after diuresis in the nephrotics, in spite of persistence of some degree of proteinuria, hypoproteinemia and hypercholesterolemia (115).

Another endocrine gland which may play a role in the development of this syndrome is the posterior pituitary. Antidiuretic activity has been detected (117) in the dialized and concentrated urine of nephrotic subjects with edema. On several occasions the material disappeared with spontaneous diuresis. Shorr and Zweifach (73) have noted greatly increased VDM, recently found to be identical with ferritin, in the peripheral blood and edema fluid in the nephrotic syndrome of glomerulonephritis. This substance is antidiuretic, via the posterior pituitary, and may play some role in the accumulation of fluid in this condition.

Since the nephrotic syndrome is most frequently seen in infants and children, it may be of interest to examine sodium metabolism in these subjects. A number of reports have indicated that sodium metabolism is quite different in children from that in adults. Cooke, Pratt and Darrow (87) have found the average sodium concentration in sweat in infants of five to sixteen months below the normal adult level. Bongiovanni (118) noted that salivary sodium was below the normal adult level in children of one to eight years. Therefore, if the assumption that reduced sweat and

salivary sodium concentration reflects increased circulating sodium-retaining hormone is valid, one may conclude that there is an increase in activity of this particular phase of adrenal cortical function in infancy and childhood. It is possible, however, that differences in urinary electrolyte metabolism in infants may be due to renal immaturity, as suggested by lower urea and inulin clearances in this condition (119). The reduction in sweat and salivary sodium may be a concommitant of growth, as indicated by the work of Whitney, Bennett and Li (120) who found that growth hormone caused retention of both sodium and potassium in normal female rats. They suggested that the retention of sodium is associated with an expansion of the extracellular fluid volume.

Cirrhosis of the Liver

The accumulation of fluid which often accompanies cirrhosis of the liver has been attributed to many causes, such as hypertension of the venous portal system, decreased plasma albumin, increased tubular reabsorption of water, decreased glomerular filtration rate, and increased tubular reabsorption of salt.

There is no doubt that the production and circulating level of albumin is reduced in this condition (121). However, hypoalbuminemia does not seem to be the only factor of importance in edema formation, because the administration of salt-poor serum albumin does not correct deficient salt excretion (122), and a spontaneous diuresis may occur despite continued hypoalbuminemia (123).

Ralli and her co-workers (124,25) and Dochios and Deifus (96) have detected an antidiuretic substance (ADS), possibly of posterior pituitary

origin, in the urine of patients with hepatic cirrhosis. Lloyd and Lobotsky (61) have found increased quantities of serum ADS in three cirrhotics who were in positive fluid balance. VDM, or ferritin, which has a vasodepressor and antidiuretic effect, has been found in a saline extract of liver prepared from animals with experimental cirrhosis of the liver (126). Although it has been shown that the liver can inactivate the antidiuretic principle of posterior pituitary extract (127), White et al have shown that there is no defect in the mechanism of destruction of pitressin in cirrhosis (128).

Decreases in glomerular filtration rate have been noted by some investigators (129,130) but not by others (131,132,133) in this condition. Leslie et al (129) found that the effective renal plasma flow and tubular maximum of para amino hippuric acid (Tm PAH) were decreased during the fluid accumulating phase. Epstein (131) noted no disturbances in glomerular filtration rate and renal plasma flow during the accumulating phase, and has suggested that there must be an increase in tubular reabsorption of sodium. Farnsworth and Krakusin (130) found that urinary electrolytes, other than sodium, exhibited normal responses to variations in intake, suggesting a specific increased tubular reabsorptive behaviour toward sodium. Goodyer et al (133) studied the effect of an infusion of saline in normal subjects and cirrhotics, and observed that the cirrhotics retained the sodium much longer than normal individuals, although they had normal renal plasma flow and glomerular filtration rate. They interpreted these results as evidence of specific impairment of the mechanism for the excretion of administered sodium, which was the result of increased tubular

reabsorption.

Goldman (79) has studied the diurnal variations in water and electrolyte excretion, in 13 patients with cirrhosis of the liver, with ascites. In normal individuals, maximum excretion of sodium, potassium and water occurred during the day. In 11 of the cirrhotics, the sodium excretion cycles were reversed, and in eight the water excretion cycles were reversed. This could not be explained on the basis of increased renal circulation because there was no consistent increase in creatinine clearances during nocturnal diuresis. He suggested that increased hepatic circulation in the horizontal position (135) might affect some humoral agent which acts on renal tubular function.

The importance of the level of intake of sodium in the accumulation of edema has been pointed out on many occasions (137,122,130,133). Eisenmenger has found extremely low serum levels of sodium during maximum formation of ascites, and that an increase in serum and urinary sodium indicated decreased production of ascites long before the associated changes in fluid balance were observed. Reduced sweat and salivary sodium levels have been noted in this condition (137,138). The level of both sweat and salivary sodium can be depressed by adrenal cortical hormones of the "desoxycorticosterone" type (24,139).

In a recent study of adrenal cortical metabolism in this condition, Bongiovanni and Eisenmenger (138) observed that the urinary 17-ketosteroids were depressed, the chemical corticoids were elevated, the glycogenic corticoids were normal and, in two of eight cases, the "sodium-retaining" corticoids were increased. They found that, after long-term

sodium restriction, there was a further rise in chemical corticoids, giving support to the view that the elevated corticoids in cirrhosis are physiologically related to DOC-like hormones of the adrenal cortex.

Essential Hypertension

Although most observers believe that the cause of "essential hypertension" is unknown, a number of investigators have suggested that the kidney is primarily responsible for the condition. Some of the evidence for this belief is based on the work of Goldblatt (140) and his group, who have shown in dog and other animals that if the renal artery is partially constricted, a permanent increase in the blood pressure may be produced. Trueta (141) has stated that there is increased sympathetic-adrenal-medulla reactivity, or an exaggeration of the "adaptation syndrome" as described by Selye in those individuals predisposed to hypertension. This causes frequent transient vasoconstriction of the arterioles of the renal cortex with subsequent release of pressor substances.

Vaso-excitor material (VEM), descfibed by Shorr and Zweifach (142), has been found in the blood of patients with "essential hypertension". It has been shown that this substance is released by the kidney in anoxic conditions. It does not, however, have a direct pressor activity since its main site of action is the meta-arteriole.

"Pherentasin", a highly active pressor substance, has been isolated from the blood by Schroeder and Olsen (143,144) in patients with "renal" hypertension, but not in those cases of "neurogenic" or "endocrine" hypertension.

A widely held view of hypertension is that it is of diverse and

probably multiple causation including such factors as can be classified as neurogenic, cardiovascular, renal and endocrine (145,146,141,147). There is considerable evidence that the adrenal cortex is necessary for the development of hypertension in animals (148), but its importance in hypertensive disease in man has been questioned (147). Selye has suggested that "essential hypertension" may be a "disease of adaptation" in which there is a relative overabundance of "mineralocorticoids" (149). This suggestion is supported by the finding that some patients tend to retain administered sodium somewhat unduly (150). Locke and his associates (139) have noted reduced sweat chloride levels in two of four patients, but this was not confirmed by Eisenberg and his associates (151), who studied 35 patients.

No studies on the urinary excretion of "sodium-retaining" corticoids in this condition are available, although two reports of the "formaldehydogenic" urinary corticoids have been published. Tobian (152) found that the urinary corticoids in eight of nine patients were in the normal range. However, Corcoran, Page and Dustan (153) noted that hypercorticoiduria was a common finding in patients with "essential" and "malignant" hypertension. Hetzel and his associates (154) have investigated the glucocorticoid (bio-assay) and 17-ketosteroid excretion in a group of 180 patients with "essential hypertension", and have found that the values were in the normal range.

Corcoran et al (204) have suggested that both the kidney and the adrenal are involved in experimental hypertension. This concept is based on the finding that renal hypertensive rats, or rats treated with

renin show hypertrophy of the zona glomerulosa of the adrenal cortex which is considered, by some, to be the site of origin of DOC-like corticosteroids (155). Thus these observers suggest that renal hypertension proceeds in two phases, the first is associated with increased liberation of renin, which is natriuretic (156,157), and the second with an increased liberation of DOC-like adrenal hormone, which is sodium-retaining.

Rheumatic Diseases

Disturbances in adrenal cortical function have been postulated as an etiological factor in rheumatic diseases by Selye (158,159,149), who was able to produce lesions similar to those found in rheumatoid arthritis and rheumatic fever in sensitized animals by the administration of DCA or lyophilized anterior pituitary (LAP). This suggestion has met with opposition because the lesions produced in these animals were similar to, but not identical with those found in man, and because large, "unphysiological" doses were necessary for their development.

The report that ACTH or cortisone administration may alleviate the symptoms of rheumatic conditions (160) suggests that there may be a relative deficiency of "glucocorticoids". Studies on the level of urinary "formaldehydogenic" and biologically active glucocorticoids in rheumatoid arthritis have indicated that they are, in fact, below normal.

One difficulty in the understanding of rheumatic fever is the fact that it is a highly variable disease with manifold signs and symptoms, which occur in numerous combinations and with a wide range of intensities. A widely held concept of the mechanism of the disease is that which holds

that the initial event is a hemolytic streptococcal infection of the upper respiratory tract. This infection initiates a series of events involving the action of streptococcal products and the complex host reaction, leading to the disease which may occur in the absence of demonstrable streptococcus.

The fact that infection by group A hemolytic streptococci is a preliminary to the development of rheumatic fever has been well established. However, it is not agreed that rheumatic fever is invariably preceded by infection, since it may be initiated by trauma, cold or fatigue (161,162). Streptococcal products such as streptokinase and streptococcal hyaluronidase, and auto-antibodies to damaged host tissue have been implicated in this condition, as well as anti-streptococcal antibodies.

The role of the adrenal cortex in the pathogenesis of this disease is obscure. The level of urinary "formaldehydogenic" corticoids is above normal during periods of high fever, and normal after the fever has subsided (pers. comm. Dr. F. McCall, Children's Memorial Hospital). No studies have appeared on the urinary "salt-retaining" corticoid excretion in this condition.

Post-Operative State

Weil and Browne (163) have reported the presence, by the cold protection test, of increased quantities of urinary cortin following surgery. This was confirmed by Venning, Hoffman and Browne by means of the liver glycogen deposition test (164).

Although renal retention of sodium during the post-operative period had been observed, it had been attributed to circulatory changes

consequent to the shock of operation. Johnson, Conn and Iob (165) have recently investigated the electrolyte and nitrogen changes in sweat, in the post-operative state. They noted, in 14 patients, an average decrease of 38.1% in sodium concentration and 39% in chloride, with maximum effect from the fifth to eighth day. There was an average increase of 73.3% in potassium concentration with maximum effect on the second to fourth days, as well as an increase of 58.1% in nitrogen concentration. The circulating eosinophils of these patients fell immediately after operation, but returned to normal from the second to fifth post-operative day.

Toxemia of Pregnancy

Eclampsia is a specific disease of pregnancy, of unknown origin. Although disturbances in renal function have not been observed in normal pregnancy (166), reductions in glomerular filtration rate have been noted in pre-eclampsia and eclampsia. According to Smith (73) the data are inadequate to establish that the changes in renal function are significant. The deviations in renal function in toxemia of pregnancy generally disappear after delivery, in those patients who do not develop persistent hypertension.

Most authors have attributed the hypertension, edema and albuminuria, characteristic of pre-eclampsia, to some endocrine disturbance during late pregnancy. The posterior pituitary gland is believed by many to play an important part in the edema formation of eclampsia. This view is based on the fact that antidiuretic substances have been detected in the urine and placentae of patients with toxemia of pregnancy (167), that there was a delayed excretion of urine in eclamptics (168), and that posterior pituitary extract caused convulsions in pre-eclampsia (168).

The finding that DOC intoxication is associated with hypertension, edema and albuminuria has suggested that there may be an excess of this type of activity in patients who develop toxemia of pregnancy. Urinary chemical corticoid excretion is frequently elevated in this condition (152, 169,170), but is not always correlated with increases in blood pressure, or with other clinical symptoms. Lloyd (69, p.480) has noted that the "water-insoluble" fraction of the chemical corticoids was particularly elevated. Since DOC is relatively water-insoluble, this finding is consistent with the suggestion that the increase in urinary corticoids may be due to DOC-like substances. Chart, Shipley and Gordon (171) have reported that the "sodium-retaining" activity of urine in patients with toxemia of pregnancy was considerably above normal in seven patients. They did not note a parallel increase in the 11-oxysteroids (water-soluble chemical corticoids). In one patient, the increase in sodium-retaining activity preceded the appearance of clinical symptoms. The activity of the urine of these patients returned to normal levels following delivery.

In a recent review on the toxemias of pregnancy, Ordman (172) has suggested that this condition is a "disease of adaptation". He noted that during an intense cold winter spell several cases of toxemia were admitted to hospital in quick succession. He suggested that a non-specific insult caused the increase in the secretion of adrenal cortical hormones of the salt-active type. The author explained the small adrenals observed by the Smiths (173) as being in the "stage of exhaustion" of Selye's "adaptation syndrome".

Evidence of disturbances in tissue electrolytes have recently been

reported by Parviainen and his group (174,175), who studied a case of pre-eclampsia in which sudden death ensued as a result of severe cerebral hemorrhage. They noted that the sodium:potassium ratio was generally higher in this case of pre-eclampsia than in a control case, especially in the brain and striated muscle tissue. They noted also that the sodium content was usually higher, and the potassium cnntent usually lower than normal in the plasma of 18 cases of toxemia of pregnancy. The greatest changes were in the most severe toxics or in patients who had just been admitted to the hospital.

Garrett (176) noted four types of non-specific responses to stress in association with eclamptic toxemia. These are fibrinolysin in blood, hemorrhagic diathesis, gastro-intestinal ulceration, and pituitary-adrenal hyperactivity. He suggested that the source of alarm in this condition may be psychological or mechanical.

Masson, Corcoran and Page (177,178) have reported on the experimental production of a syndrome resembling toxemia of pregnancy in established DCA-hypertensive rats, by the injection of a renin-containing renal extract. They concluded from their experiments that the concurrence of prolonged sodium retention and of briefly sustained renin injections resulted in a syndrome which had some of the aspects of toxemia of pregnancy.

Carbohydrate and nitrogen metabolism in toxemia of pregnancy has been studied by Mukherjee, Govan et al (179,180). Patients with typical toxemia of pregnancy were found to have a reduced sensitivity to insulin, suggesting hyperactivity of the anterior pituitary gland. The authors did not feel that their evidence permitted them to state which pituitary hormone

was responsible, since ACTH, growth hormone and "glycostatic" hormone may have anti-insulin effects. They found that there was a negative nitrogen balance in this condition, and that even if the albuminuria were not present the positive nitrogen balance would not be as great as in normal pregnancy. These results indicate that there may be an increase in adrenocorticotrophic hormone, while still allowing for an increase in growth hormone.

The frequent finding of low urinary estrogens and progesterone, and of elevated chorionic gonadotrophins in these patients has led the Smiths (173) to suggest that premature senility of the placental syncytium, and premature withdrawal of placental steroid hormones was the final intermediary pathology in this condition. They suggested that the primary disturbance involved metabolic abnormalities affecting the placenta, or a decrease in blood supply to the placenta, or both. This syncytial steroid aberration was assumed to be contributory to, but not the sole precipitating cause of the toxemia syndrome. The authors suggested that a toxin may be released by the placenta which may be the final cause of toxemia of pregnancy.

A substance has been extracted from the villi of the placentae, which caused the development of symptoms of toxemia of pregnancy in pregnant animals, but which hardly affected males and non-pregnant females (181). This substance, extracted by methods used for the separation of polysaccharides from bacteria, is water soluble and alcohol insoluble. The mechanism of the reactive phenomenon caused by this substance was not regarded as allergy, by the author, but rather as "pathergy", as defined by Roessle.

Large amounts of both VEM and VDM were present in peripheral

blood during the period of pre-eclamptic hypertension, the one or the other predominating (142). These factors disappeared on return of the pressure to normal levels. The importance of VEM in the development of hypertension, or of VDM in the retention of fluid is not known.

Acromegaly

Acromegaly, a condition believed to be due to an excess of growth hormone, has not, generally, been associated with disturbances in electrolyte metabolism. Recently, however, Selye (182) has produced nephrosclerosis, periarteritis nodosa and hypertensive disease in animals with this substance, as he had done previously with DCA or lyophilized anterior pituitary. He has suggested that growth hormone may sensitize the tissues to "mineralocorticoid" activity or that it may increase the production of this substance by the adrenals.

Whitney, Bennett and Li (120) have studied the effect of growth hormone on urinary sodium and potassium in normal female rats. They noted that the administration of this substance produced a retention of both sodium and potassium, and suggested that the retention of sodium was due to an expansion of the extracellular fluid volume. This was in accord with a previous observation of this group that growth hormone administration to hypophysectomized rats resulted in an increase in the thiocyanate space but no change in muscle or serum sodium concentrations.

White, Heinbeck and Rolf (183) have studied the effect of several preparations of growth hormone (prepared by the method of Wilhelmi et al, or modifications thereof) on the renal function of normal and hypophysectomized dogs. They noted that some of the preparations produced

greatly increased para amino hippuric acid and inulin clearances. They believed that the effects were due to growth hormone, and that these functions are reduced following hypophysectomy through the loss of growth hormone.

Stack-Dunne and Young (184) have recently reported the presence of two separable factors from ACTH preparations. One causes adrenal ascorbic acid depletion while the other stimulates adrenal weight gain. The above-mentioned investigators, as well as Selye (182), have observed that growth hormone preparations contained an adrenal weight stimulating factor similar to that observed in ACTH preparations.

Methods of Evaluating "Salt-Retaining" Corticoids

Thermal Sweat

In April 1949, Conn (22) reported that under standard conditions of testing the concentration of sodium and chloride in the sweat of normal adults was within the range of 15-60 m.equiv./l with a mean value of 42. Cooke and co-workers (87) have found that the values were much lower in infants. DCA and ACTH markedly reduced the sweat sodium and chloride values. Patients with Addison's disease had concentrations considerably above normal, which fell with DOC or lipoadrenal cortex therapy, and rose once more on cessation of treatment. Conn predicted that some pathologic conditions not yet linked to the adrenal cortex might be found to be associated with a preponderant activity of desoxy-like corticosteroids. In a further study he and his associates (23) found that after sustained administration of ACTH to normal humans, cessation of such

treatment resulted in temporary adrenocortical insufficiency, followed by temporary adrenal cortical hyperactivity, followed by return to normal function.

Locke, Talbot, Jones and Worcester (139) have noted that certain non-endocrine factors play a major role in the determination of sweat electrolyte composition. Intensity of thermal stimulus, skin temperature and rate of sweating were all found to bear an important relation to sweat electrolyte concentration. They found that hormone activity modifies the sweat electrolyte content, and that this activity is best appraised by use of an index which expresses sweat chloride concentration values relative to rate of sweating. They have shown, as did Conn and his group, that the chief determinant of the sweat chloride-rate index is an adrenal cortical hormone which, like DOC, acts chiefly upon electrolyte and water metabolism. Cortisone induced a slight rise in the index values in these studies, as opposed to a marked fall produced by DOC or ACTH. Estrogens and androgens did not produce a consistent effect, which suggested to these authors that their influence on sweat electrolytes may have been accomplished indirectly by stimulating or inhibiting pituitary ACTH production, rather than by direct action on the sweat glands.

Elevated sweat sodium and chloride values have been noted in hypoadrenal states (22,139), and reduced values in adrenalcortical carcinoma (22) and patients with cirrhosis and ascites (137). Conflicting reports have appeared regarding sweat electrolyte values in essential hypertension (139,186), and normal sweat electrolyte concentrations have been noted in patients with congestive heart failure (89).

Salivary Sodium

In June 1951, Frawley and Forsham (24) investigated the factors which cause variations in the salivary sodium:potassium ratio and noted that this ratio varied inversely with the state of adrenal cortical activity. The ratio was found to be 1.3 in normal subjects, 5.0 in patients with untreated Addison's disease, and 0.5 in patients with Cushing's syndrome. Both ACTH and DOC produced a fall in the salivary sodium:potassium ratio. This fall in salivary sodium:potassium ratio, due to ACTH, has been confirmed by Grad (187). Subnormal salivary sodium levels have been observed in patients with cirrhosis with ascites (137).

Methods Based on the Urinary Sodium Excretion in Laboratory Animals

a) Hartman, Thatcher and Lewis A method for the assay of "salt-retaining" hormones in biological fluids was developed by Hartman and his associates (188) in 1941. It was based on the use of normal dogs on constant diet, and measured the effect of an unknown substance on the urinary excretion of sodium over a six-hour period as compared with that produced by DOC in the same dog. Under these conditions they were able to detect 700 micrograms (γ) of DOC. The method cannot be used to detect the minute quantities of active material present in small volumes of blood or urine.

b) Dorfman, Potts and Feil The first step in the development of a more sensitive assay occurred in December 1947 when Dorfman and his group (189) reported on a method which detected as little as 1γ of DOC. This assay was based on measurement of the urinary excretion of a dose of injected sodium-24 by adrenalectomized rats, in a six-hour period. The

sodium-24 excretion of the DOC-treated animals was compared with that of non DCA-treated control animals. Although the dosage-response relations of this assay were not consistent, the results suggested that the method had a much more desirable range of sensitivity than that of Hartman et al. The publication of this assay procedure stimulated new interest in the measurement of adrenal cortical hormones with sodium-retaining activity.

c) Deming and Luetscher Several modifications of the method of Dorfman et al have been published during the course of the present investigation. The first of these was presented by Deming and Luetscher (51) in February 1950. These workers used adrenalectomized rats under standard conditions, and determined total urinary sodium in a five-hour period, using a flame photometer for the determination of sodium. The first day of testing was used as a control for subsequent days on which test materials were studied. In a more recent study (106), three sub-groups were rotated from day to day. A sub-group that received the test substance on one day received a standard dose of 5γ of DOC on the second day and served as a control on the third day. The values for the three days were pooled for the final results. Still more recently, they noted that alcohol, the injection vehicle for the test substances, had some sodium-retaining activity (100), indicating the necessity for its use in control animals. The results obtained with this method were similar to those obtained by Dorfman's group, in spite of the fact that total sodium, not radioactive sodium, was measured. They detected a sodium-retaining effect with 2γ of DCA, and a maximum effect with 25γ. Dosage-response curves have not been published. To obtain the same sodium-retaining effect, estradiol,

progesterone and testosterone were required in dosages 25 to 50 times greater than that of DCA.

Deming and Luetscher were the first to report the presence of sodium-retaining substances in human urine. They used crude chloroform extracts of urine, which had been acidified to pH 1.5 immediately before extraction, and injected the extract equivalent of 20 minutes of urine into each test animal. Although they could detect no sodium-retaining activity in such small quantities of normal human urine, they did note quite marked activity in the urine of patients with congestive heart failure and nephrosis. A 40-minute urine-extract did not always result in a greater effect. In fact, a larger time-dose was sometimes associated with a smaller response. The significance of this behaviour was not clear, but it suggested the presence of interfering substances in the urine-extracts. Significant sodium-retaining activity was noted in 30-minute urine-extracts in two of eight patients with cirrhosis and ascites (138), by using a method similar to that of Deming and Luetscher.

d) Spencer The next modification of the assay was published in July 1950 by Spencer (190), who further increased the sensitivity of the assay by using adrenalectomized mice. Spencer found a very good dose-response relation between one-half and four micrograms of DCA. The test is based on the determination of total urinary sodium measured by the flame photometer, in the first six hours following the injection of a salt solution. Three groups of animals were used concurrently on three consecutive days, and were given either the injection vehicle (5% glucose), a standard dose of DCA, or the unknown substance. By the end of the test,

each animal had received the standard dose of DCA, the unknown, and the solvent vehicle. This method of rotation was later adopted by Luetscher and Deming (106), as mentioned above.

Spencer studied dog blood from various sources and observed that adrenal vein blood gave the greatest quantity of sodium-retaining activity, i.e. the equivalent of 4γ of DCA per ml. of serum. He detected 0.04γ of DCA equivalents in each milliliter of serum in an extract of carotid artery blood. He could detect no DCA-like activity in human blood taken from the inferior vena cava by catheter at the level of the 12th thoracic vertebra.

e) Simpson and Tait The next paper on this subject appeared in October 1950. Simpson and Tait (191) studied dose-response relations of DCA on the sodium excretion of adrenalectomized rats, using sodium-24. Sodium-retaining activity was determined by comparing the sodium-24 excretion/hr. for two hours before injection of the test substance with the sodium-24 excretion/hr. for four hours after the injection. They reported good dosage-response relations between one and thirty-two micrograms of DCA. They noted diurnal variations in the rates of excretion of sodium, and that the sodium-retaining period was lengthened with increasing quantities of DCA. The sodium-retaining period, regardless of its duration, was inevitably followed by a "sodium-excreting" period, during which the sodium excretion rose sharply above that of the controls.

More recently, these investigators (192) have altered their method somewhat. The weight of the animals was reduced from 130-150 grams to 30-40 grams. The ratio of urinary sodium-24 and potassium-42 was

used as an index of mineral activity of corticoids. The radioactive material was injected one hour after the hormone was injected, and the urine was collected for two hours. They found that the ratio was linearly dependent on the log of the dose of DCA for a range of 0.8 to 4.0γ, Slopes of adrenal cortical extract and other adrenal cortical hormones were found to be linear and parallel to that of DCA. Although there was variation in the actual ratios obtained from test to test, a standard curve could be constructed for the approximate assay of substances by expressing the ratios of the compounds tested as the percentage of concurrent control values. The activities of beef adrenal extract and DCA were considerably higher than those observed with corticosterone, 17-Hydroxy-corticosterone, 11-dehydro-corticosterone, cortisone and 17-hydroxy-11-desoxycorticosterone.

f) Marcus, Romanoff and Pincus In March 1952, Marcus, Romanoff and Pincus reported on the electrolyte-excreting activity of adrenal cortical substances (193). Their method was based on the sodium excretion of adrenalectomized rats, as determined by the flame photometer. The animals were given a standard salt load, and the urine was collected for the first four hours following the injection of the test substance. They observed that there was a sodium-retaining effect over a range of from 6 to 60γ of. DCA. Cortisone, 17-hydroxy-corticosterone, corticosterone, 11-dehydro-corticosterone, had a less marked but reverse effect, although all of these compounds had sodium-retaining activity at the lowest dosage studied, i.e. 10γ. The effect of dehydro-corticosterone was as great as that of DCA, at this dosage level.

g) Kagawa, Shipley and Meyer A method based on the urinary sodium excretion of adrenalectomized rats, as measured by a microchemical procedure, has recently been developed by Kagawa, Shipley and Meyer (194). The test material was divided into two parts and injected subcutaneously, in oil, 24 and 27 hours following adrenalectomy. At the 24th hour the animals received 2.5 ml. of 0.85% sodium chloride by subcutaneous injection. Urine was collected between the 28th and 30th post-operative hours. In order to obtain accurate urine collections, they ligated the urethra of the rats for the duration of the collection period. A good dosage-response relation was observed between 2 and 9γ (total dose) of DCA. They used the average excretion of the oil-injected control animals as 100% excretion, and differences in sodium excretion in the experimental group were considered as sodium retained. Using this method of assay, Chart, Shipley and Gordon (171) have found markedly elevated sodium-retaining activity in 15-20 minute equivalents of crude lipid urine-extracts of seven patients with toxemia of pregnancy. The values returned to normal following delivery, and regression of symptoms.

SECTION B - EXPERIMENTAL WORK

THE PROBLEM

There is evidence to indicate that ingested sodium is retained in abnormal amounts in certain pathological conditions. This has led to the suggestion that the adrenal gland may be secreting an excess of salt-retaining hormone under these circumstances. The object of the present investigation was to develop a method for the biological assay of small quantities of "sodium-retaining" substances, and apply it to the determination of sodium-retaining activity in human urine.

The procedure adopted for this assay was dependent on the measurement of the urinary excretion of sodium-24 by adrenalectomized rats, based on principles suggested by Dorfman, Potts and Feil (189). These investigators found that the urinary excretion of sodium-24 during the first six hours after its subcutaneous injection could be reduced by pretreatment with as little as 1 microgram of DCA. Since the quantitative aspects of this method had not been investigated, studies have been carried out to determine what factors effect the assay. Such variables as weight of animals, room temperature, timing, solvent vehicles, and method of urine collection have been modified in an effort to standardize the method and improve reproducibility.

The method has been applied to the study of crude lipid extracts of urine from normal individuals and a number of pathological states. The effect of storage and of alkali washing on the activity of the extracts has been investigated, and dosage-response curves of several extracts have been

determined. Two urinary extracts prepared and assayed by Drs. Deming and Luetscher have also been assayed by the present method.

METHODS

Animals

Hooded male rats on a standard diet of purina fox chow were used in all experiments. Dorfman et al had used animals ranging in weight from 118 to 175 grams. As more reproducible results are generally obtained with animals of similar age and weight, assays were performed to determine the "optimum weight" for the experimental conditions. In the early experiments, young animals of approximately 100 grams were used in the hope of increasing sensitivity, but they did not stand the adrenalectomy and post-operative treatment as well as the larger animals. The most satisfactory weight was found to be from 160 to 170 grams. Standard curves and urine assays were performed on rats within this weight range.

When the animals were transferred from stock cages to the smaller experimental cages, they were healthy but slightly dehydrated. This was due to overcrowding and lack of drinking water throughout the night, the period of greatest thirst in the rat. The animals gained considerable weight during their first day of stay in the less crowded experimental cages. The weight changes of a typical experiment are tabulated below:

Experiment	No. of rats	Aver. wt. when transferred to experimental cages	Aver. weight gain in 1 day
45(a)	38	159.1 grams	7.8 grams

This marked weight gain was associated with a large intake of fluid which was not maintained on subsequent days. Another group of animals studied at this time gained an average of 3.3 grams during the second day. To avoid fluctuations in the "state of Hydration" due to adjustment to laboratory conditions, the animals were transferred to the experimental cages only one day before the adrenalectomies were performed. Food was placed on the floor of the cages, as the animals were accustomed to feeding in this manner.

Seasonal Variation

Experiments were carried out throughout the year, except for July and part of August when it was impossible to obtain satisfactory temperatures in the laboratory. Consistent results were obtained throughout this period except for a few days at the beginning of both May and December. These irregularities were related to seasonal changes and were noted on more than one occasion. On these days, the sodium-24 excretion within each group, including the control animals, covered a much wider range than usual. In some cases, the animals excreted little urine during the entire collection period. Assays performed during these periods were not accepted as valid and were repeated.

Room Temperature

Optimum temperatures, particularly for the post-operative period, were found to be between 78° and 80°. It was difficult to maintain this temperature in the earlier experiments, as an air-conditioned room was not available. Later, facilities for heating, but not cooling the animal room became available. Higher temperatures (85°) were generally

associated with increased sodium-24 excretion rates, whereas lower temperatures (78°) were associated with relatively decreased rates. If the temperature fell to 75° or below, the adrenalectomized animals which were not receiving saline as drinking fluid drank very little water, lost considerable weight, and excreted small amounts of urine and sodium during the test period. Many of these animals were cold and lethargic and had to be discarded. A fall in temperature of this magnitude did not have such damaging effects on adrenalectomized animals on saline therapy, or on non-adrenalectomized animals.

Adrenalectomy

Bilateral adrenalectomies were performed under light ether anaesthesia by a single midline dorsal incision, or by two small lateral incisions. Anaesthetic ether was used for these operations. A great number of the adrenalectomies were performed by Miss H. Stone.

Radioactive Sodium

The radioactive material used in this study was the isotope of sodium with atomic mass 24. This substance was obtained from the atomic pile at Chalk River, first through the National Research Council of Canada, and later through the Eldorado Mining and Refining Ltd. On two occasions the material was prepared in the Cyclotron at the Radiation Laboratory of McGill University, through the courtesy of Prof. J.S. Foster.

The characteristics of sodium-24 are as follows: (134)

Half-life	Radiation	Energy of Rays	Product of Disintegration
14.8 hrs.	beta ray	1.44 MEV	Mg-24
	gamma ray	1.4 MEV	
	gamma ray	2.8 MEV	

One of the important features of this isotope is its short half-life. In 24 hours approximately 30% of the original activity is left, and after four days only 1% of the original activity remains (Fig. 1). One advantage of the short half-life is that any contamination through spilling will be short-lived. Another is that disposal of the animals treated with the material involves no difficulties.

Production of Sodium-24

In the chain reacting pile, the sodium was prepared by the bombardment of sodium-23 with neutrons by the following reaction: (134)

$$_{11}Na^{23} + {}_{0}n^{1} \longrightarrow {}_{11}Na^{24} + \gamma$$

The first lot of radiosodium used in these experiments was prepared from sodium chloride. This method proved to be unsatisfactory, as a small quantity of the chloride was converted to sulphur-35 during the irradiation procedure. Although this contamination was slight at the time of irradiation, the long half-life of sulphur-35 (88 days), compared with that of sodium-24, caused marked alterations in the decay curve within a few days (Fig. 1). All other shipments were prepared from sodium carbonate, which was converted to sodium chloride before use.

In the cyclotron, the material was prepared by the bombardment

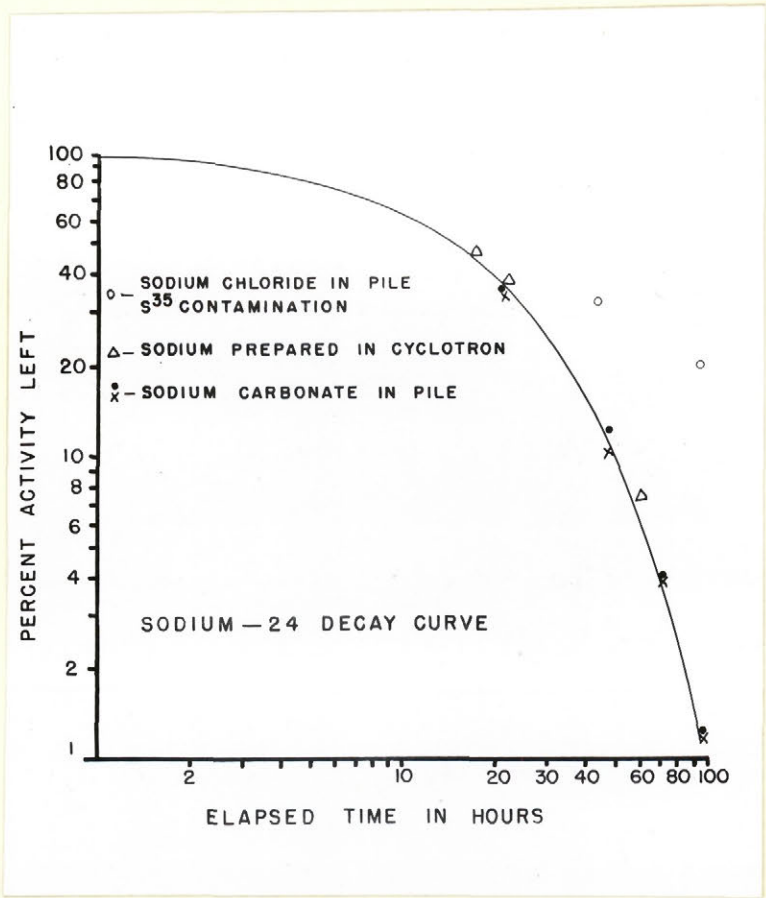

Fig. 1

Sodium-24 reference plot showing percent of initial activity left after elapsed time from 1 to 98.5 hours.

of aluminum with protons by this postulated reaction:-

$$_{13}Al^{27} + _{1}p^{1} \longrightarrow _{11}Na^{24} + 3_{1}p^{1} + _{o}n^{1}$$

A small quantity of F1-18, with a half-life of 112 minutes, was formed during the course of the reaction. A period of approximately 15 hours was therefore allowed between preparation and processing, to allow for almost complete disintegration of this isotope.

Preparation of Sodium-24 for Injection

A number of factors, such as availability of laboratory animals, the nature of sodium-24, and the plan of the proposed biological assay, determined the choice of chemical form and frequency of the shipments of radioactive material.

The sodium was received in 5 millicurie (mc) lots, approximately every two weeks. Each shipment contained 154 mg. of sodium carbonate (2.9 m.eq.) in 45 mls. of water. On arrival, this material was converted to sodium chloride by the addition of 1 N Hydrochloric acid.

$$Na_2CO_3 + 2\,HCl \longrightarrow 2\,NaCl + H_2O + CO_2$$

The hydrochloric acid was added until neutrality, as indicated by pH paper, and the carbon dioxide released by this reaction was blown off with a stream of nitrogen. The final volume of the solution was adjusted to 48.55 mls. by the addition of water, giving a solution which contained 3.5 mg. of sodium chloride per milliliter.

During the entire procedure, the radioactive material was left in its original lead container. Each day an aliquot was removed from the

container so that the investigator was never exposed to more than a few microcuries at a time.

In order to make the best use of this material, only a small quantity was used on the first day. This was diluted with a non-radioactive salt solution of the same concentration. On subsequent days, larger aliquots were used with appropriate reductions in dilutions, as indicated below:

Day	Volume of Active solution ml.	Volume of Inactive solution ml.	Final volume ml.
1	0.5	35.5	36
2	1.5	34.5	36
4	10.0	24.0	36
5	36.0	0.0	36

In this way, the work was spread over five days, with experiments on the first, second, fourth and fifth days. The solutions used each day contained roughly the same concentrations of radioactive sodium.

The two lots of sodium prepared at the cyclotron were processed in a fume cupboard, behind lead bricks two inches thick. A mirror was used as an aid in following the process. The material was received as approximately 5 gms. of aluminum. It was brought into solution in four and a half hours with concentrated boiling hydrochloric acid. On the second occasion, a platinum crucible, which acted as a catalyst, was used for this reaction. Approximately one and a half hours of reaction time was saved by this procedure.

$$2Al + 6HCl \xrightarrow{Pt} 2AlCl_3 + 3H_2 (\uparrow)$$

The radioactive sodium was converted to sodium chloride in this reaction.

$$2Na + 2HCl \longrightarrow 2NaCl + H_2\uparrow$$

When the aluminum was completely converted to aluminum chloride, the solution was brought to neutrality with concentrated ammonium hydroxide:-

$$AlCl + 3NH_4OH \longrightarrow Al(OH)_3\downarrow + 3NH_4Cl$$

The radioactive sodium chloride was converted to sodium hydroxide during this reaction.

$$NaCl + NH_4OH \longrightarrow NaOH + NH_4Cl$$

The aluminum hydroxide was filtered off with the aid of a filtration accelerator.

The precipitate was washed several times with water, but complete recovery of the radioactive sodium was not possible in the short time allowed for filtering and washing. Roughly 75% of the original radioactive material present in the aluminum chloride solution was recovered in this way.

The filtrate was heated almost to dryness and concentrated nitric acid was added, drop by drop, until all the ammonium chloride disappeared:

$$NH_4Cl + HNO_3 \longrightarrow N_2O\uparrow + 2H_2O + HCl\uparrow$$

The "carrier-free" sodium chloride was dissolved in water and adjusted to the proper volume and salt concentration for injection.

Measurement of Radioactive Sodium

a) Units Quantitative measurements of radioisotopes are usually expressed as curies, which are defined as those quantities of isotopes which have 3.7×10^{10} disintegrations per second. However, accurate measurement of absolute quantities of radioisotopes is difficult because, for a number of reasons, the Geiger counter may not record one count for each disintegrating atom.

On the basis of the data accompanying the shipments of sodium, each animal received about 0.35 microcuries, a quantity which is probably low enough to avoid serious irradiation effects, and high enough to give satisfactory counts.

It was not necessary to determine absolute amounts of radioactivity in the assay, since all test animals were compared with similarly treated control animals. The percentage of the injected material appearing in the rat urine could be determined by the comparison of a sample of rat urine with one of the injected radioactive solution.

b) The Geiger Counter The instrument used for the measurement of the radioactivity was a Geiger-Muller (G-M) counter, attached to a scaling circuit which recorded the impulses detected by the counter. A self-quenching Tracerlab TGC-1 G-M tube with a thin mica end-window (less than 2 mg/cm^2) was used. This tube was mounted in a castle, and was connected to a Berkeley Decimal Scaler (Model 1000 B) with a scale of 1000. Attached to this instrument was a stop-clock which started and stopped with

the counting.

The instrument required a half-hour for warming up before use, and was satisfactory for the intermittent counting which was required by the assay. Before each counting session, the performance of the instrument was checked by means of a "standard" of uranium oxide. Since this material has a half-life of 4.56×10^9 years, the count should remain constant for any one tube. The uranium standard was also used in determining the voltage at which each tube could be used for counting. One of the properties of the G-M tube is that with increasing voltage a "plateau" is reached on which the counting is independent of the voltage. Using the uranium standard, counts were taken at increasing voltage levels, and the plateau region determined. Operating voltage was in the first third of the plateau range. It was never necessary to count greater activities than 10,000 counts per minute, which is well within the resolving time of the instrument.

c) Preparation of Samples The radioactive urine was plated on small watch glasses during the early experiments, and on cupped metal planchets in later experiments. It was dried in a small oven under an infra-red lamp to prevent splattering. The volume of all samples was 1.5 ml. Drying time was approximately one hour.

The change from watch glasses to metal planchets was made because the shape of the planchets was such that more of the radioactive material remained under the window of the G-M tube, resulting in higher counts per sample, and more economical use of the radioactive material. The cupped planchets fitted into a grooved sample holder in the castle, which assured

consistent geometry for counting purposes. The shape of the sample container and the presence of urine residue affected the number of counts obtained. This is illustrated in the following table:

Experiment 10a

In each instance, 0.04 mls. of the radioactive solution was plated onto the container.

Method of Dilution Before Drying		Count/min Watch Glasses	Count/min Metal Planchets
Added 1.46 ml. of water		3999	5175
		3969	5373
		3856	4973
	Aver:	3941	5174
Added 1.46 ml. of urine		4101	4815
		4167	4807
		4307	
	Aver:	4192	4811

It can be seen that, regardless of the method of dilution of the radioactive material, the counts were higher when the metal planchets were used. Since the solids present in the urine might absorb some of the radiation, the slightly lower counts obtained with the planchets were not unexpected, when the urine rather than water was used as the diluting fluid. The fact that this absorption effect was not obtained with the watch glasses suggests that the solids of this urine settled at the centre, facilitating the entry of the rays through the mica window.

The variations between triplicate samples noted here are actually

larger than those obtained in the counting of urine samples, since in that case larger volumes are pipetted.

d) Counting Technique A five-minute background count was taken at the beginning and end of each counting session, and the average background count per minute (cpm) was determined. This background count is due to cosmic radiation and varies with the individual G-M tube. In this study it varied between 25 and 30 cpm. In order to obtain the true cpm, the background cpm was subtracted from the total cpm.

The samples were placed in a groove on the sample changer attached to the lead castle. This was pushed into a fixed position so that the sample rested just beneath the end window of the G-M tube. The counts were recorded as follows:

Initial Reading	Final Reading	Count	Time (min.)	Counts/min.
	4440			
4440	5574	1134	1	1134
5574	7269	1695	1	1695
7269				

The results show that with this type of scaler the counts can be obtained by simple subtraction. This manner of recording saves time since it is not necessary to clear the instrument between samples.

Approximately 3% of the injected radioactive material was excreted within the 5 or 6 hours of the assay procedure. Sufficient radioactivity was injected so that approximately 3000 cpm appeared in the urine in this time period. Since the total volume of the urine plus washings was usually under 3 ml., and an aliquot of 1.5 ml. was used for counting purposes,

most samples contained approximately 1500 cpm. If any sample had less than 1000 cpm, the counting-time was increased until 1000 counts were recorded. The samples of one set of experiments were generally counted within a half-hour period. The samples of the control animals were counted in the middle of this period.

Procedure of Biological Assay

Post-Operative Management

The immediate post-operative treatment was designed to permit optimum recovery of the animals from surgery by the replacement of their drinking water with saline. Two days were allowed between operation and testing. If the animals were deprived of saline on the second day after operation, their sensitivity to DCA was increased, as indicated in the following table:-

Exp't	Post-op. Conditions	%Sodium-24 Exc'd by Controls in 6 hrs	Urinary Sodium-24 as % of Controls (Animals given 10Y DCA)
2 a	2 days saline	5.19	74.0
2 c	1 day saline, 1 day water	2.89	36.6

The final procedure adopted for post-operative treatment was a) saline on the first post-operative day and b) distilled water on the second. This allows for recovery from surgery and for a satisfactory level of sensitivity.

Procedure on Day of Assay

The animals were weighed on the morning of the experiment, i.e. on the second day after adrenalectomy. Any animal that had lost 10 grams or more, or gained more than a few grams from the time of operation was discarded. The most efficient use of the sodium and animals was made if experiments were performed on 35 rats on each of four days during the first five days after arrival of the radioactive sodium. These animals were divided into three groups of eight, and one control group of eight to eleven animals. Groups of this size gave more satisfactory results than were obtained earlier with six animals.

Five cages of seven rats each were prepared for each day. The groups were divided throughout all the cages to avoid variations due to the time of handling. Food and water was removed from each cage one hour before it was handled.

The procedure of the test, as originally suggested by Dorfman and his associates, included the following steps: 1) Subcutaneous injection of test substance in 0.25 ml. of corn oil. 2) Subcutaneous injection of one or two millilitres of an aqueous solution containing 3.5 mg. of sodium chloride per hundred grams body weight and tracer quantities of sodium-24, one hour later. 3) Urine collection for a period of six hours, begun immediately after the injection of radioactive material, with animals in individual metabolism cages.

During the course of the present investigation a number of modifications have been introduced into the original procedure, although the general principles of the assay have been retained.

a) Solvent Vehicles Absolute alcohol was found to be the most satisfactory solvent for injection of urinary extracts. Earlier experiments with corn oil and 25% alcohol had indicated that they were satisfactory for DCA but that emulsions were formed with residues of urinary extracts. Since alcohol causes tissue damage at the site of subcutaneous injection, the volume administered to each animal was reduced from 0.25 to 0.1 ml. Relatively aldehyde-free alcohol was prepared by the addition of p-phenylenediamine (4 gms/l) to alcohol which was allowed to stand for one week, with frequent shaking. The alcohol was then triple-distilled and refrigerated.

The radioactive material was injected in an aqueous solution containing 3.5 mg. of sodium chloride per millilitre and tracer quantities of sodium-24. Each animal received a subcutaneous injection of 1.0 ml. of this solution.

b) Period of Urine Collection In the early experiments, the radioactive solution was injected one hour after the administration of the test substance, and urine was collected for the first six hours after the second injection.

In the later experiments, when the solvent had been changed to absolute alcohol, the one hour wait allowed for absorption of the test material was eliminated and the urine collection period was reduced to five hours.

The effect of these variations on the sensitivity of the assay will be discussed later.

c) Method of Urine Collection Since the radioactivity was to be

counted on dry samples, it was desirable to keep the total volume of urine plus rinsings as small as possible. This was accomplished in the following manner:

The animal was placed on a board on its back, and held in position at the limbs and mouth with loops of cord. The bladder was emptied with gentle abdominal pressure, although it was generally emptied involuntarily while the animal was being attached to the board. The foreskin was drawn back with the aid of forceps and the urethra was ligated with heavy thread (button and carpet), just below the glans penis. After the loose threads were cut, the foreskin was returned to its original position. The rat was then released from its fixed position on the board and returned to its cage for the collection period.

At the end of the test period, the animals were killed at three minute intervals by the intraperitoneal injection of 1 ml. of nembutal. The abdominal cavity was opened and the bladder exposed. The urine was removed directly through the bladder wall by means of a fine (no. 25) needle and syringe. Urine volumes were generally between one and two ml. The needle was left in position while the syringe was emptied into a small graduated centrifuge tube containing a drop of caprylic alcohol to prevent foaming. A small quantity of water (about 0.5 ml.) was drawn up into the syringe, which was reattached to the needle, and used for rinsing the bladder. This bladder rinse, plus a syringe rinse of similar magnitude, was added to the urine in the centrifuge tube. The bodies of the animals were kept on the roof of the building for a day or two to allow for decay of the radioactivity, and then incinerated.

d) Determination of Urinary Sodium-24 The volumes of urine plus washings were recorded, and aliquots of 1.5 ml. of each sample were plated, dried and counted on the same day. The background cpm was subtracted from the cpm of each sample. The total urinary cpm for each rat was calculated on the basis of the recorded total volumes, and the average of each group was then determined. Occasionally, an extremely low count, accompanied by a low urine volume was observed. It is believed that an animal displaying these deviations was either in poor condition before operation or damaged during adrenalectomy. Since the rats were being used in an assay for the determination of salt-retaining activity, such false positives had to be eliminated. The vast majority of the animals eliminated throughout the entire study excreted considerably less than one-quarter of the radiosodium totally excreted in their respective groups. In doubtful cases, an animal was only eliminated if the radioactivity in the urine was less than one-half or greater than double the mean of that excreted by the other animals in the group.

The average total cpm of the control animals was considered as 100% excretion. The average total cpm of a test group was expressed as its percent of the value obtained for the control animals. If this was less than 100% it was considered to be "sodium-retaining", and could be expressed in terms of approximate DCA equivalents.

Variability of Control Animals

In order to determine what percentage of the injected radioactivity was present in the urine of the rats, four percent (0.04 ml.) of the radioactive solution used for injections was plated in triplicate. These

samples were diluted to 1.5 ml., the volume used for counting aliquots of rat urine, and counted at the same time as the samples obtained in the rats. The value obtained in this manner was multiplied by 25 to give the total number of counts injected per animal.

The concentration of the diluting urine affected the count slightly. In order to overcome this difficulty, comparisons were made only between groups whose counts were determined with the same inactive urine. One urine served for all experiments carried out with one shipment of sodium.

The percentage of injected sodium-24 excreted by five consecutive sets of controls is presented below:

Experiment	Date	No. of Control Animals	% of Na-24 excreted in 5 hrs.
32 a	Nov.20/51	10	2.2
32 b	21	14	2.53
32 c	22	7	2.05
32 d	23	6	2.54
32 e	24	10	2.8

Although the animals were maintained under almost identical conditions, it was not possible to further decrease daily variations below those observed in this group of experiments. Fluctuations of this type led to difficulty in interpreting the effects of DCA, but this was overcome by expressing the results in relation to the sodium-24 excretion of the control animals.

Although the percentage of injected radioactivity recovered in the urine was not used in the calculation of results, this value was useful as a check on the method. Under optimum conditions, control animals excreted

approximately 2.5% of the injected sodium-24 in five hours. If they excreted much less than two or much more than three percent, it could generally be shown that some condition of the test had not been adhered to.

Effect of Desoxycorticosterone on Urinary Excretion of Radioactive Sodium in Assay

DCA was chosen as a standard of reference in this assay because it is particularly active with respect to sodium retention and because it is easily available and relatively stable.

DCA in Oil

The earliest method used for the assay of DCA was based on the original procedure as suggested by Dorfman, Potts and Feil (189). The test substance was injected subcutaneously in 0.25 ml. of corn oil. One hour later the animals received a subcutaneous injection of radioactive sodium, and were placed on urine collection in the manner already described. The effects of 1, 2, 5, 10, 15 and 20 micrograms (γ) of DCA are presented in Table I.

The sodium-24 excretion of the animals receiving 1γ of DCA was 88.8% of the control values. Higher dosages were associated with greater sodium-retaining effects. The test animals receiving 20γ of DCA, the highest dosage studied, excreted only 142% of the control values. Significant sodium retention occurred between the 2 and 5γ dosage levels. The dosage-response relation obtained with rats studied in this manner is plotted in Fig. 2. The shape of the curve indicates that it is steepest between 2 and 15 γ and that the effect produced by 20γ is quite close to maximum. If the

TABLE I

EFFECT OF DCA AND DOC, INJECTED IN CORN OIL, IN URINARY RADIOSODIUM IN RATS

Dosage in γ	No.test rats	Urinary Na-24* (% of controls)	S.E.**	No.control animals	S.E. of control rats	P
		Effect of DCA				
1	11	88.8	9.74	7	17.44	< 0.6
2	10	67.5	8.00	8	18.00	< 0.2
5	5	46.1	4.07	6	13.12	< 0.01
10	5	36.6	12.30	6		
15	12	17.3	1.70	13		
20	5	14.2	4.50	5		
		Effect of DOC				
2	5	79.4	7.25	5		
20	5	15.5	5.20	5		

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

** S.E. (Standard Error) = $\sqrt{\frac{\varepsilon(x)^2 - \varepsilon^2(x)}{n(n-1)}}$

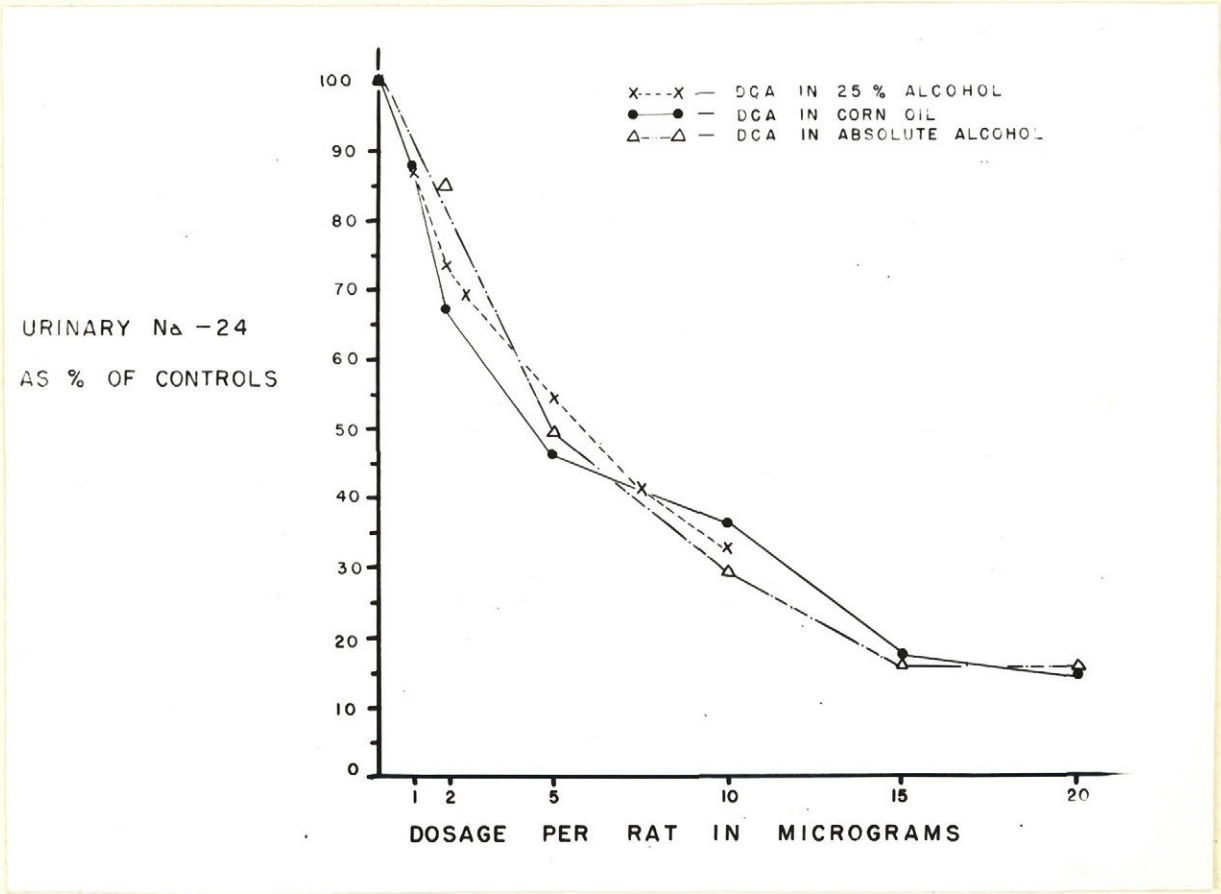

Fig. 2

Effect of DCA on urinary radiosodium excretion in rats. Curve for DCA in absolute alcohol obtained with the five-hour assay. Other curves obtained with the six-hour assay. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium-retention.

dosage-response relations are plotted in terms of the log of the dose and the urinary sodium-24 ratio of test and control animals, a straight line relation is observed. The regression line obtained in this manner is presented in Fig. 3. The free steroid, DOC, was assayed at the 2 and 20γ dosage levels. The values were not significantly different from those obtained with the acetate esters.

DCA in 25% Alcohol

Urine extracts were considerably less soluble in corn oil than DCA. For this reason, the sensitivity of the assay was repeated using 0.25 ml. of 25% alcohol as the solvent vehicle.

Assays were performed with dosage levels of 1, 2, 2.5, 7.5 and 10γ. The results are presented in Table II, and the dosage-response relation obtained is plotted in Fig. 3. It is apparent that the change in solvent did not have a marked effect on the assay, since the dosage-response curve obtained in this manner was almost identical with that obtained with DCA injected in corn oil. The difference between the coefficients of the regression lines (136) obtained with both solvents is not significant (P is <0.8).

DCA in Absolute Alcohol

For purposes of urine assay, the best solvent was absolute alcohol. For this reason, DCA was assayed with this solvent vehicle. The volume used for each animal was reduced from 0.25 ml. to 0.1 ml. The one hour wait between injections of the test substance and radiosodium was eliminated, and the collection period was reduced to five hours. The effects of 2, 5, 10, 15 and 20γ of DCA are presented in Table III. The sodium-retaining effects of DCA injected in this manner were similar to

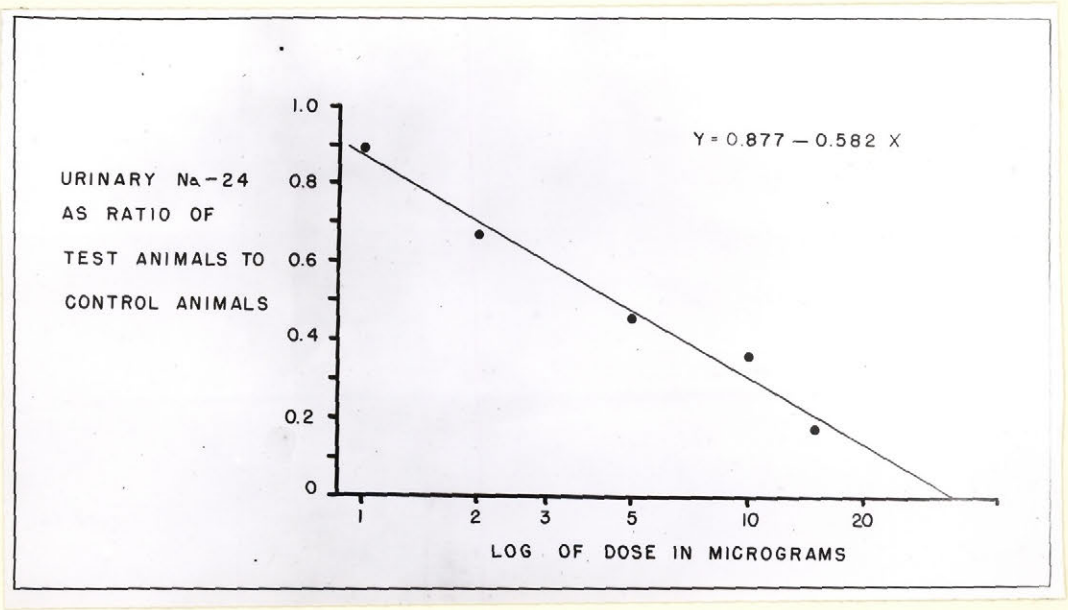

Fig. 3

Effect of DCA in corn oil on urinary excretion of radiosodium in rats. Log-dose-response relation.

TABLE II

EFFECT OF DCA, INJECTED IN 25% ALCOHOL, ON URINARY RADIOSODIUM IN RATS

Dosage in γ	No. test rats	Urinary Na-24* (% of controls)	S.E.**	No. of control animals
1	12	87.0	6.2	10
2	12	73.8	6.4	10
2.5	7	69.8	7.4	5
5	6	54.0	7.4	6
7.5	4	41.3	13.6	5
10	5	32.8	1.7	6

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

**S.E. (Standard Error)

TABLE III

EFFECT OF DCA, INJECTED IN ABSOLUTE ALCOHOL, ON URINARY RADIOSODIUM IN RATS

Dosage in γ	No. test rats	Urinary Na-24* (% of controls)	S.E.**	No. control animals
2	5	85.5	14.60	6
5	6	49.0	8.06	7
10	4	28.8	7.87	8
15	6	16.0	9.33	6
20	6	15.5	2.50	7

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention"

**S.E. (Standard Error)

those noted with DCA in corn oil (Fig. 2), and the coefficients of the regression lines (136) obtained with both solvents are not significantly different (P is <0.2). The regression coefficient is, however, significantly different from that obtained with DCA in 25% alcohol.

Effect of Desoxycorticosterone on Urinary Excretion of "Total Sodium" in Assay

DCA in 25% Alcohol

The effect of 10 and 20γ of DCA on total urinary sodium was studied by the method of assay employed for the measurement of radioactive sodium. The results are listed in Table IV. (Sodium determinations were done on the flame photometer by Miss H. Thomson, of the Department of Experimental Surgery of McGill University). The sodium-retaining effects were less marked than those obtained with radioactive sodium with the same dosages. The effects of 10 and 20γ were similar to those observed with 5 and 7.5γ respectively, when DCA was injected in 25% alcohol (see Table II).

Effect of Cortisone on Urinary Excretion of Radioactive Sodium in Assay

Cortisone in Oil

The effects of 10, 30, 48 and 50γ of cortisone, injected in corn oil, are listed in Table V. A sodium-retaining effect was not observed at any dosage level, but a reverse effect was noted with 10, 30 and 48γ. The dosage-response relations have been plotted in Fig. 4. A line was used to join the points determined under identical conditions, i.e. on the same day. Although the results obtained at the 10, 30 and 48γ levels suggest that

TABLE IV

EFFECT OF DCA ON TOTAL URINARY SODIUM IN RATS

Dosage in γ	No. of rats	Urinary Na/6 hrs (m.eq.)	S.E.*	Urinary sodium (% of controls)
0	7	0.2852	0.035	100%
10	5	0.1555	0.023	54.4
20	5	0.1231	0.021	43.2

*Standard Error

TABLE V

EFFECT OF CORTISONE, INJECTED IN CORN OIL, ON URINARY RADIOSODIUM IN RATS

Expt.	Dosage in γ	No. test rats	Urinary Na-24* (% of controls)	S.E.**	No. control animals
6a	10	6	117.0	11.0	6
6a	30	6	126.2	12.9	6
6e	48	9	169.8	10.2	5
7e	50	6	100.5	6.5	6

*Sodium-24 excretion of control animals was considered as 100%. Values greater than this indicate a "sodium excreting" effect.

**Standard error.

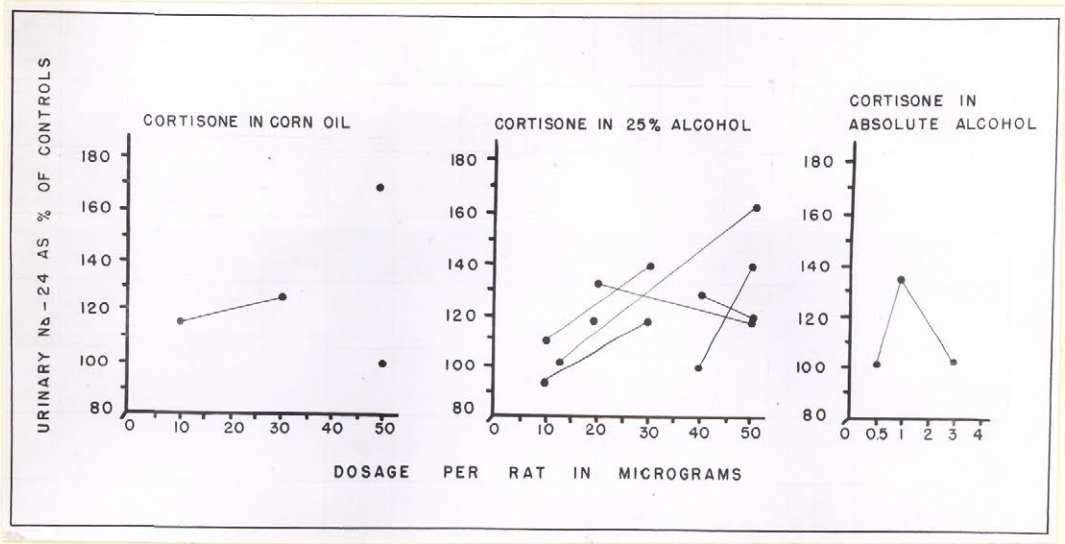

Fig. 4

Effect of cortisone on urinary excretion of radio-sodium in rats. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium-retention. Experiments performed on one day are joined by lines. Each point represents the mean value for one group of rats. Studies with cortisone in absolute alcohol were done with the five-hour assay, the others were done with the six-hour assay.

cortisone produces a sodium-excreting effect which increases with dosage, the sharp decrease in activity from 48 to 50γ indicates that the activity may vary from day to day.

Cortisone in 25% Alcohol

The results of thirteen assays of cortisone in 25% alcohol, in dosages ranging from 10 to 52.5γ, are listed in Table VI. Sodium-excreting effects were obtained with 20, 30, 40, 50 and 52.5γ on one but not every occasion. A sodium-retaining effect was observed twice, at the 10 and 40γ levels, but even then it was not great since the test animals excreted over 90% as much radiosodium as the control animals. The dosage-response relations have been plotted in Fig. 4. Points determined on the same day have been joined. The lines formed in this manner indicate that the shape of the dosage-response curve of cortisone, in 25% alcohol, varies from day to day, as already suggested by the studies of cortisone in corn oil.

Cortisone in Absolute Alcohol

Since absolute alcohol was found to be the best solvent for urinary extracts, experiments on the effect of cortisone dissolved in this solvent have been carried out. The dosage range studied was reduced to 0.5-3.0γ because most of the urine assays were performed on 20-minute urine samples, which contain amounts of cortisone-like material in this range (185).

The five-hour assay described for DCA in absolute alcohol has been used in these studies. The results are listed in Table VII.

Although a sodium-excreting effect was observed with 1γ of cortisone, no effects were observed with 0.5 or 3γ. In spite of the fact that the effect was not significant (P was < 0.1), the mild sodium-excretion

TABLE VI

EFFECT OF CORTISONE, INJECTED IN 25% ALCOHOL, ON URINARY RADIOSODIUM IN RATS

Expt.	Dosage in γ	No. test rats	Urinary Na-24* (% of controls)	S.E.**	No. control animals
6b	10	5	93.7	11.7	6
7b	10	6	108.9	24.3	4
6c	13	5	101.6	26.5	5
6d	19	9	117.5	28.0	7
7a	20	5	132.2	15.0	5
6b	30	6	117.9	15.6	6
7b	30	6	138.4	12.5	4
7c	40	5	97.7	10.3	5
7d	40	6	128.0	8.3	4
7a	50	6	118.6	16.0	5
7c	50	6	139.5	15.7	5
7d	50	4	117.7	12.0	4
6c	52.5	5	162.4	16.9	5

*Sodium-24 excretion of control animals was considered as 100%. Values greater than this indicate a "sodium excreting" effect.

**Standard Error

TABLE VII

EFFECT OF CORTISONE, INJECTED IN ABSOLUTE ALCOHOL, ON URINARY RADIOSODIUM IN RATS

Expt.	Dosage in γ	No. test rats	Urinary Na-24* (% of controls)	S.E.**	No. control animals	S.E.	P
46c	0.5	6	100.5	11.8	9	11.8	
46c	1.0	7	134.7	14.0	9	11.8	<0.1
46c	3.0	7	102.3	17.3	9	11.8	

*Sodium-24 excretion of control animals was considered as 100%. Values greater than this indicate a "sodium excreting" effect.

**Standard Error.

caused by 1Y of cortisone might mask a sodium-retaining effect due to some other compound. The effect of cortisone in absolute alcohol has been plotted in Fig. 4.

Preparation of Human Urine Extracts for Assay

Extraction Procedures

The methods used for the extraction of urine were based on those generally employed for the determination of biological or chemical corticoids (195,196,197,198). The extracts were prepared in the following manner:-

a) Collection of urine Complete 24-hour urines were collected without preservatives, refrigerated after collection, and extracted within one or two days.

b) Acidification of Urine Prior to Extraction Most methods for determining corticoids now include acidification of the urine prior to extraction. This procedure, presumably a mild hydrolysis of esterified steroids, results in the extraction of greater quantities of corticoids by lipid solvents. The urine was adjusted to pH 1.5 with sulphuric acid. Extraction was done within a half-hour of acidification.

c) Extraction with Lipid Solvent Redistilled reagent grade chloroform was used as an extraction solvent for the bulk of the urines. In some of the earlier experiments redistilled ethylene dichloride was used, but this was replaced because a high temperature was required for its distillation. The urine was extracted three times with one half volume of chloroform in the first extraction and quarter volumes in the second and third.

The three extracts were pooled, placed in a round-bottom distilling flask, and evaporated to dryness in a still attached to a water pump. The water bath was kept at 40°C throughout this procedure. The dry residue, which remains in the flask, is referred to as the "crude lipid" fraction.

d) Alkali Washing of Lipid Extract The dried "crude lipid" extract was dissolved in 50 ml. of chloroform and washed five times with 15 ml. portions of 0.1 N sodium hydroxide. Each lot of sodium hydroxide was back extracted with an equal volume of chloroform, which was added to the rest of the extract. This procedure removes alkali-soluble steroids such as the estrogens, and other impurities, from the lipid extract.

e) Water Washing of Lipid Extract The chloroform extract was washed several times with 15 ml. portions of distilled water, and each lot of water was back extracted with an equal volume of chloroform. This procedure removes the alkali left in the chloroform by the previous step. The water washings were continued until they were neutral to pH paper. The washed chloroform extract was evaporated to dryness as described in step C. The remaining residue is considered the "neutral lipid" fraction.

f) Preparation of "Water-insoluble" Fraction The dried "neutral lipid" extract was dissolved in 50 ml. of reagent grade redistilled benzene. The benzene extract was washed ten times with equal volumes of distilled water. The purpose of this procedure was to separate the more water-soluble compounds, such as cortisone, from the water-insoluble compounds. The benzene was evaporated to dryness with a stream of nitrogen in a water bath at 40°C. The residue is referred to as the "water-insoluble" fraction.

After extraction procedures for any fraction were completed, the residue was dissolved in a small quantity of absolute alcohol (1 ml.) which was evaporated under a stream of nitrogen. This was done to assure the removal of traces of chloroform from the residue.

Storage of Urine-Extracts

Since shipments of sodium-24 were received at two-week intervals, and since only 12 assays or fewer were performed with each shipment, some urine-extracts were stored for periods up to a month or longer before assay. Two methods of storage, described as "dry" and "wet" storage, have been used in these studies.

a) "Dry" Storage When the final extracts were ready for storage they were dissolved in chloroform and transferred to test tubes. The chloroform was removed under a stream of nitrogen. Remaining traces were removed with a little absolute alcohol, as previously described. The test tubes were stored in a desiccator, at room temperature. When the processing was not completed in one day, the dried residues were stored in the refrigerator. This method of storage is referred to as "dry" storage. The urinary extracts were stored in this manner for some time until it was noted that many extracts which had a sodium-retaining effect when first assayed. no longer displayed this activity when they were reassayed. two or four weeks later. Several instances of this apparent deterioration of the extracts are shown in the following table. A time-dose of 20 minutes of "crude lipid" extract was used for each rat:-

Experiment Date	Date of Urine Collection	No. of Test Rats	% of Controls	No. of Controls
E.V. (Acromegaly)				
Mar. 2/51	Feb.28/51	5	60.4	5
Mar.16/51	"	5	94.9	5
R.B. (Acromegaly)				
Mar. 3/51	Feb.28/51	5	49.8	7
Mar.16/51	"	6	77.7	5
P.W. (Nephrosis)				
May 25/51	May 13,14/51	5	42.8	7
June 23/51	"	6	89.2	6

A decrease in activity with "dry" storage was not, however, observed with a normal urine to which DOC had been added, nor with a urine from a patient with nephrosis. This patient, P.W., was studied on a previous occasion when a decrease in extract activity with time was noted.

A time-dose of 20 minutes of "crude lipid" extract was used for each rat:-

Experiment Date	Date of Urine Collection	No. of Test Rats	% of Controls	No. of Controls
R.M. (Normal, DOC added)				
Stored "wet" 5 days				
Jan.13/52	Jan. 7/52	6	39.1	10
Stored "dry" 16 days				
Jan.24/52	Jan. 7/52	6	31.6	5
P.W. (Nephrosis)				
Oct.21/51	Sept.20/51	5	46.2	6
Nov. 9/51	"	5	52.1	4

b) "Wet" Storage Changes in the storage procedure were made in an effort to avoid the decrease in activity noted in some extracts on "dry" storage. The storage procedure was changed to the following and referred to as "wet" storage:-

i) The extract was dissolved in alcohol
ii) It was transferred to a glass ampoule which was sealed under light vacuum
iii) The sealed ampoule was refrigerated

The changes were introduced after Drs. Deming and Luetscher had forwarded two extracts dissolved in alcohol in sealed glass ampoules.

A urinary extract was divided into three parts which were stored in different ways for approximately the same length of time. The effect of the different methods of storage on the activity of the extract was studied. A time-dose of 20 minutes of "crude lipid" extract was used for each rat:-

Experiment Date	Type of Extract Method of Storage	No. of Test Rats	% of Controls	No. of Controls
	S.L. (Nephrosis) Dec. 28-31/51			
A. Jan. 10/52	"wet" storage	7	68.4	7
B. Jan. 13/52	"dry" storage until Jan.9, when alcohol was added and extract was refrigerated	5	90.7	10
C. Jan. 11/52	"dry" storage	8	113.3	8

If the sodium-retaining activity detected in Experiment A was due to "activation" by the addition of alcohol, a sodium-retaining effect should have been observed when alcohol was added to a "dry" extract in Experiment B.

The fact that this was not noted suggests that the difference in activity of the "wet" and "dry" extracts was due to losses on "dry" storage.

The effect of "wet" storage on the activity of three extracts have been studied. A time-dose of 20 minutes of "crude lipid" extract was used for each rat:-

Experiment Date	Length of Storage	No. of Test Rats	% of Controls	No. of Controls
	A.P. (Toxemia of Pregnancy) Jan. 24-25/52			
36c Jan. 27/52	2 days	8	66.4	10
37d Feb. 10/52	16 days	6	67.6	6
	H. MacD. (Nephrosis) May 13-14/52			
43a May 18/52	4 days	5	57.1	7
45b June 13/52	30 days	6	62.7	10
46a June 26/52	43 days	7	68.2	4
	D.L. (Nephrosis) June 3-4/52			
45b June 13/52	9 days	8	57.0	6
46a June 26/52	22 days	7	59.0	4

The above results suggest that this storage procedure prevents serious losses of activity for a period of at least six weeks. All values reported in the section of "Results" were obtained with extracts stored in the "wet" state unless otherwise indicated.

c) Method of Sealing of Ampoules The extract was transferred to a small glass ampoule in one or two ml. of alcohol. It was evacuated with light suction until the alcohol vapour flame was seen condensing on the sides of the ampoule. A narrow flame was applied to the neck, just below the end of the rubber tubing, until softening and sealing occurred. The end of a fine pipette, used as a "micro-burner", gave the required narrow flame. Care was taken to avoid accidental heating of the alcohol during the sealing procedure, as this can destroy the activity of the extract.

Effect of Variations in "Time-Dosage" on Sodium-Retaining Activity of Urinary Extracts

For routine purposes assays have been performed on a 20 minute time-dose of urinary extract for each rat. The choice of this time-dosage was based on the fact that Deming and Luetscher (51) had published positive results with this quantity of urine.

Two extracts, prepared from nephrotic urine, were studied in dosages ranging from 4.4 to 40 minutes. The activities of the different dosages are presented in Table VIII.

In one case (H. MacD.) the effects of the 4.4, 10, 20 and 40 minute dosage levels are presented. The greatest effect was observed at the 10-minute dosage level. This was maintained at the 20-minute level, but decreased at 40 minutes. In the other case (D.L.), the 10, 20 and 30 minute dosages were studied. The greatest effect was obtained at the 20-minute level. For optimum results, values should be read along the steep part of the dosage-response curve, i.e. between 4.4 and 10 minutes in the first case, and between 10 and 20 minutes in the second (Fig. 5).

TABLE VIII

EFFECT OF VARIATION IN TIME-DOSAGES ON SODIUM-RETAINING ACTIVITY OF URINARY EXTRACTS

Subject	Condition	Time dose (minutes)	Urinary Na-24* (% of controls)
H.MacD.	Nephrosis	4.4 min.	85.8
		10 "	54.2
		20 "	57.1
		40 "	77.6
D.L.	Nephrosis	10 min.	72.6
		20 "	49.7
		30 "	68.3

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

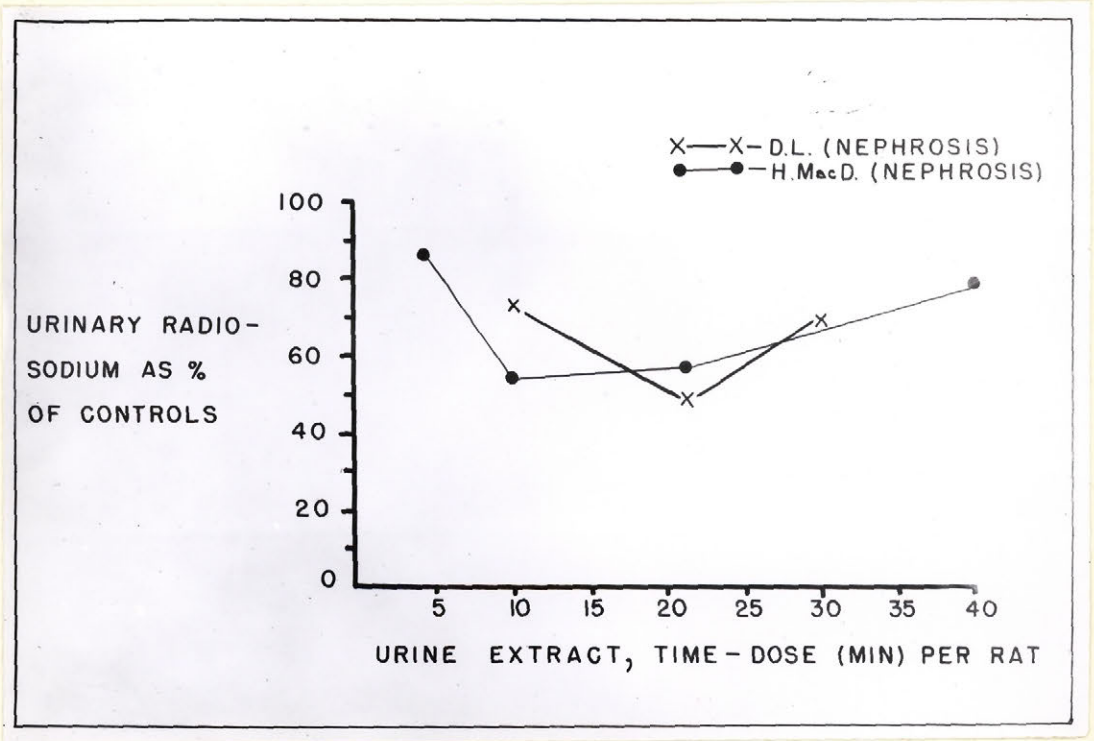

Fig. 5

Effect of different time-dosages of urinary extract on radiosodium excretion in rats. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium-retention.

Effect of Alkali and Water Washings on Sodium-Retaining Activity of Urinary Extracts

The bulk of the assays carried out in this investigation have been done with "crude lipid" extracts. A few studies, however, have been performed on "neutral lipid" extracts in an effort to further characterize the solubility properties of the active material found in some urines. Most assay procedures for biological and chemical corticoids include alkali washing of the "crude lipid" extracts, as this step has been shown to remove phenols, acids, and possibly other substances. The results obtained with "crude" and "neutral lipid" fractions of two urine extracts are presented in Table IX.

Both extracts were obtained from patients with nephrosis. Sodium-retaining effects were observed in the "crude" and "neutral" fractions of both extracts. In terms of approximate DCA equivalents, a recovery of 111.4% in one case and 84.0% in the other, was obtained after the alkali and water washings. These changes in activity are not great when one considers the degree of manipulation to which the extracts were subjected. Although the matter requires further study, the results suggest that the bulk of the active material of these extracts was alkali-insoluble.

Comparison of Method of Deming and Luetscher with Present Assay

Drs. Deming and Luetscher kindly forwarded two urine extracts from patients with nephrosis in which sodium-retaining activity had been detected by their assay procedure. The extracts were prepared by chloroform extraction of urine acidified to pH 1.5 immediately prior to extraction.

TABLE IX

EFFECT OF ALKALI AND WATER WASHINGS ON SODIUM-RETAINING ACTIVITY OF CRUDE LIPID URINARY EXTRACTS *

Expt.	Name	Fract.	No.test patients	No.control animals	Urinary Na-24** (% of controls)	Approx.DCA equiv. (γ)
43(c)	H.MacD.	Crude	5	7	57.1	4.4
44(b)	"	Neutral	6	8	50.0	4.9
					Recovery	111.4 %
45(b)	D.L.	Crude	8	10	49.7	5.0
45(c)	D.L.	Neutral	6	6	58.5	4.2
					Recovery	84.0 %

*Studies performed on urine of patients with nephrosis.

**Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium-retention".

They were stored in alcohol (in test tubes) and refrigerated. Just before mailing they were transferred to glass ampoules. The results obtained with both procedures are tabulated in Table X.

The urinary extracts had marked sodium-retaining effects in both methods of assay. One extract, No. 4401, was more active in the assay described here than it was in the method of Deming and Luetscher, i.e. 10γ of DCA equivalent as compared with 6.2γ. Extract No. 2216 had less sodium-retaining activity in the present assay, i.e. 4.4γ as compared with 7.3γ, but since the extract was 13.5 months old at the time of assay, this was not unexpected. Although some variation in the methods has been noted, the results indicate that the order of sensitivity is within the same range in both assay procedures.

Discussion of Methods

The method suggested by Dorfman et al. (189) has been modified and standardized as a method for the biological assay of DCA-like substances in human urine. During the course of the present study, five groups of investigators have published modifications of the same method. The leading features of the original method and the six modifications thereof are tabulated in Table XI.

In the original procedure, rats of 118 to 175 gms. were used. A much narrower weight range has been used by all other groups of investigators. Four of the methods employ adult rats, while two others use 35 gm. mice or 30-40 gm. rats with resultant increase in sensitivity. All groups but one (51) have used male animals.

TABLE X

SODIUM-RETAINING ACTIVITY OF TWO URINARY EXTRACTS BY THE METHOD OF DEMING AND LUETSCHER AND THE PRESENT ASSAY

Subj.	Age	Sex	Age of extract	Urine vol. (ml/dy)	No.test rats	No.control animals	Urinary Na-24* (% of control)	Approx. DCA equiv. (γ)
				Results obtained by Deming and Luetscher**				
2216	2	M		170				7.3
4401	5.5	M		500-600 (pool)				6.2
				Results obtained in present assay				
2216			13.5 mths.		13	10	56.1	4.4
4401			1 month		14	14	27.6	10.0

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium-retention".

**Personal communication Dr. Q.B.Deming, November 6, 1951.

TABLE XI

METHODS FOR THE ASSAY OF SODIUM-RETAINING SUBSTANCES

Feature of assay	Method of Dorfman et al (189)	Method of Deming & Luetscher (51)	Method of Spencer (190)	Method of Simpson & Tait (192)	Method of Marcus, Romanoff & Pincus (193)	Method of Kagawa, Shipley & Meyer (194)	Present Study
Animals weight	Male albino rats 118-175 gm.	Female rats 150 gm.	Male swiss mice 25 gm.	Male albino rats 30-40 gm.	Male rats 150-175 gm.	Male albino rats 150-155 gm.	Male hooded rats 160-170 gm.
Post-op. period	1 to 11 days	3 to 4 days	4 to 7 days	4 days	varied	1 day	2 days
Post-op. Drinking Fl.	Saline	Saline	5% glucose in 1% saline. Fasted last 8 hrs. but given 10% glucose	1% saline in 5% glucose	Saline up to 24 hrs before assay, tap water for 1 day.	5% cerelose	Saline 1 day. Distilled water 1 day
Manner of studying Electrolyte Effect	Urinary Na-24	Urinary total Na-flame photo.	Urinary total Na-flame photo.	Na-24/K-42 urinary	Total urinary Na-flame photo.	Total urinary Na-microchemical	Urinary Na-24
Solvent Vehicle	Corn oil 0.25 ml.	95% ethanol	5% glucose 0.2 ml.	20% alcohol 0.1 ml.	olive oil	0.05 ml. corn oil (twice, 3 hrs apart)	Absolute alcohol 0.1 ml.
Absorption Time	1 hour	30 min.	no wait	1 hour	no wait	4 hrs and 1 hr	no wait
Salt and Water Load for Test	3.5 mg NaCl/100 gm. in 1 or 2 ml.	5 ml. distilled water	6.35 mg Na and other ions in 2 ml.	27Y Na & 38lY K in 0.5 ml.	5 ml 0.9% NaCl	2.5 ml 0.85% NaCl	3.5 mg NaCl in 1 ml.
Length of urine collection-method	6 hrs. - cages	5 hrs-cages (repeated on 2nd & 3rd days)	6 hrs-beakers (repeated on 2nd & 3rd days)	2 hrs-beakers	4 hrs-cages	2 hrs-bladder	5 hrs-bladder
Expression of Results	Relation to controls	Relation to control day for same animals	Relation to control day for same animals	Relation to concurrent controls	Absolute sodium excretion levels	Relation to concurrent controls	Relation to concurrent controls
Ranges, Dose-response Relations (DCA)	.98-250Y. No dose-response curve	1-25Y. No dose response curve	0.5-4Y. Linear relation with log dose.	0.8-4Y. Linear rel'n with log dose	2.4-60Y. Linear rel'n with log dose	1-12Y. Linear rel'n with log dose	1-20Y. Linear rel'n with log dose
Na retaining effect of other hormones	-	Estradiol, progesterone and testosterone, mild activity	-	Beef adr. extr.-marked effect;Comps.B,S,F,A,E-mild eff: progesterone, estradiol,testosterone-very mild effect	Lipoadrenal cortex-marked effect;Comps. A,B,E,F eff. at 10Y but reversed at higher dosages	-	Cortisone zero to Na excreting effect, varying from experiment to experiment.

Comp. B - corticosterone; Comp.S - 17-hydroxy-11-desoxycorticosterone; Comp.F - 17-hydroxycorticosterone; Comp.A - dehydroxycorticosterone; Comp.E - 17-hydroxy-11-dehydrocorticosterone

In the method suggested by Dorfman et al., the length of time between adrenalectomy and assay varied from one to eleven days, and the animals were maintained on saline until the morning of the experiment. In the modifications, assays were performed from the first to the seventh post-operative days. In three of these, including the present study (192, 194), the length of the post-operative period was rigidly adhered to. Experience obtained in the present study indicates that greater sensitivity was achieved when the animals were deprived of saline for one day before assay. This modification has been introduced by two other groups of investigators (193,194), while a third deprived the animals of saline for the last eight hours before assay (190).

The use of radioactive sodium has been retained only in the present study. Another modification employs the urinary Na-24 to K-42 ratio (192). Three of the methods employ the measurement of total urinary sodium, as determined by the flame photometer (51,190,193), and a fourth uses the measurement of total urinary sodium, determined by a microchemical method (194). The determination of total urinary sodium has a certain advantage over methods using radioactive materials, since experiments can be performed at a convenient time. In the present study, the sodium-retaining effect of DCA was greater when the radioactive material was followed than when total urinary sodium was studied. This is probably related to the timing of the method, since the urine collecting period is begun immediately after the subcutaneous injection of the radioactive material, when a large fraction of the injected sodium is being absorbed into the blood stream and presented to the kidney for excretion. The use of the Na-24 to

K-42 ratio requires the chemical separation of sodium and potassium by a physical absorption procedure, to be published. This method is probably less time-consuming than those now in use for the separation of these materials, otherwise the determination of this ratio would be very time-consuming. A possible objection to this method is the fact that steroids which increase the potassium output without necessarily affecting the sodium output may contribute to the reduction in the ratio.

Very similar results have been observed in the present investigation with DCA in 0.25 ml. of corn oil, 0.25 ml. of 25% alcohol, and 0.1 ml. of absolute alcohol. In the last case the urine collection period was reduced to five hours and the hour allowed for absorption of the test substance was eliminated. Examination of the regression lines obtained with these solvents reveals that there was a significant difference in the regression coefficients of the lines obtained with 25% alcohol and absolute alcohol. On closer inspection, this difference appears to be related to the reduced effect of 2γ of DCA in absolute alcohol. As this dosage was studied once, in a group of five animals, it should be repeated before conclusions on this point are reached. Absolute alcohol has been retained as the solvent vehicle for the urine studies because of the solubility of urine-extracts in this substance. Corn oil has been retained as the solvent vehicle in only one of the modifications (194). One group has employed olive oil (193), another 5% glucose, and the rest have used various concentrations of alcohol (51,192).

The effect of elimination of the one-hour wait between administration of test substance and radioactive material cannot be assessed from

the present investigation because it was introduced at the same time as the solvent was changed to absolute alcohol and the urine collection period was reduced to five hours. As mentioned above, these changes did not affect the sensitivity of the assay except for the 2γ dosage level. In the method of Kagawa, Shipley and Meyer (194), one half of the hormone, or unknown, was injected at the 24th post-operative hour, the other half at the 27th hour, and the urine collected from the 28th to 30th hours. In the other modifications, the absorption time ranges from zero to one hour. The six-hour urine collection period of the original method has been retained by one group (190). Others reduced it to five hours (51), four hours (193) or two hours (192,194).

In the original method, the animals received 3.5 mg. of NaCl per 100 gm. of body weight, at the start of the urine collection period. All the other workers have modified this slightly, although it is difficult to tell what effect, if any, this change had on the sensitivity of the methods. Marcus, Romanoff and Pincus (193) found that a salt load of 5 ml. of 0.9% saline was necessary for good results, in spite of the fact that Dorfman et al. (189) found the method more sensitive when the salt load was low. It must be remembered, however, that the animals of the latter workers had received saline up to the morning of the experiment whereas those of the former had not received any added salt for 24 hours. Spencer (190) injected a rather heavy salt load. Nevertheless, his method is more sensitive than most due, probably, to the fact that 35 gm. mice were used as test animals. In the assay of Deming and Luetscher, the animals did not receive salt at the start of the assay, but were allowed to drink saline up to the morning of

the experiment. The method of Kagawa, Shipley and Meyer (194) combines a 24-hour period of salt deprivation with a relatively high salt load at the start of the test, while the present method uses a 24-hour salt deprivation period as well as a relatively low salt load. The method of Simpson and Tait (192) uses the smallest salt load, i.e. 27γ of sodium and 381γ of potassium per animal. These workers do not, however, deprive the animals of saline before the experiment. The relatively high salt loads used by some of these investigators are probably related to the fact that total urinary sodium is determined, rather than radioactive sodium.

In the method of Dorfman et al. (189), the urinary sodium excretion of the test animals was expressed in terms of its relation to that of control animals, not necessarily studied at the same time. In three of the modifications, including the present study (192,194), the sodium excretion of the test animals has been expressed in terms of its percentage of that excreted by concurrently studied controls. Spencer (190) and Deming and Luetscher (51) have used three rotating subgroups on three successive days, so that each subgroup had served once as controls by the end of the three-day period. Results were expressed in relation to the average sodium excretion of the control animals studied over the three-day period. Marcus, Ramonoff and Pincus (193) were the only investigators who used the absolute urinary sodium output as a method of expressing their results.

One of the weaknesses of the method of Dorfman et al.(189) was the fact that a consistent dosage-response relation was not obtained. In the present study, the results were found to be more consistent. There was a linear relation between the log of the dose and the response in the range

of 1 to 20γ of DCA. Similar relations were noted by Spencer (190) between 0.5 and 4γ, by Simpson and Tait (192) between 0.8 and 4γ, by Marcus, Romanoff and Pincus (193) between 2.4 and 60γ, and by Kagawa, Shipley and Meyer (194) between 1 and 12γ of DCA. The most sensitive methods are those employing the smallest animals. The fact that one of these sensitive assays is based on the measurement of total urinary sodium and the other the Na-24/K-42, suggests that the size of the test animals is probably more important in determining the sensitivity of the assay than any other single feature.

Cortisone did not have a consistent activity in the present investigation. Mild to marked "sodium-excreting" effects were noted on some but not all occasions throughout the range of 1 to 52.5γ. For this reason, a dosage-response curve could not be obtained. The results indicate that only those points determined on the same day are comparable, and that the compound does not always produce an increase in sodium excretion.

Between the range of 10 to 52.5γ increasing dosage was generally associated with an increased sodium-excreting effect, however, the slopes of the "curves" varied considerably from day to day. Two groups of investigators have found that adrenal cortical extract was highly active with respect to sodium retention (192,193). Marcus, Romanoff and Pincus (193) obtained a "sodium-excreting" effect above the 10γ level with dehydrocorticosterone, corticosterone, cortisone and 17-hydroxycorticosterone. Slight sodium-retaining effects were observed with all of these compounds at the 10γ level except dehydrocorticosterone, which was as active as DCA. Simpson and Tait noted mild sodium-retaining effects with corticosterone, 17-hydroxy-

11-desoxycorticosterone, 17-hydroxycorticosterone, dehydrocorticosterone and cortisone (192). The curves obtained with these compounds were parallel to those obtained with DCA. The effects of estradiol, progesterone and testosterone were studied by Deming and Luetscher (51) and Simpson and Tait (192), who found them to be almost negligible.

Although all of these methods are rather difficult and tedious, they have the advantage of sensitivity as well as the ability to characterize substances on the basis of their "electrolyte" effect.

A decrease in activity of urinary extracts was frequently observed in the present investigation following storage in the "dry" state at room temperature (Fig. 6). This was prevented for a period of at least six weeks by refrigerating the extracts in alcohol in sealed glass ampoules. It has recently been reported that certain common molds can be used for the oxygenation of DOC, 17-hydroxy-11-desoxycorticosterone and progesterone (199,200). If the sodium-retaining material detected in urine-extracts is a compound of this type, the report may have some bearing on the lability of the extracts stored in the "dry" state.

Study of "crude" and "neutral lipid" extracts of the urine from two patients with nephrosis indicated that alkali and water washing left most of the active material. This suggests that the sodium-retaining substance in these extracts may be different from Hartman's "sodium factor", which has been shown to be very soluble in dilute alkali (36).

When two urinary extracts were assayed at increasing dosages from 4.4 to 40 minutes, interfering substances were detected at the 20-minute level in one case and the 30-minute level in the other (Fig. 5). These

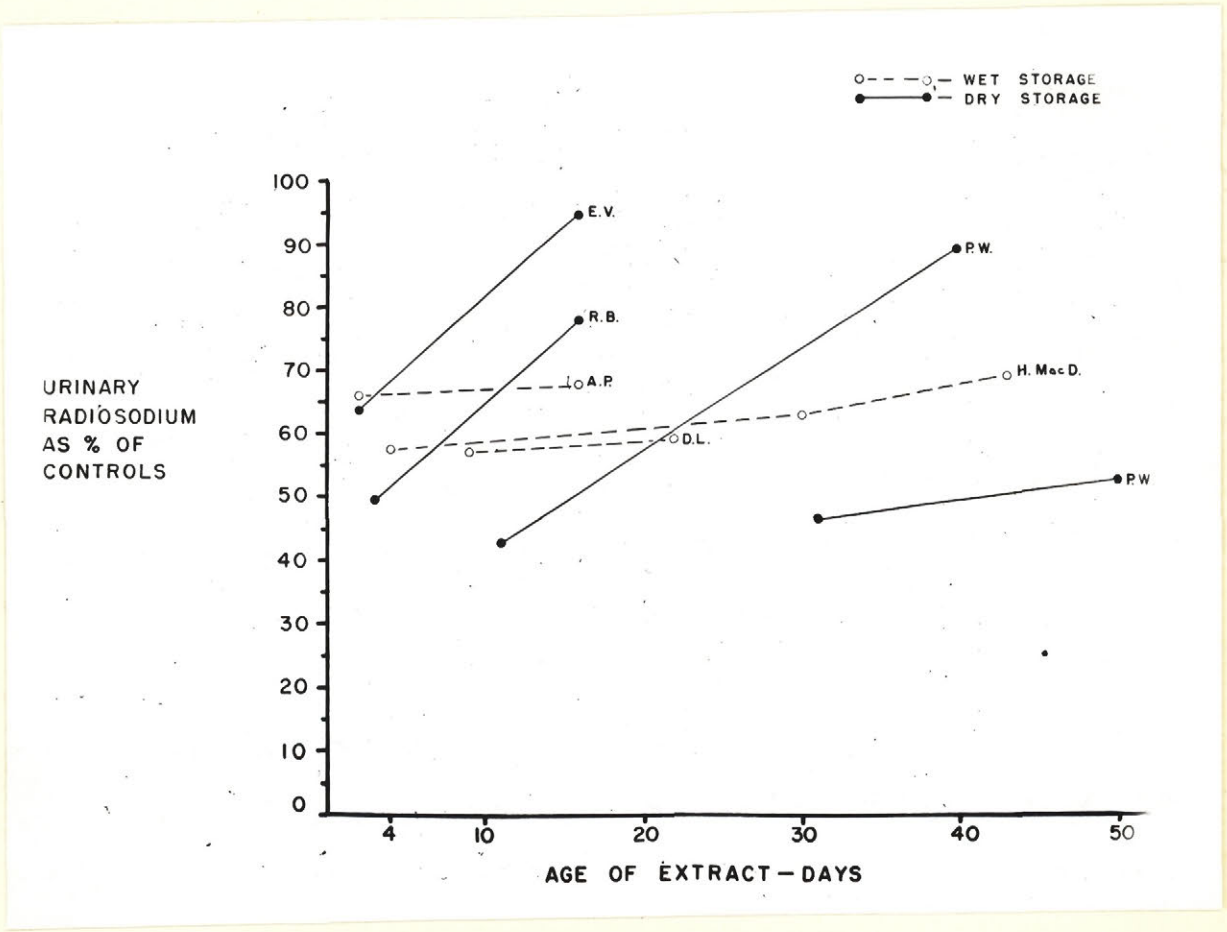

Fig. 6

Changes in activity of urinary extracts with time: Effect of different methods of storage. Radiosodium excretion of control animals was considered as 100%. Values under 100% indicate sodium-retention.

results suggest that values obtained in routine assays at the 20-minute dosage may not be quantitative. The fact that 1γ of cortisone had a "sodium-excreting" effect in this assay indicates that it may be one of the substances responsible for interferences noted in the above-mentioned extracts, since small quantities of cortisone have been detected in human urine (33). Deming and Luetscher have observed similar interferences at higher dosages in their assay (51).

Summary of Methods

A method for the assay of small quantities of sodium-retaining substances in human urine has been standardized. It is based on the urinary excretion of a dose of sodium-24 by adrenalectomized rats.

a) Hooded male rats on a standard diet of purina fox chow were used. The test animals weighed between 160 and 170 grams at the time of adrenalectomy.

b) The animals were adapted to laboratory conditions for one day prior to adrenalectomy.

c) Room temperature was maintained as close to 80° as possible.

d) The post-operative period lasted two days.

e) Drinking fluid following operation was saline on the first day and distilled water on the second. One hour before the assay was started, food and water were removed from the cages.

f) Animals which lost 10 grams or more following adrenalectomy, or gained more than a few grams, were not used.

g) The animals were attached to a board and given subcutaneous

injections of test substance or solvent vehicle (0.1 ml. of absolute alcohol) and 1 ml. of an aqueous solution containing 3.5 mg. of sodium chloride and tracer quantities of sodium-24.

h) The animals were returned to their cages after ligation of the urethra with heavy thread.

i) The various groups studied on one day were divided between all the cages to avoid variation due to the time of handling. A group of control animals was required every day.

j) Five hours after receiving the radioactive sodium, the animals were killed by intraperitoneal injection of nembutal. The body was opened and the bladder exposed. A fine needle and syringe were used to remove the urine. Bladder and syringe rinses were added to the urine. Care was taken to insure a full five-hour collection period for each rat.

k) The mean sodium-24 excretion of each group of animals was determined. The average count per minute obtained in the test animals was expressed in percentage, that of the control animals being 100%. Values under 100% indicated sodium retention.

l) DCA was found to be sodium-retaining between the dosages of 1 to 20γ. A linear relation was noted between the log of the dose and the urinary sodium-24 ratio of the test and control animals.

m) Cortisone had a mild to marked sodium-excreting effect in the range of 1 to 52.5γ which was not always reproducible. A consistent dosage-response relation was not observed.

n) Urinary extracts were stored in absolute alcohol in sealed glass ampoules and refrigerated. This prevented, for periods of at least six weeks,

the serious losses of activity frequently observed in extracts stored in the "dry" state at room temperature.

o) The sodium-retaining effects of two "crude lipid" extracts of urine were not significantly altered by dilute alkali and water washings.

p) Dosage-response curves of human urine extracts indicated the presence of interfering substance at the 20-minute level in one case and the 30-minute level in another.

q) Two "sodium-retaining" urinary extracts forwarded by Drs. Deming and Luetscher had a sodium-retaining effect in the present assay. Minor differences were noted between the two methods, in terms of DCA equivalents, but the order of sensitivity was similar.

RESULTS

Introduction

The edema or ascites of such conditions as toxemia of pregnancy, nephrosis, cirrhosis and congestive heart failure has frequently been attributed to a DCA-like substance of adrenal cortical origin. It has also been suggested that certain rheumatic conditions may be associated with a relative excess of DCA-like "mineralocorticoids". In animals, growth hormone has been reported to cause pathological changes similar to those noted with an excess of DCA, suggesting that this hormone may have an action on the adrenal gland. To test the validity of these suggestions, quantitative determinations of substances having a sodium-retaining effect in animals were made on the urine of normal individuals and of patients with the above-mentioned diseases. The effect of surgical trauma and the intravenous administration of growth hormone on the excretion of these substances has also been investigated.

In the early studies, an extract of a 12-hour urine sample was administered to each rat. This large aliquot was chosen on the assumption that the sodium-retaining factor, if present, must be there in minute amounts. "Water-insoluble" fractions were used in these studies because the "benzene-water" partitioning in the preparation of these fractions had been shown to remove a large part of the "cortisone-like" material in the extract, and because cortisone may interfere with the detection of sodium-retaining material in this method. Twenty-eight normal individuals and four patients with panhypopituitarism were studied in this manner. The results are

presented in Table XII.

Wide variation in the excretion of sodium-retaining substances was noted in normal individuals. The values ranged from no effect to an equivalent sodium-retaining effect of 15γ of DCA per day. The average value for males was 6.1γ/day and for females 4.3γ/day. The results of studies on four patients with panhypopituitarism showed a similar range and average to that found in normal individuals. Since many of these extracts were stored in the "dry" state for a number of weeks before assay, and since this method of storage was later shown to result in marked loss of sodium-retaining activity, the significance of these values may be questioned. It is possible that some of the sodium-retaining effect noted in these large and relatively crude urinary extracts might be due to the presence of toxic substances.

At this point in the study, the dosage of extract used for each animal was reduced from approximately 12 hours to 20 minutes. This change was instituted when Deming and Luetscher (51) reported the presence of a sodium-retaining factor in "crude lipid" extracts of 20-minute urine samples in nephrosis and congestive heart failure. The "crude lipid" fraction studied by Deming and Luetscher has also been used in the present investigation for routine assays.

Unless otherwise stated, all values presented in the following sections were detected at the 20-minute dosage level with "crude lipid" extracts which had been stored in alcohol, as described. The sodium-24 excretion of the control animals was considered as 100% excretion, and the sodium-24 excretion of the test animals was expressed as a percent of the

TABLE XII

DCA-LIKE SUBSTANCES IN "WATER INSOLUBLE" FRACTIONS OF URINARY EXTRACTS, BY BIO-ASSAY

Subject	Time-Dose/rat (hrs.)	Approx. DCA Equiv. (γ) per Sample	Approx. DCA Equiv./Day (γ)
		Normal Subjects	
Males			
B.J.K.	10.3	0.0	0.0
J.B.	10.3	5.0	11.7
L.T.	10.3	1.0	2.3
W.Z.	10.3	1.5	3.4
S.S.	10.3	6.7	15.4
F.B.	10.3	1.8	4.1
H.S.	11.25	2.5	5.3
R.P.	11.25	5.0	11.7
C.Y.	11.25	1.5	3.1
M.F.	11.25	0.8	1.7
P.P.	11.25	2.5	5.3
K.P.	11.25	1.0	2.1
N.O.	11.25	4.9	10.3
J.S.	13.5	1.9	3.4
N.N.	11.25	5.8	12.2
			Average: 6.1
Females			
B.S.	10.3	2.1	4.83
H.E.	11.25	3.4	7.1
S.N.	11.25	0.8	1.7
B.Z.	11.25	1.3	2.7
N.A.	11.25	5.9	12.4
M.T.	11.25	1.7	3.6
J.E.	11.25	0.75	1.6
E.M.	11.25	0.4	0.85
J.S.	11.25	4.0	8.5
S.C.	11.25	0.5	1.07
R.M.	11.25	1.0	2.1
E.C.	11.25	1.0	2.1
M.L.	11.25	3.5	7.5
			Average; 4.3
		Patients with Panhypopituitarism	
B.K.	9.6	5	12.5
E.C.	9.6	3.5	8.5
F.N.	10.5	0.5	1.1
R.R.	11.25	1.7	3.6
			Average: 6.4

controls. Approximate DCA equivalents were determined from the curve obtained with DCA in absolute alcohol.

Normal Subjects

Adults

Thirteen subjects, eight males and five females, have been studied. This group consisted of normal volunteers between the ages of 20 and 35 years, and was composed of university students and members of the hospital laboratory staff. The results are presented in Table XIII.

The sodium-24 excretion of the test animals was 104.2% of the control values, which is not a significant effect. Although there was a greater excretion of radioactive sodium in the animals treated with female urine, the results are not significantly different from those observed in males (at the 2% level of confidence). The normal urine having the greatest activity produced a sodium-retention similar to that obtained with 2.1Y of DCA equivalents.

Children

Six children ranging from 4 to 13 years of age have been studied. The results are presented in Table XIV.

The sodium-24 in the urine of the test animals was 97.1% of that amount excreted by the control animals, which is equivalent in effect to less than 1Y of DCA per aliquot. This result is not significantly different from that observed in normal adults (P is < 0.5). Here again, some of the extracts were found to cause a slight increase in the excretion of urinary sodium-24, relative to the control animals. The greatest sodium-

TABLE XIII

EFFECT OF URINARY EXTRACTS OF NORMAL ADULTS ON THE EXCRETION OF SODIUM-24 IN RATS

Subject	No. of test rats	No. control animals	Urinary Na-24* (% of controls)
		Females	
R.M.	13	15	117.9
R.C.	8	9	128.5
M.I.	8	8	116.5
Y.K.	7	9	98.8
L.S.	6	9	98.0
			Average: 111.9 ± 5.92**
		Males	
R.S.	9	8	92.8
M.S.	7	10	105.8
H.R.	8	8	78.9
E.E.	6	8	98.0
F.J.	6	9	106.1
G.J.	7	5	92.7
W.W.	5	4	104.5
D.K.	5	4	92.6
			Average: 96.4 ± 3.27**
			Overall Average: 102.4% ± 3.61**

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

**Standard Error

TABLE XIV

EFFECT OF URINARY EXTRACTS OF NORMAL CHILDREN ON THE EXCRETION OF SODIUM-24 IN RATS

Name	Age	Sex	No. test rats	No. Control Animals	Urinary Na-24* (% of Controls)
I.L.	4	M	7	8	81.1
I.G.	5	M	7	9	94.6
S.C.	7	F	8	8	99.7
L.C.	9	M	7	8	72.2
J.S.	11	M	8	12	112.8
M.M.	13	M	7	8	122.1
				Average:	97.1 ± 7.68**

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

**Standard Error

retaining effect was obtained in a child of nine. In this case, the sodium-24 excretion of the test animals was 72.2% of that of the control animals, which is equivalent to approximately 2.7γ of DCA.

Discussion

The urinary extracts of the normal adults and children studied in this investigation caused small fluctuations, both positive and negative, in the excretion of sodium-24 in test animals, as compared with the controls. The values obtained in the adult group are similar to those reported by Luetscher and Deming (106) who found that normal subjects and a variety of non-edematous hospital patients excreted from 0 to 2.3γ of DCA equivalents, or an average of 1.4γ per 20-minute aliquot of urine. No other studies on normal adults have been published.

Although there is some evidence that sodium is metabolized differently in children than in adults, particularly with respect to sweat sodium concentrations in infants (87) and salivary sodium concentrations in young children (118), the present studies have not shown this to be associated with a difference in the amount of "sodium-retaining" lipids in the urine in children of 4 to 13 years of age. Whitney, Bennett and Li (120) have suggested that the renal retention of sodium following the administration of growth hormone to rats in their experiments is related to an expansion of the extracellular fluid volume. If growth hormone is secreted in normal children, it may be responsible for the aforementioned tendency to salt retention.

Congestive Heart Failure

Seven patients with congestive heart failure have been studied. These patients have been divided into two groups, depending on their clinical condition at the time of urine collection. One group consists of patients who showed symptoms of severe congestive heart failure, while those in the second group were maintained in a relatively controlled state. The results are listed in Table XV. (Patients were under the care of Drs. J. Wener, L. Horlick and P. Pare).

One patient, A.M., was studied in the severe and controlled states. While in the severe state, he did not respond to the administered diuretics and had gained four pounds in the five days before urine collection was completed. His weight at this time was 172 pounds. Six months later this patient was again investigated. At this time he was in a more controlled state, was feeling much better and weighed 162 pounds. He was no longer on treatment with digitalis. He was, however, receiving a cation exchange resin. On each occasion urine collections were started three days after the last mercurial diuretic injection. Etiology and treatment were similar in the two groups of patients. Two of the severely ill patients were on mercurial diuretic treatment but were responding poorly. A third patient, (H.S.) was no longer on this treatment because of failure of response. The congestive failure in the fourth patient (A.B.) was of recent onset and was complicated by diabetes. All the patients were on a salt-restricted diet, all but one (A.B.) were ambulatory, and all but two were receiving digitalis (A.M. in the controlled state, and L.M.).

The urine of patients in severe congestive failure showed marked

TABLE XV

DCA-LIKE SUBSTANCES IN THE URINE OF PATIENTS WITH CONGESTIVE HEART FAILURE, BY BIO-ASSAY

Subj.	Age	Sex	Etiology	Urine Vol. (patient)	No.days after Diuretic	Other Treatment	Urinary Na-24* (% of controls)	Approx.DCA Equiv. (γ)
					Severe			
H.S.	29	F	RHD	270 ml/dy		digitalis	57.7	
A.B.**	56	F	ASHD	2240		digitalis	65.3	
A.M.	53	M	ASHD	525	3	digitalis	64.1	
A.A.	61	M	HCVD	695	6	digitalis	64.2	
							62.3 ± 1.72***	3.6
					Controlled			
H.S.	60	M	RHD	750	7	digitalis	89.0	
E.V.	53	M	ASHD	915	6	dig. & NH_4Cl	87.6	
L.M.	54	M	HCVD	1480	3	NH_4Cl	77.7	
A.M.	53	M	ASHD	600	3	cation exch. resin.	89.0	
							85.8 ± 2.73***	1.5

*See table XIV. **Diabetic. *** Standard Error

sodium-retaining activity. All the values were greater than those obtained in the normal adults. The average sodium-24 excretion of the test animals was 62.3% of that of the control animals. The difference between these results and those obtained in normal adults is highly significant (P is < 0.001). In terms of approximate DCA equivalents, the sodium retention produced by these extracts was similar to that produced by 3.6γ of DCA.

The four patients who were in mild failure did not show as marked sodium-retaining activity as was noted in the severely congested patients. The average sodium-24 excretion of the test animals in this group was 85.8% of the control values. In terms of approximate DCA equivalents, the sodium retention produced by these extracts was similar to that caused by 1.5γ of DCA. Although these results are not as striking as those noted in the other group of patients with congestive heart failure, the activity obtained in this group was significantly higher than that found in the normal adults (P is < 0.01).

Discussion

The finding of increased sodium-retaining activity in the urine of patients with congestive heart failure, particularly in uncontrolled patients, suggests that the accumulation of fluid in this condition might be related to an excess of circulating salt-retaining hormone (Fig. 7). The present findings are in agreement with those of Deming and Luetscher (51) who found, with a similar method of assay, increased quantities of urinary sodium-retaining substances in five of six patients with uncompensated congestive heart failure.

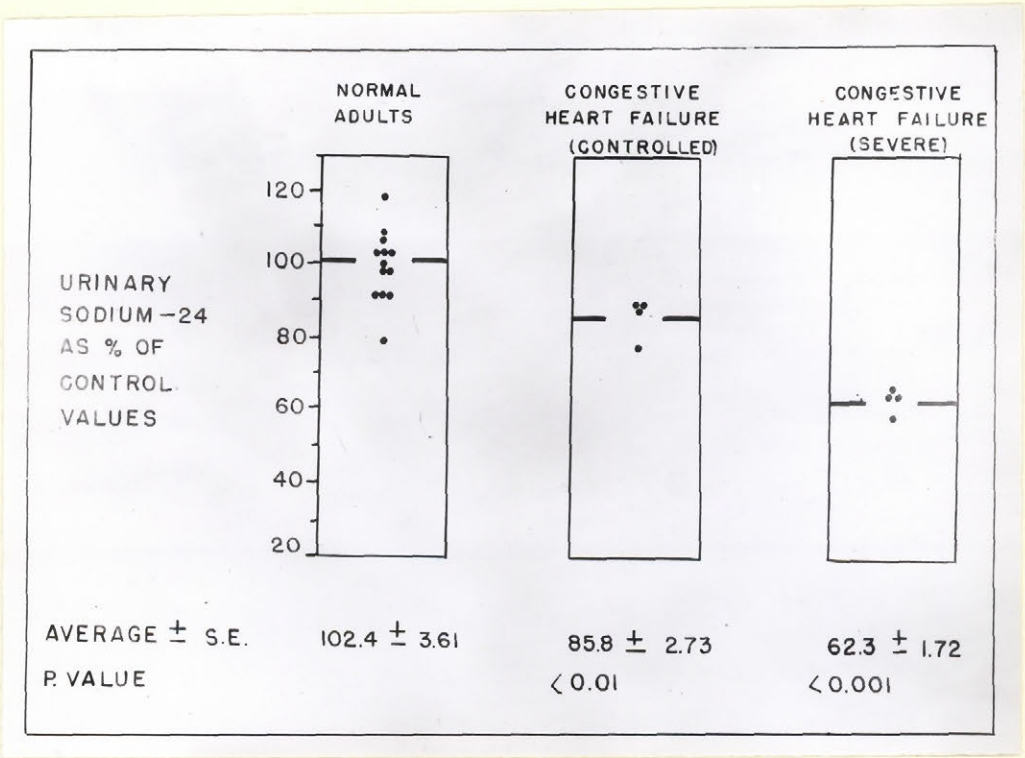

Fig. 7

Effect of urinary extracts obtained from normal individuals and patients with congestive heart failure on the urinary excretion of radiosodium in rats. An extract of a 20-minute sample of urine was used for each rat. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium retention.

Lasche et al. (93) noted "hypoglycemic unresponsiveness" and a decreased fall in eosinophils following a test dose of insulin in this condition, suggesting that there is a decreased ability of the adrenal gland to secrete "glucocorticoids". The 11-oxysteroid excretion was below normal in most of the patients. The finding of increased circulating ACTH levels in this condition by Bornstein and Trewhella (94) suggests that there may be a compensatory oversecretion of this substance on the part of the anterior pituitary. The report that salivary sodium levels are below normal in congestive heart failure (88) lends support to the suggestion that there is a relative oversecretion of "sodium-retaining" corticoids in this condition.

Some reports, however, are not in keeping with this view. Parrish (91) has found increased urinary "glucocorticoids" (biologically active) in these patients, and Reynolds (89) has detected normal sodium concentrations in the sweat. Some of the variations observed may be due to the fact that the patients were studied at different stages of the disease and that some were ambulatory.

The possibility that some of the mercurial diuretic administered to the patients may be present in the urinary extract and may be responsible for the differences noted between the patients with controlled and uncontrolled congestive heart failure seems very unlikely. Grossman et al. (201) have studied the urinary excretion of mercury following the administration of mercurial diuretics to normal individuals and patients with congestive heart failure. About 60 to 95% of the injected mercury was excreted in the urine during the first 24 hours. Mercury continued to

appear in the urine for the next day or two in highly variable amounts. Neither ammonium chloride which enhances, nor DCA which inhibits the diuretic response to mercurials affects the renal excretion of mercury. Thus, it is hardly likely that much mercury would be present in a 20-minute aliquot of urine excretion, since none of the urine collections were started less than 72 hours after the last injection of the diuretic. Whether mercury is excreted in a "diuretic" form and whether it would be extracted from the urine by chloroform, is not known.

Clinically, a reduced glomerular filtration rate has been observed in most patients with congestive heart failure (74,75,76,77). Many investigators assumed that tubular function was normal in this condition but this has recently been questioned by White and his associates (82,83). They were able to show that a reduced glomerular filtration rate in hypophysectomized dogs, supported by DCA therapy, is not necessarily accompanied by electrolyte disturbances. Also, Goldman (79) has recently studied diurnal variations in the output of electrolytes and water in congestive heart failure and found evidence that there is an alteration in renal tubular function in this condition. It would appear, therefore, that reduced salt excretion in congestive heart failure may be influenced not only by ischemia, increased venous pressure and diminished glomerular filtration, but also by increased tubular reabsorption due, possibly, to an increase in circulating salt-retaining corticoids.

It is becoming increasingly evident that congestive failure and the development of edema is a complicated process due to many interacting factors. From recent studies it appears that not only the heart and kidney

but also the antidiuretic factor of the posterior pituitary and the "sodium-retaining" factor of the adrenal cortex may play a role in this condition. The importance of each factor, however, cannot be ascertained with the data available at the present time.

Nephrosis

Eleven nephrotic children, between the ages of 18 months and seven years, have been studied. One patient (J.P.S.) was at the Royal Victoria Hospital, all others were under the care of Dr. F. McCall, of the Children's Memorial Hospital. The results are presented in Table XVI.

These patients have been studied at three different stages: i) while actively accumulating edema fluid, ii) while edematous but not accumulating any more fluid, and iii) during diuresis. Most of the patients were studied in more than one phase.

Of the nine patients who were observed during the period of "accumulation", eight showed marked sodium-retaining activity in their urinary extracts. The ninth patient, D.W., was very ill and excreted very little urine with reduced urinary creatinine values. The results obtained in this group of children have been compared with those obtained in normal children in Fig. 8. It is noted that the sodium excretion of the test animals treated with urinary extracts obtained from the nephrotic children was 61.2% of the control values, as compared with 97.1% obtained from normal children. The difference between the groups is significant (P is < 0.01). The effect of these extracts was equivalent to that obtained with 3.8γ of DCA.

Six determinations have been made in four nephrotic children

TABLE XVI

DCA-LIKE SUBSTANCES IN THE URINE OF PATIENTS WITH THE NEPHROTIC SYNDROME, BY BIO-ASSAY

Subj.	Date	Age	Sex	History	Extent of Edema	Course	Urine Vol. (Ml./day)	Urinary Na-24* (% of controls)	Approx. DCA Equiv.

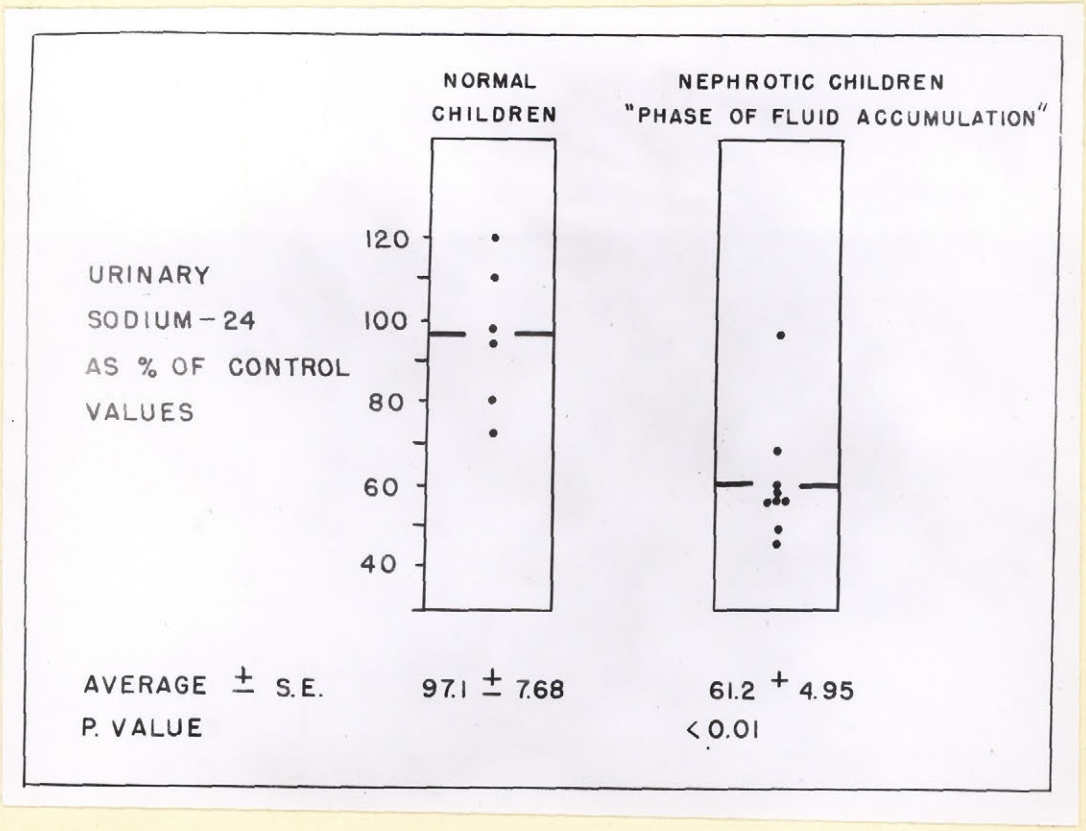

Fig. 8

Effect of urinary extracts obtained from normal children and patients with nephrosis during the "fluid accumulation" phase on the excretion of sodium-24 in rats. An extract of a 20-minute sample was used for each rat. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium-retention.

who were in a "static" state with respect to their edema. These patients apparently had stopped accumulating fluid at different edematous stages. Marked sodium-retaining activity was detected on only one occasion. This patient, A.C., was very edematous and excreted very little urine. His weight had been stationary for some time and continued to remain at the same level for a few days after completion of the urine collection. It is possible, however, that he was actually gaining edema fluid during this period since the losses in weight due to urinary albumin may have been replaced by increases in body water.

The patient J.S. was studied during the "accumulation" phase and in the stationary phase while on intravenous ACTH. His urine output increased while on ACTH partly because his water intake was increased and partly because the patient was no longer accumulating fluid. The marked sodium-retaining effect (radiosodium excretion was 56.2% of control values) observed before ACTH treatment was no longer evident after he had stopped accumulating fluid while on intravenous ACTH therapy.

Three patients, S.L., J.M. and H.McD., were studied during the fluid accumulating phase and during diuresis while still on ACTH or immediately following ACTH therapy. In the patient S.L., the water diuresis was accompanied by an increased salt excretion as well. In the second case (J.M.), a water diuresis, occurring on the 13th day of ACTH, preceded the salt diuresis. The urinary sodium values were not determined in the third case (H.McD.) The urinary sodium-retaining activity in the urine of these three nephrotic patients, before and after diuresis, is presented in Fig. 9. It is seen that the marked sodium-retaining effect observed when

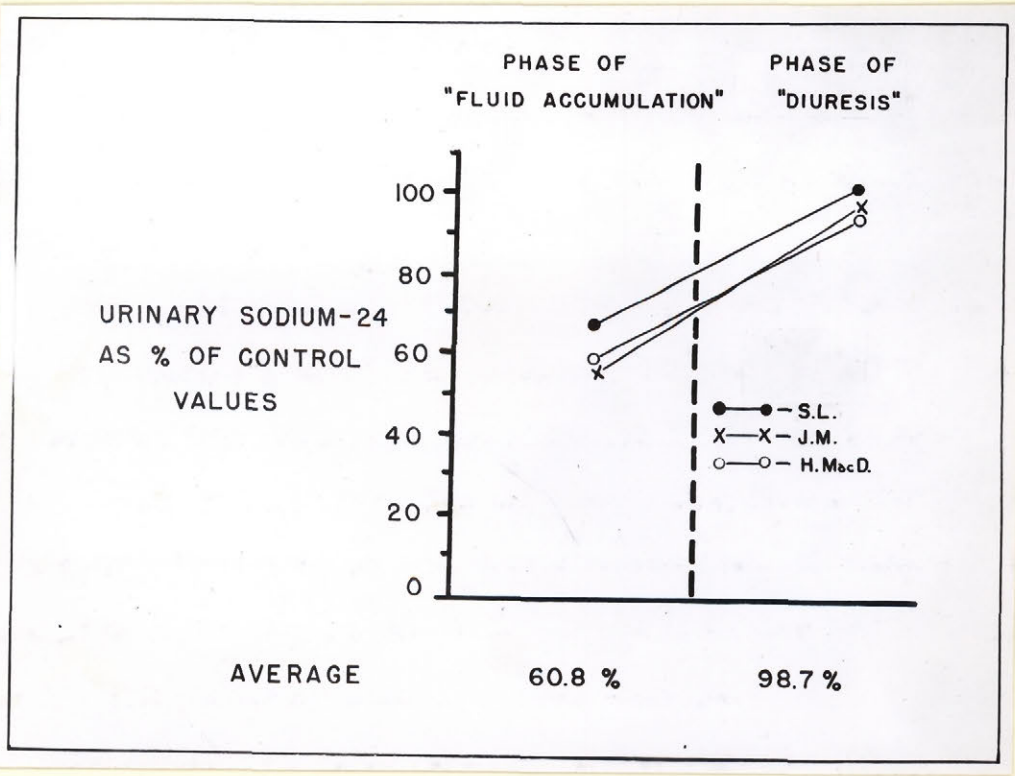

Fig. 9

Sodium-retaining activity in the urine of children with nephrosis, before and after diuresis. An extract of a 20-minute sample was used for each rat. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium retention.

the patients were accumulating fluid (radiosodium excretion was 60.8% of the control values) had decreased to the normal range (98.7% of the control values) during the phase of diuresis.

Discussion

This finding of increased urinary sodium-retaining activity during the phase of edema formation, and disappearance of activity during the static phase or the phase of diuresis, suggests that there is an increased secretion of "salt-retaining" corticoids during the phase of edema formation in the nephrotic syndrome, and adds to the growing body of evidence that adrenal cortical function is in some way related to the salt and water disturbances of this condition. The results are a confirmation of the work of Deming and Luetscher (51,100,106) who, using a similar assay, found increased amounts of DCA-like substances in the urine of most patients with nephrosis. These values fell to normal during a diuresis produced by the administration of ACTH, cortisone or salt-poor serum albumin.

Some of the strongest evidence in support of the theory that disturbed adrenal cortical function may play an important role in the nephrotic syndrome is based on the fact that the administration of ACTH or cortisone frequently results in loss of edema fluid. The frequent remissions following infection with malaria (112,113) and measles (111) have also been attributed to the release of ACTH with subsequent activation of the adrenal cortex. These findings, plus the fact that the urine of the patients contained a high degree of sodium-retaining activity, suggests that the beneficial effects of these substances or conditions are due to a counteraction of "salt-retaining" hormones by "glucocorticoids". This is

further supported by evidence that the urinary "formaldehydogenic" steroids are reduced in this condition (personal communication Dr. F. McCall) although the possibility that this is due to renal retention of the substances has not been eliminated.

Cirrhosis with Ascites

Three patients with cirrhosis and ascites have been studied. The results are presented in Table XVII. Two of these patients (C.C. and E.D.) were under the care of Dr. R. Laing of the Montreal General Hospital, while the third (S.G.) was studied at the Royal Victoria Hospital. Although all the patients had cirrhosis with ascites at the time of urine collection, they were not by any means at the same stage of the disease.

Patient P.J.W. was studied within a few days of admission to hospital while he was still severely ill and before any special treatment had been instituted. Death occurred two months later. The extract of his urine caused a marked sodium retention in the assay, i.e. the sodium excretion of the test animals was 62.7% of that of the control animals.

Patient S.G. had been placed on a low salt, low fat, high caloric diet for two weeks, and was considerably improved at the time of urine collection. The extract of this patient's urine was mildly "sodium-retaining". The sodium-24 excretion of the test animals was 76.1% of the control values, which is just below the lower limit observed in normal adults.

Patient E.D. was studied ten days after admission to hospital while he was on a high protein, low fat diet. He improved continuously in the two and a half weeks he was in hospital and lost ten pounds in this period.

TABLE XVII

EFFECT OF URINARY EXTRACTS OF PATIENTS WITH CIRRHOSIS AND ASCITES ON THE EXCRETION OF SODIUM-24 IN RATS

Subject	Age	Sex	Treatment	Course of Disease	Urine Vol./day (patients)	Urinary Na-24* (% of controls)
P.J.N.	57	M		Died 2 months later	705 cc	62.7
S.G.	57	M	Diet	Improved	960	76.1
E.D.	52	M	Diet	Improved	1360	206.4

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention"

The extract of this patient's urine was markedly "sodium-excreting", i.e. the radiosodium excretion of the test animals was 206.4% of the control values.

Discussion

A recent report by Bongiovanni and Eisenmenger indicated that there was a high degree of sodium-retaining activity in the urine of two of eight patients with Laennec's cirrhosis (138). Urines of the other six patients also showed these effects but their importance could not be determined because of difficulty with controls. These patients had subnormal sodium concentrations in sweat and saliva (137,138) which is regarded by some to be an indication of increased "salt and water" hormonal activity of the adrenal cortex (22,23,24,139,187).

From the three values obtained in the present study, it is apparent that urinary extracts of patients with cirrhosis and ascites may have different effects in the assay. The results are consistent with the view that the period of accumulation of ascites is associated with increased levels of circulating "sodium-retaining" substances, and that the period of improvement is associated with a relative increase in the level of circulating sodium-excreting substances.

Although further study is indicated, the finding of a marked sodium-retaining effect in the urine of a patient who was accumulating ascitic fluid suggests that a salt-retaining substance, possibly of adrenal cortical origin, may be a factor contributing to the retention of salt and water in cirrhosis. How this is related to such factors as the Presence of excessive quantities of antidiuretic hormones, the portal

hypertension and hypoproteinemia observed in this condition remains to be determined.

Essential Hypertension

Four patients, three females and one male, with "essential hypertension" have been studied. The results are presented in Table XVIII. (The patients were under the care of Dr. J. Wener).

Two of the patients, B.B. and A.P., were receiving treatment with an ion exchange resin and the other two were untreated except for a salt-restricted diet. The values obtained with the urinary extracts of this group of patients ranged from 87.2 to 107.0% of the control values, with an average of 95.6%. The effect of these extracts on the sodium-24 excretion of test animals was similar to that obtained with extracts of urine from normal individuals. In terms of the effect of DCA, the sodium-retention produced by these extracts was equivalent to less than 1Y.

Discussion

Although there is considerable evidence that the adrenal cortex may play a role in the development of experimental hypertension in animals (148), the etiology of "essential hypertension" in man is not known. Some evidence exists for a slight retention of sodium in man (150) and in animals (202) but this has not been found by all investigators. Eisenberg et al. (186) found normal sweat electrolyte concentrations in 35 patients with "essential hypertension" and Stamler and co-workers (203) have observed no disturbance in the pattern of strong electrolyte excretion in Goldblatt hypertensive dogs. Urinary chemical corticoid studies in

TABLE XVIII

EFFECT OF URINARY EXTRACTS OF PATIENTS WITH ESSENTIAL HYPERTENSION ON THE EXCRETION OF SODIUM-24 IN RATS

Subj.	Sex	Age	No.test rats	Urinary Na-24* (% of controls)	No. Control Animals
B.B.	F	46	8	107.0	7
A.P.	F	65	8	93.9	7
R.A.G.	M	51	6	94.2	8
G.M.	F	50	6	87.2	8
				Average: 95.6%	

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

"essential hypertension" have indicated that they may be either elevated (153) or"normal" (152,154). This type of corticoid determination does not, however, differentiate between the "sodium-retaining" or other corticoids which may be secreted by the adrenal gland.

The results of the present study do not suggest that there is a relative increase in the excretion of "salt-retaining" lipids in "essential hypertension". Although two of the patients were on ion exchange therapy, it is not believed that this affected the assay because the resin is not absorbed from the gut. The values obtained with the urinary extracts of these patients were similar to those noted in the untreated patients. The fact that the studies were performed on "crude lipid" extracts at only one dosage level introduces some difficulty in the interpretation of the negative findings noted here. Studies of the dosage-response curves of two extracts of nephrotic urine have indicated that interfering substances may be present at the 20- or 30-minute dosage levels. As similar studies have not been performed in this condition, the possibility that interfering substances may have masked the activity of these extracts has not been ruled out. The suggestion that interfering substances may be present in these extracts is supported, in this instance, by the theory of Corcoran et al. (204) which postulates that experimental renal hypertension is associated with excesses of both renin which is "sodium-excreting", and DCA-like corticoids which are "sodium-retaining". If this theory is applicable to "essential hypertension" in humans, and if renin is present in the urinary extracts of these patients, the two effects may cancel each other out, explaining the findings of the present study.

Before conclusions on this subject can be reached, a more detailed study should be carried out at different stages of the disease using various time-dosages other than the 20-minute level and more highly purified urinary extracts.

Acute Rheumatic Fever

Eleven cases of rheumatic fever in children of 4.5 to 14 years have been studied. The results are presented in Table XIX.

Although a diagnosis of acute rheumatic fever was made in every case, there was considerable variation in the clinical state of these patients. The disease has been characterized as very mild, mild, moderately severe and severe on the basis of the severity of symptoms. Temperatures taken at the time of urine collection did not always parallel the severity of the condition of the patient. The results show that there is a wide variation in the effect of these urinary extracts on the excretion of sodium-24 in test animals. The values range from 55.4% to 110.7% of the control values, with a mean of 82.4%. Although the mean value is lower than that found in normal children, the difference is not significant (P is <0.2). The variations in results cannot be correlated with the severity of the rheumatic condition or degree of fever of the patient at the time of study, nor do the results appear to have any direct relation to the urine volumes.

Discussion

The predominantly negative results in this group of patients (Fig. 10) are difficult to interpret because of the finding that the urinary

TABLE XIX

EFFECT OF URINARY EXTRACTS OF PATIENTS WITH ACUTE RHEUMATIC FEVER ON THE EXCRETION OF SODIUM-24 IN RATS

Subject	Age	Sex	Condition (Rheumatic)	Temp.	Urine Vol. (patient)	Urinary Na-24* (% of controls)
G.R.	4.5	M	severe	100-101°	450 ml/dy	55.4
A.B.	10	M	mild	no fever	468	63.1
C.C.	10	M	mod.severe	103°	825	70.0
D.M.	10	F	mild		534	73.4
A.L.	10	M	mod.severe	103°	750	77.8
E.R.	7	F	mild	no fever	950	78.6
A.B.	13	M	mod.severe	100°	480	81.0
E.C.	11	M	very mild	103°	592	84.7
R.T.	11	M	mod.severe	103°	4110	102.6
J.P.Y.	10	M	severe	101°	770	109.6
D.L.	14	M	severe	103°	650	110.7
						82.4 ± 5.49**

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".
**Standard Error.

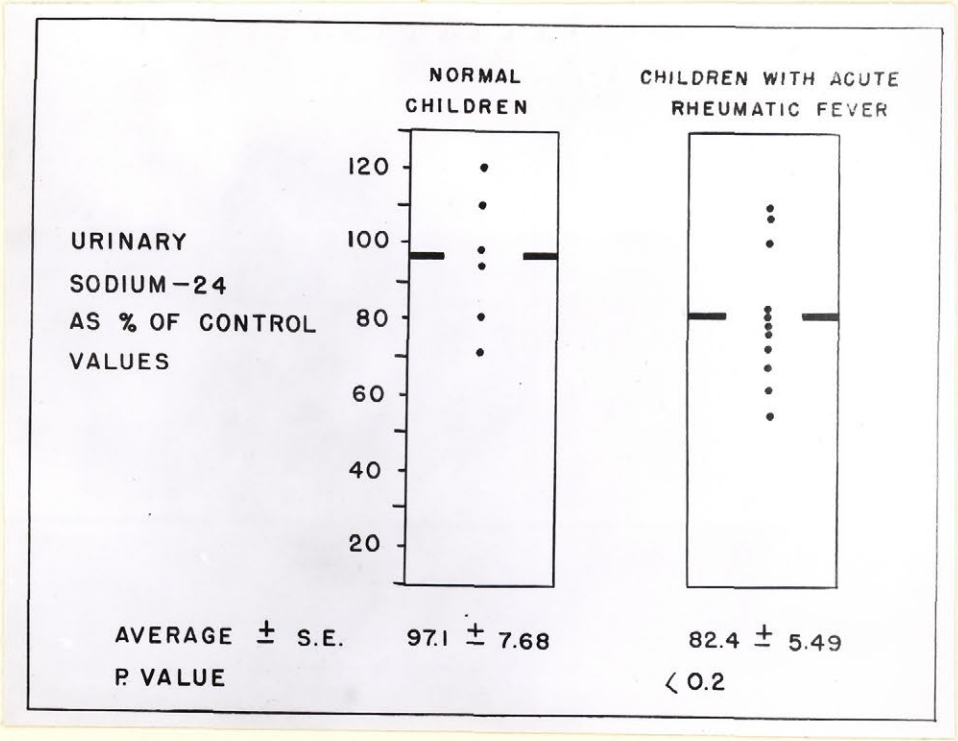

Fig. 10

Effect of urinary extracts obtained from normal children and children with acute rheumatic fever on the excretion of sodium-24 in rats. An extract of a 20-minute sample was used for each rat. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium retention.

"formaldehydogenic corticoids" are frequently elevated in rheumatic fever patients who are febrile (personal communication Dr. F. McCall, Children's Memorial Hospital). If these "corticoids" are predominantly of the "glucocorticoid" type there is evidence, from studies described elsewhere in this report, that they may mask the effects of sodium-retaining substances. Thus, a patient with rheumatic fever with elevated temperature may have increased amounts of both types of corticoids in his urine, resulting in a negative effect in the assay. The relative excess of "salt-retaining" corticoid postulated in this condition may, if present, be detectable only at lower time-dosages such as 5 or 10 minutes, in which the concentration of interfering substances is too low to have a masking effect in the assay.

Post-Operative State

Six patients with gastric ulcer have been studied during the first eight days following gastrectomy. Random assays from the fifth to the eighth days were performed on five patients, while a sixth was studied before operation and on five occasions during the first eight post-operative days. The results are listed in Table XX.

Of the five patients studied at random, the urinary extracts of two showed marked sodium-retaining effects on the sixth post-operative day. The sodium-24 excretion of the test animals was, respectively, 43.8 and 60.4% of the control values. In the sixth patient, the greatest sodium-retaining effect was observed on the seventh post-operative day. In this instance, the radiosodium excretion of the treated animals was 59.5% of that of the control animals. The results obtained in this patient are

TABLE XX

DCA-LIKE SUBSTANCES IN THE URINE OF PATIENTS FOLLOWING GASTRECTOMY, BY BIO-ASSAY

No.	Subj.	Sex.	Day	Urine Vol. (patient)	Urinary Na (patient)	Urinary Na-24* (% of controls)	Approx. DCA Equiv. (γ)
1	E.K.	M	5th post-op.	795 ml/dy	1.89 gm/dy	110.4	
2	J.T.	M	" "	1010	2.27	126.0	
3	R.M.	M	6th "	770		100.0	
4	B.McB.	M	" "	605		43.8	6.0
5	L.O'H.	M	" "	893	0.05	60.4	4.0
	"		8th "	720	0.24	116.7	
6	G.J.	M	1st pre-op.	1800	2.92	92.7	0.6
	"		2 & 3 post-op.	1996	0.21	117.5	
	"		5th "	625	0.53	91.8	0.8
	"		6th "	920	1.55	76.5	2.4
	"		7th "	850	2.02	59.5	4.1
	"		8th "	1140	3.32	73.8	2.6

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

illustrated in Fig. 11. On the first pre-operative day, the sodium-retaining effect was within the range obtained in normal individuals. On the second and third post-operative days, the extract produced a mild increase in the urinary sodium-24 excretion. By the fifth day the "sodium-excreting" effect was no longer present; on the sixth day a mild sodium-retention was observed; on the seventh day the sodium-retention was quite marked and on the eighth post-operative day the "sodium-retaining" effect appeared to be wearing off.

The levels of urinary sodium in the urine of these patients did not always correlate with the "sodium-retaining" effects of the urinary extracts. However, the dietary sodium of these patients could not be controlled in the post-operative period.

Discussion

The "sodium-retaining" activity which was demonstrated on the sixth and seventh post-operative days in three patients of this group is in keeping with the findings of Johnson, Conn and Iob (165) who showed that the most marked decrease in sweat sodium concentration occurs between the fifth and eighth post-operative days. The increase in "glucocorticoid" excretion which has been demonstrated following operational stress (163,164) may be responsible for the delay in the appearance of these substances in both studies. The final appearance of the effect may be related to a decrease in the secretion of "glucocorticoids" from the very high levels of the immediate post-operative period. This is indicated by the return of the circulating eosinophils to normal levels in the patients studied by Johnson and his associates.

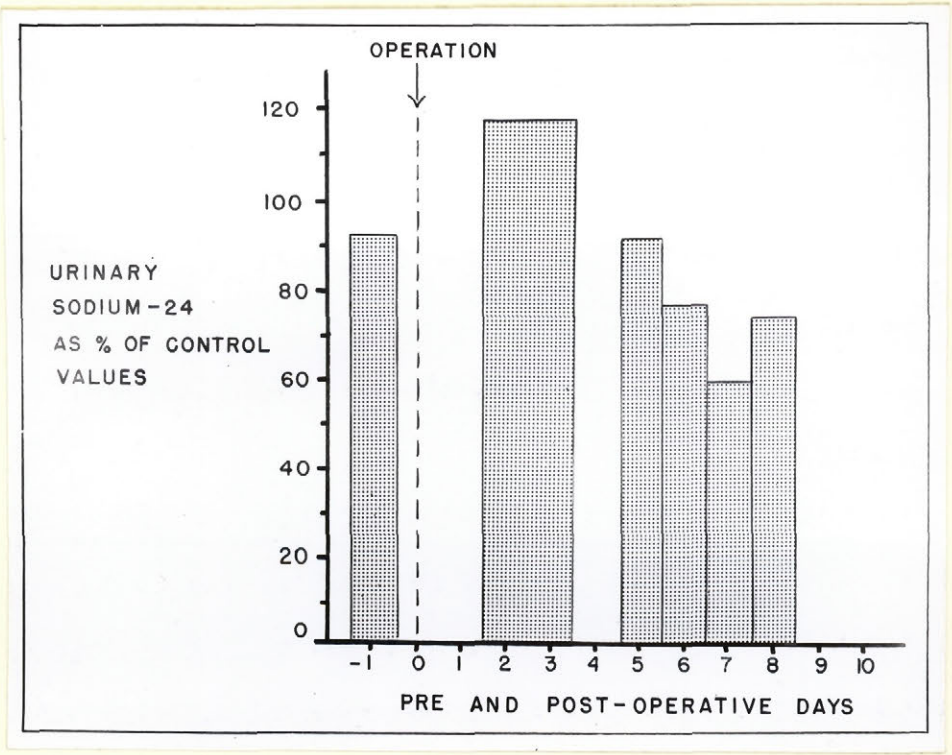

Fig. 11

Effect of urinary extracts obtained following gastrectomy on the excretion of radiosodium in rats. An extract of a 20-minute sample of urine was used for each rat. Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate sodium retention.

The present findings suggest that following the stress of surgery in man there is not only an increase in the secretion of "glucocorticoids" but also an increase in the secretion of "salt-retaining" substances.

Late Pregnancy

Toxemia of Late Pregnancy

Five patients with toxemia of pregnancy have been studied. Urine was collected from these patients as soon as possible after admission to hospital, and while symptoms were still present. The results are listed in Table XXI.

Albuminuria, edema and hypertension were present in all cases with the exception of A.P., who had no visible edema but was admitted in convulsions. The patient F.F. had been suffering from diabetes for a number of years and had shown signs of toxemia in a previous pregnancy. Each of the patients was in her eighth or ninth month of pregnancy. All the urinary extracts obtained from these patients showed a marked "sodium-retaining" activity in comparison with normal women. The average sodium-24 excretion of the test animals of this group was 61.7% of the control values, which is equivalent in effect to 3.8γ of DCA.

The findings on one patient where the diagnosis of toxemia of pregnancy was questionable have been included at the bottom of the same table. This woman (M.Mu.) had ankle edema from the onset of pregnancy, a symptom not generally found in patients with true toxemia of pregnancy. The results on this patient differ considerably from those noted in the patients with toxemia of pregnancy but are, in fact, similar to those noted

TABLE XXI

DCA-LIKE SUBSTANCES IN THE URINE OF PATIENTS WITH TOXEMIA OF PREGNANCY, BY BIO-ASSAY

Subject	Symptoms	Urine Vol. (patient)	Urinary Na-24* (% of controls)	Approx. DCA Equiv.(Y)
F.F.	Diabetes,Edema,4+ Albumin, Hypertension.	370 ml/dy	52.5	4.7
M.L.	Edema,Urine Albumin, Hypertension.	785	64.1	3.6
A.P.	Convulsions,3+ Urine Albumin, Hypertension.	750	66.4	3.3
M.M.	Edema,3+ Urine Albumin, Hypertension, Jaundice	650	56.3	4.3
S.B.	Hypertension,Urine Albumin, Edema	720	69.2	3.1
			61.7 ± 3.16**	3.8
	Questionable Toxemia of Pregnancy			
M.Mu.	Ankle Edema since onset of Pregnancy, Nausea.	520	114.0	

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".
**Standard Error.

with urinary extracts obtained from normal non-pregnant women. This patient was included because she is typical of the vast majority of patients who were admitted to hospital for investigation of possible toxemia of pregnancy. Very few of them have more than one, or at most two, of the symptoms of toxemia and frequently these are mild and transitory.

Normal Late Pregnancy

Four normal women, in their eighth and ninth months of pregnancy, have been studied. The results are presented in Table XXII.

None of the urinary extracts obtained from these patients had a "sodium-retaining" effect in the assay. The average sodium-24 excretion of the test animals was 98.9% of the control values. Since the urinary extracts obtained from non-pregnant women produced a "sodium-excreting" effect in the assay (test animals excreted 111.9% as much sodium-24 as the control animals), the effect of the extracts obtained from pregnant women is relatively "sodium-retaining". However, the difference between the groups is not significant (P is < 0.1).

Discussion

A comparison of the effects of extracts of urine obtained from normal women, normal pregnant women and women with toxemia of pregnancy,on the excretion of sodium-24 in rats,reveals that the urinary extracts (Fig.12) obtained from normal pregnant women tend to cause a mild "sodium-retention" in the assay, and that this is greatly exaggerated in toxemia of pregnancy. This confirms the work of Chart et al. (171) who noted, with a similar method of assay, a marked increase in the excretion of a "sodium-retaining" factor in toxemic pregnancies. Bush et al. (205) have recently reported

TABLE XXII

EFFECT OF URINARY EXTRACTS OF NORMAL PREGNANT WOMEN ON THE EXCRETION OF SODIUM-24 IN RATS

Date of Study	Date of Delivery	Subj.	Urine Vol. (patient)	Urinary Na-24* (% of controls)
Feb. 5/52	Mar.5/52	R.R.	1920 ml/dy	91.6
Feb.28	Apr.7	R.B.	1100	110.0
Mar. 3	Mar.5	R.M.	665	96.9
Mar. 6	Mar.9	S.K.	520	97.1
				98.9 ± 3.92**

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

**Standard Error.

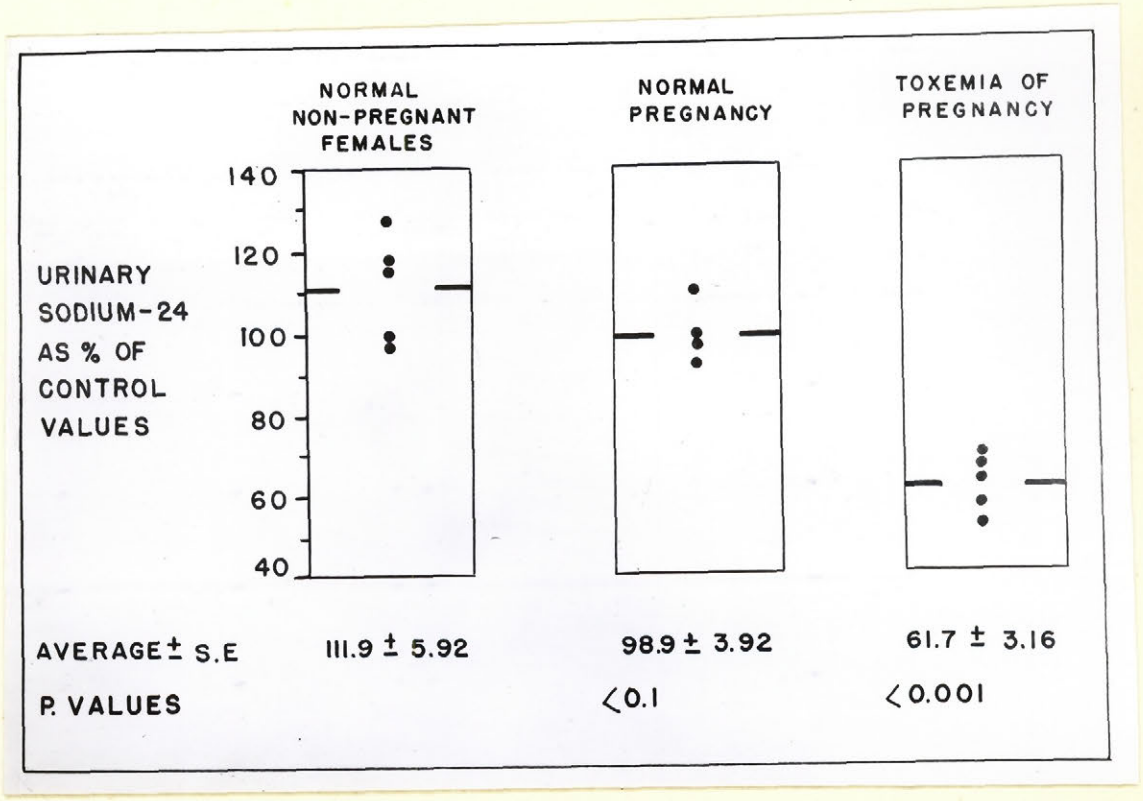

Fig. 12

Sodium-retaining activity in the urinary extracts of patients with toxemia of pregnancy: a comparison with non-pregnant women and normal pregnant women. An extract of a 20-minute sample of urine was used for each rat. Sodium-24 excretion of the control animals was considered as 100%. Values under 100% indicate sodium retention.

the presence of two unidentified compounds in extracts of pregnancy urine which appear to be identical with two compounds present in the "amorphous fraction" of adrenal extracts. This is of particular interest because the amorphous fraction is most active with respect to sodium retention. It would be of great value to know whether the output of these compounds is increased in patients with toxemia of pregnancy.

Studies of protein and carbohydrate metabolism have led Mukherjee, Govan et al. (179,180) to believe that there may be an increase in "corticotrophic" hormone and, possibly, growth hormone in toxemic pregnancies. These conclusions were based on the finding of a reduced sensitivity to insulin and a relatively reduced nitrogen balance which could not be accounted for solely on the basis of the albuminuria. It is possible that the increased excretion of salt-retaining substances noted in the present study and in that of Chart and his associates may be related to a disturbance in pituitary function with respect to ACTH and/or growth hormone secretion.

Acromegaly, and Effect of Growth Hormone

Acromegaly

Four patients with acromegaly, two men and two women, have been studied. The results are presented in Table XXIII.

A high degree of sodium-retaining activity has been observed in the extracts of three of the four patients. The average sodium-24 excretion of the test animals was 68.4% of that of the control animals. One patient, J.G., was on estrogen therapy (3 mgm. of stilbestrol per day) at the time

TABLE XXIII

EFFECT OF URINARY EXTRACTS OF PATIENTS WITH ACROMEGALY ON THE EXCRETION OF SODIUM-24 IN RATS

Subject	Age	Sex	No.of rats	No.Control Animals	Urinary Na-24* (% of controls)
E.V.	44	M	5	5	60.6**
R.B.	33	M	5	7	49.8**
J.G.	33	F	8	9	67.9
M.O.	44	F	7	11	95.3
				Average:	68.4

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

**Stored in "dry" state for two or three days

of study. It is unlikely that this treatment was responsible for the effect observed with this patient's urinary extract, since the excretion of estrogenic material following the administration of stilbestrol is not significantly increased (unpublished work - McGill University Clinic).

Effect of Growth Hormone

The effect of the intravenous injection of growth hormone on the activity of urinary extracts of two normal male subjects has been studied. The growth hormone experiments were part of a larger study conducted by Drs. Carballeira, Elrick et al. (in press), at the McGill University Clinic. The results are presented in Table XXIV.

Pretreatment values were obtained with urine collected two days before hormone therapy. Post-treatment values were obtained with urine excreted in the first six hours following the intravenous injection of 2 mg./kilo (D.K.) or 1 mg./kilo (W.W.). In both cases, after administration of growth hormone, there was a marked sodium-retaining effect in the urinary extracts which was not present before treatment. The effect of 1 mg./kilo was as great as that noted with 2 mg./kilo. The sodium-24 excretion of the test animals following the administration of growth hormone was 62.5% of the control value in one case (D.K.), and 57.0% in the other (W.W.). Before treatment, values of 92.6% and 104.5%, respectively, had been obtained with the urinary extracts of these subjects.

Discussion

It is thought that the increase in urinary sodium-retaining lipids observed in these experiments actually resulted from the treatment with growth hormone. This assumption is supported by the fact that the growth

TABLE XXIV

EFFECT OF GROWTH HORMONE ON THE URINARY EXCRETION OF SODIUM-RETAINING MATERIAL IN NORMAL ADULT MALES

Expt.	Subject	Treatment	No. test rats	No. Control Animals	Urinary Na-24* (% of Controls)
46 a	D.K.	Pretreatment	5	4	92.6
45 d	"	2 mg/kilo Growth Hormone	7	8	62.5
46 a	W.W.	Pretreatment	5	4	104.5
45 c	"	1 mg/kilo Growth Hormone	6	6	57.0

*Sodium-24 excretion of control animals was considered as 100%. Values under 100% indicate "sodium retention".

hormone used in these experiments was relatively free of ACTH and other impurities, and that neither of the two subjects showed a fall in circulating eosinophils following its administration. The detection of "sodium-retaining" activity in the urine of three of four patients with acromegaly may be taken as additional evidence for this view, if it is agreed that this condition is associated with an increased secretion of growth hormone.

These results are of interest in view of recent studies on growth hormone by several groups of investigators. Stack-Dunne and Young (184) have found an "adrenal-weight-stimulating factor" in growth hormone and ACTH preparations. The adrenal-weight-stimulating effect of growth hormone preparations has also been observed by Selye (182) who postulated that this substance may stimulate the adrenal cortex to secrete "mineralocorticoids" or may sensitize the body to the action of these substances. Whitney, Bennett and Li (120) have noted that growth hormone produces a retention of sodium as well as of potassium in normal female rats. They believe that this is related to an expansion of the extracellular fluid space.

The present findings, plus those of others mentioned here, suggest that growth hormone administration or secretion may, in some way, result in the increased release of "sodium-retaining" substances from the adrenal cortex. Further work on this point is indicated.

GENERAL DISCUSSION

Results of the present investigation show that the excretion of "sodium-retaining" substance was above "normal" in a number of patients with congestive heart failure, nephrosis, cirrhosis and toxemia of pregnancy while they were accumulating edema or ascitic fluid. This would suggest that an increased secretion of this substance may actually be directly related to the accumulation of fluid observed in these patients.

Studies of the solubility properties of the active material which was extracted from acidified urine with chloroform indicate that it is a lipid which is relatively insoluble in dilute alkali. Although these properties are characteristic of many steroid hormones, it is not known whether the active substance is, in fact, related in structure to these compounds. The development of newer methods for the detection of steroids, such as adrenal-gland-perfusion and paper partition chromatography indicate that the predominant secretory products of the adrenal cortex are 17-hydroxy-corticosterone and cortisone (29,46,47,205) which do not have marked "sodium-retaining" effects. Small quantities of substances with many of the chemical properties of DOC and 17-hydroxy-11-desoxycorticosterone have been detected in beef-adrenal extracts (31), beef-adrenal-gland perfusates (29), pooled normal human urine treated with β-glucoronidase (32, p. 506), and "quick-frozen" hog adrenal glands (29, p. 241). However, it is not known whether they were present in sufficient quantity to be of physiological importance. Bush and his associates were unable to detect either of these compounds, but have found two steroids in dog adrenal-vein blood and pregnancy urine whose Rf values corresponded with two similar compounds in the "amorphous" fraction which is highly active with respect

to sodium-retention (47,205). It is possible that one of these compounds may be similar to a highly active "mineralocorticoid" recently separated from adrenal extract by Grundy et al. (39) using paper chromatographic procedures. This substance, or a similar one, has also been detected in extracts of adrenal venous blood taken from a monkey and a dog (206). Studies should be carried out to determine whether the activity observed in the present study was due to the effect of one of these substances.

Although the urinary extracts obtained from normal individuals did not show a significant sodium-retaining effect in the present assay, a high degree of activity was found in the urine of normal subjects following the intravenous administration of growth hormone. This effect of growth hormone suggests that the excretion of an increased amount of "sodium-retaining" substances in some conditions may be related to an oversecretion of growth hormone. This is further supported by the fact that sodium-retaining activity was observed in the urine of three of four patients with acromegaly. Unfortunately, little is known about the mechanism of the release of this hormone or about its presence in the circulation in man. Although it has never been demonstrated, it is possible that the secretion of growth hormone may, in some way, result from a relative decrease in "anabolic" functions within the body. If this is so, then there are certain disturbances in the aforementioned conditions which may act as stimuli for the release of growth hormone. These include the negative nitrogen balance following operational stress, the albuminuria of nephrosis, the hypoalbuminemia of cirrhosis and the "relatively" negative nitrogen balance (179) in toxemia of pregnancy.

Although an increased excretion of the "salt-retaining" factor was not detected in "essential hypertension" and in most cases of acute rheumatic fever, the possibility exists that this was due to an interfering substance in the extract. Dosage curves of several urinary extracts prepared from nephrotic patients have indicated that the dosage level at which interferences are detectable may be different for each extract. In view of this, and until more highly purified extracts can be investigated, it would be advisable to study various time-dosages in those cases where activity was not detected at the level used for routine assays, i.e. 20 minutes.

SUMMARY AND CONCLUSIONS

A method for the biological assay of small quantities of sodium-retaining substances in human urine has been developed. It is based on the excretion of a dose of sodium-24, within a five-hour period, by adrenalectomized rats kept under standard conditions.

DCA was found to be sodium-retaining between the dosages of 1 to 20γ per rat. A linear relation was noted between the log of the dosage and the urinary sodium-24 excretion expressed as the ratio of test to control animals studied at the same time. When results were expressed in this manner variation from test to test was reduced to a minimum. With small groups of animals (circa 6 to 10 per group) significant sodium-retention occurred between the 2 and 5γ dosage levels.

Cortisone caused mild to marked sodium-excreting effects on some but not all occasions between the dosage range of 1 to 52.5γ per rat.

Urinary extracts were prepared by extraction with chloroform. The urine was acidified to pH 1.5 before extraction. Storage of the extracts before assay in the "dry" state, at room temperature, frequently resulted in losses of "sodium-retaining" activity with time. Refrigeration of the extracts in absolute alcohol in sealed ampoules prevented these losses in activity for at least six weeks, the longest period studied.

An extract of a 20-minute urine sample was used for each rat in routine assays. Assays of various time-dosages of two extracts of urine obtained from patients with nephrosis gave maximum sodium-retaining effects at the ten and twenty-minute dosage levels in one case and the twenty-minute level in the other. Higher dosage levels were associated

with reduced effects in both cases. The possibility exists that cortisone may act as one of the interfering substances in these assays.

Studies on the effect of alkali washing on the activity of the urinary extracts of two nephrotic patients indicated that the active material was relatively insoluble in dilute-alkali.

The urinary extracts of thirteen normal adults, eight males and five females, caused mild fluctuations, both positive and negative, in the excretion of sodium-24 of test animals. The sodium-24 excretion of the test animals was 104.2% of that amount excreted by the control animals. The difference between males and females was not significant although the urinary extracts of the females had a greater "sodium-excreting" effect than those of the males. Significant quantities of "sodium-retaining" material were not detected in the urine of six normal children, ranging in age from 4 to 13 years. The test animals treated with extracts prepared from the urine of these subjects excreted 97.1% as much sodium-24 as the control animals.

Eight assays have been performed on urinary extracts obtained from seven patients with congestive heart failure. Marked "sodium-retaining" effects were noted in the "controlled" and "uncontrolled" patients. The sodium-24 excretion of the test animals was 85.8% and 62.3% of the control values, respectively. Both values were significantly different from those obtained with urine of normal adults. In terms of DCA equivalents these extracts had an average effect equivalent to 1.5 and 3.6Y respectively.

Eleven nephrotic children, from eighteen months to seven years old, have been studied. Eight of nine patients who were retaining fluid

at the time of study had marked "sodium-retaining" activity in their urinary extracts. The test animals treated with these extracts excreted 61.2% as much urinary sodium-24 as the control animals. This value is significantly different from that obtained with extracts of urine of normal children, and is equivalent to that obtained with 3.8γ of DCA. Six determinations were performed on the urinary extracts of four patients who were edematous but static with respect to their edema. Marked sodium-retaining activity was detected only in one instance. The activity detected in the urine of three patients before therapy was no longer evident when the patients were studied during diuresis following ACTH therapy.

Three patients with cirrhosis and ascites were studied. A marked sodium-retaining effect was obtained with the urinary extract of the one patient of this group who was accumulating fluid at the time of study. A mild sodium-retaining effect and a marked sodium-excreting effect was obtained with the urinary extracts of two other patients who were studied during a period of improvement with respect to ascites.

Extracts of urine from four patients with essential hypertension did not have a different effect on the excretion of sodium-24 in rats from that noted with urinary extracts obtained from normal individuals.

Three of eleven children with acute rheumatic fever had marked "sodium-retaining" activity in their urinary extracts, while the others showed values within the normal range.

A high degree of "sodium-retaining" activity was noted in the extracts of urine of three patients on the sixth or seventh day following

gastrectomy. In three other patients, assays carried out on the fifth and sixth post-operative days did not show any sodium-retaining activity.

The urinary extracts obtained from four normal women in their eighth and ninth months of pregnancy were slightly, though not significantly more "sodium-retaining" than those of normal non-pregnant women. The urinary extracts of five patients with toxemia of pregnancy contained substances which were very active in their effect on the urinary sodium-24 excretion of test animals. The quantity of urinary sodium-24 in these animals was 61.7% of the control values, and was equivalent to that observed with 3.8γ of DCA.

In two normal adults there was a marked increase in the excretion of "sodium-retaining" substance following the intravenous administration of 1 or 2 mg./kilo of growth hormone. The urinary sodium-24 excretion of the test animals fell from 104.5% of the control values, before treatment, to 57.0% and from 92.6% to 62.5% of the control values, respectively.

BIBLIOGRAPHY

1. Baumann, E.J. and Kurland, S. J. Biol. Chem., 71; 281; 1927.

2. Marine, D. and Baumann, E.J. Am. J. Physiol., 81; 86; 1927.

3. Swingle, W.W. and Remington, J.W. Physiol. Rev., 24; 89; 1944.

4. Kendall, E.C. Vitamins and Hormones, 6; 277; 1948.

5. Sayers, G. Physiol. Rev., 30; 241; 1950.

6. Loeb, R.F. Science, 76; 420; 1932.

7. Loeb, R.F. Proc. Soc. Exper. Biol. & Med., 30; 808; 1933.

8. Harrop, G.A., Soffer, L.J., Ellsworth, R. and Trescher, J.H. J. Exper. Med., 58; 17; 1933.

9. Harrison, H.E. and Darrow, D. Am. J. Physiol., 125; 631; 1939.

10. Anderson, E. and Joseph, M. Proc. Soc. Exper. Biol. & Med., 40; 347; 1939.

11. Anderson, E., Joseph, M. and Herring, V. Proc. Soc. Exper. Biol. & Med., 44; 482; 1940.

12. Anderson, E., Joseph, M. and Herring, V. Proc. Soc. Exper. Biol. & Med., 42; 782; 1939.

13. Stern, T.N., Cole, W.V., Bass, A.C. and Overman, R.R. Am. J. Physiol., 2; 437; 1951.

14. Swingle, W.W., Parkins, W.M., Taylor, A.R. and Hays, H.W. Am. J. Physiol., 119; 557; 1937.

15. Hegnauer, A.H. and Robinson, E.J. J. Biol. Chem., 116; 769; 1936.

16. Harrison, H.E., Darrow, D.C. and Yannet, H. J. Biol. Chem., 113; 515; 1936.

17. Steiger, M. and Reichstein, T. Helv. Chim. Acta., 20; 1164; 1937.

18. Reichstein, T. and von Euw, J. Helv. Chim. Acta., 21; 1197; 1938.

19. Ryan, E.J. and McCullagh, E.P. Cleveland Clinic Quart., 7; 19; 1940.

20. Kuhlmann, D., Ragan, C., Ferrebee, J.W., Atchley, D.W. and Loeb, R.F. Science, 90; 496; 1939.

21. Ragan, C., Ferrebee, J.W., Phyfe, P., Atchley, D.W. and Loeb, R.F. Am. J. Physiol., 131; 73; 1940.

22. Conn, J.W. Arch. Int. Med., 83; 416; 1949.

23. Conn, J.W. and Louis, L.H. J. Clin. Endocrinol., 10; 12; 1950.

24. Frawley, T.F. and Forsham, P.H. Paper #59, 33rd meeting Assocn. for the Study of Internal Secretions, June 1951.

25. Berger, E.Y., Quinn, G.P. and Homer, M.A. Proc. Soc. Exper. Biol. & Med., 76; 601; 1951.

26. Seifter, J., Baeder, D.H. and Dervinis, A. Proc. Soc. Exper. Biol. & Med., 72; 136; 1949.

27. Adrenal Cortex Conference, Josiah Macy Jr. Foundation, 1950. E. P. Ralli, Editor.

28. Davis, A.K., Bass, A.C. and Overman, R.R. Am. J. Physiol., 166; 493; (Sept.) 1951.

29. Hechter, O., Zaffaroni, A., Jacobsen, R.P., Levy, H., Jeanloz, R.W., Schenker, V. and Pincus, G. Recent Progress in Hormone Research, 6; 215; 1951.

30. Hechter, O. Federation Proc., 9; 58; 1950.

31. Zaffaroni, A. and Burton, R.B. J. Biol. Chem., 193; 749; 1951.

32. Burton, R. Recent Progress in Hormone Research, 7; 469; 1952.

33. Burton, R.B., Zaffaroni, A. and Keutmann, E.H. J. Biol. Chem., 193; 769; 1951.

34. Hartman, F.A. and Spoor, H.J. Endocrinology, 26; 871; 1940.

35. Hartman, F.A. and Lewis, L.A. Endocrinology, 29; 111; 1941.

36. Thatcher, J.S. and Hartman, F.A. Arch. Biochem., 10; 195; 1946.

37. Wells, B.B. and Kendall, E.C. Proc. Staff Meet. Mayo Clinic, 15; 133; 1940.

38. Thorn, G.W., Engel, L.L. and Lewis, R.A. Science, 94; 348; 1941.

39. Grundy, H., Simpson, S.A., Tait, J.F. and Woodford, M. 2nd International Congress of Biochemistry (abstracts), 1952,p.129

40. Thorn, G.W., Engel, L.L. and Eisenberg, H. J. Exper. Med., 68; 161; 1938.

41. Lewis, R.A., Thorn, G.W., Koepf, G.F. and Dorrance, S. J. Clin. Invest., 21; 33; 1942.

42. Ingle, D.J. J. Clin. Endocrinol., 10; 1312; 1950.

43. Slocumb, C.H., Polley, H.F., Hench, P.S. and Kendall, E.C. Proc. Staff Meeting Mayo Clinic, 25; 476; 1950.

44. Fourman, P., Bartter, F.C., Albright, F., Dempsey, E. and Carroll, E. Proc. Can. Physiol. Soc., 13th Meeting, Oct. 1949.

45. Pincus, G., Hechter, O. and Zaffaroni, A. Second Clin. ACTH Conf., J.R. Mote, Editor, 1951.

46. Reich, H., Nelson, D.H. and Zaffaroni, A. J. Biol. Chem., 187; 411; 1950.

47. Bush, I.E. Biochem. J., 50; 370 (Jan.), 1952.

48. Adrenal Cortex Conference, Josiah Macy Jr. Foundation, 1949.

49. Overman, R.R., Davis, A.K. and Bass, A.C. Am. J. Physiol., 167; 333; 1951.

50. Thorn, G. and Engel, L.L. J. Exper. Med., 68; 299; 1938.

51. Deming, Q.B. and Luetscher, J.A. Proc. Soc. Exper. Biol. & Med., 73; 171; 1950.

52. Clinton, M. and Thorn, G.W. Science, 96; 344; 1942.

53. Selye, H. Brit. M. J., 1; 203; 1950.

54. Hall, C.E., Hall, O. and McClesky, O. Acta endocrinol., 9; 199; 1952.

55. Fagans, S.S., Louis, L.H. and Conn, J.W. J. Lab. & Clin. Med., 38; 911; 1951.

56. Gaunt, R., Birnie, J.H. and Eversole, W.J. Physiol. Rev., 29; 281; 1949.

57. Gaunt, R. J. Clin. Endocrinol., 6; 595; 1946.

58. Gaunt, R. Endocrinology, 34; 400; 1944.

59. Gaunt, R. Recent Progress in Hormone Research, 6; 247; 1951.

60. Gaunt, R. and Birnie, J.H. Hormones & Body Water. Charles C. Thomas Ltd., 1951.

61. Lloyd, C.W. and Lobotsky, J. J. Clin. Endocrinol., 10; 318; 1950.

62. Gaunt, R., Cordsen, M. and Liling, M. Endocrinology, 35; 105; 1944.

63. Radcliffe, C.E. Endocrinology, 32; 415; 1943.

64. Gaunt, R., Liling, M. and Cordsen, M. Endocrinology, 37; 136; 1945.

65. Chasis, H., Ranges, H.A., Goldring, W. and Smith, H.W. J. Clin. Invest., 17; 683; 1938.

66. Toth, L.A. Am. J. Physiol., 119; 140; 1937.

67. McDermott, W.V., Fry, E.G., Brobeck, J.R. and Long, C.N.H. Yale J. Biol. & Med., 23; 52; 1950.

68. Talbott, J.H., Pecora, L.J., Melville, R.S. and Consolazio, W.V. J. Clin. Invest., 21; 107; 1942.

69. Lloyd, C.W. Recent Progress in Hormone Research, 7; 469; 1952.

70. Albright, F. Harvey Lectures, 1943.

71. McQuarrie, I., Johnson, R.M. and Ziegler, M.R. Endocrinology, 21; 763; 1937.

72. Willson, D.M., Power, M.H. and Kepler, E.J. J. Clin. Invest., 19; 701; 1940.

73. Smith, H.W. The Kidney, Oxford Univ. Press, 1951.

74. Warren, J.V. and Stead, E.A. Arch. Int. Med., 73; 138; 1944.

75. Merrill, A.J. J. Clin. Invest., 25; 389; 1946.

76. Leiter, L. Bull. N.Y. Acad. Med., 24; 702; 1948.

77. Bradley, S.E. and Bradley, G.P. J. Clin. Invest., 26; 1016; 1947.

78. Brod, J. and Fejfar, Z. Quart. J. Med., 29; 187; 1950.

79. Goldman, R. J. Clin. Invest., 30; 1191; 1951.

80. Pincus, G., Romanoff, L.P. and Carlo, J. J. Clin. Endocrinol, 8; 221; 1948.

81. Davies, C.E. and Kilpatrick, J.A. Clin. Sc., 10; 53; 1951.

82. Mueller, C.B., Surtshin, A., Carlin, M.R. and White, H.L. Am. J. Physiol., 165; 411; 1951.

83. Surtshin, A., Rolf, D. and White, H.L. Am. J. Physiol., 165; 429; 1951.

84. Heller, I. and Jacobson, W.E. Am. Heart J., 39; 188; 1950.

85. Merrill, A.J., Morrison, J.L. and Brannon, E.S. Am. J. Med., 1; 468; 1948.

86. Futcher, P.H. and Schroeder, H.A. Am. J. M. Sc., 204; 52; 1942.

87. Cooke, R.E., Pratt, E.L. and Darrow, D.C. Yale J. Biol. & Med., 22; 227; 1950.

88. White, A.G., Gordon, H. and Leiter, L. J. Clin. Invest., 29; 1445; 1950.

89. Reynolds, T. Proc. Soc. Exper. Biol. & Med., 79; 118; 1952.

90. Iseri, L.T., Boyle, A.J. and Myers, G.B. Am. Heart J., 40; 706; 1950.

91. Parrish, A.E. J. Clin. Invest., 28; 45; 1949.

92. Heard, R.D.H. The Hormones, vol. 1; chapter 13; 1948.

93. Lasche, E.M., Perloff, W.H. and Durant, T.M. Am. J. M. Sc., 222; 459; 1951.

94. Bornstein, J. and Trewhella, P. Lancet, 259; 678; 1950.

95. Bercu, B.A., Rakow, S.N. and Massie, E. Am. Heart Assoc. Trans., June3, 1949.

96. Dochios, M. and Deifus, L.S. Amer. J. M. Sc., 222; 538; 1951.

97. Eder, H.A., Chinard, F.P., Greif, R.L., Cotzias, G.C., Hiller, A., Van Slyke, D.D. and Lauson, H.D. J. Clin. Invest., 27; 532; 1948.

98. Eder, H.A., Chinard, F.P., Lauson, H.D., Greif, R.L., Hiller, A., Cotzias, G.C., and Van Slyke, D.D. J. Clin. Invest., 28; 779; 1949.

99. Barnett, H.L., Forman, C.W., McNamara, H., McCrory, W.C., Rapoport, M., Michie, A.J. and Barbero, G. J. Clin. Invest., 30; 227; 1951.

100. Luetscher, J.A., Deming, Q.B. and Johnson, B.B. J. Clin. Invest., 30; 1530; 1951.

101. Orloff, J., Welt, L.G. and Stowe, L. J. Clin. Invest., 29; 770; 1950.

102. Galan, E. Arch. Soc. estud. Clin. Habana, 38; 445; 1944.

103. Emerson, K., Futcher, P.H. and Farr, L.E. J. Clin. Invest., 20; 361; 1941.

104. Galan, E. Am. J. Dis. Child., 77; 328;,1949.

105. Emerson, K. and Dole, V.P. J. Clin. Invest., 22; 447; 1943.

106. Luetscher, J.A. and Deming, Q.B. J. Clin. Invest., 29; 1576; 1950.

107. Luetscher, J.A., Hall, A.D. and Kremer, V.L. J. Clin. Invest., 29; 896; 1950.

108. Luetscher, J.A. J. Clin. Invest., 26; 1189; 1947.

109. Burnett, C.H., Burrows, B.A. and Commons, R.R. J. Clin. Invest., 28; 773; 1949.

110. Fox, C.L. and Slobody, L.B. Pediatrics, 7; 186; 1951.

111. Rosenblum, H.H., Lander, H.B. and Fisher, R.M. J. Pediat., 35; 574; 1949.

112. Byrne, E.A.J. Lancet, 262; 844; 1952.

113. Gairdner, D. Lancet, 262; 842; 1952.

114. Heymann, W., Lund, H.Z. and Hackel, D.B. J. Lab. & Clin. Med., 39; 218; 1952.

115. Lange, K., Slobody, L., Graig, F., Ogur, G., Oberman, J. and LoCasto, F. Pediatrics, 8; 814; 1951.

116. Lange, K., Gold, M.M.A., Weiner, D. and Simon, V. J. Clin. Invest., 28; 50; 1949.

117. Robinson, F.H. and Farr, L.E. Ann. Int. Med., 14; 42; 1940.

118. Bongiovanni, A.M. Am. J. M. Sc., 222; 710; 1951.

119. Young, W.F., McCaull, R.A. and Finch, E. Arch. Dis. Child., 17; 65; 1942.

120. Whitney, J.E., Bennett, L.L. and Li, C.H. Proc. Soc. Exper. Biol. & Med., 79; 584; 1952.

121. Sterling, K. J. Clin. Invest., 30; 1238; 1951.

122. Ricketts, W.E., Eichelberger, L. and Kirsner, J.B. J. Clin. Invest., 30; 1157; 1951.

123. Faloon, N.N., Eckhardt, R.D., Murphy, T.L. and Cooper, A.M. J. Clin. Invest., 28; 583; 1949.

124. Ralli, E.P., Robson, J.S., Clarke, D. and Hoagland, C.L. J. Clin. Invest., 24; 316; 1945.

125. Stueck, G.H., Leslie, S.H. and Ralli, E.P. Endocrinology, 44; 325; 1949.

126. Shoor, E., Baez, S., Zweifach, B.W., Payne, M.A. and Mazur, A. Tr. A. Am. Phys., 63; 39; 1950.

127. Heller, H. and Urban, F.F. J. Physiol., 85; 502; 1935.

128. White, A.C., Rubin, G. and Leiter, L. J. Clin. Invest., 30; 1287; 1951.

129. Leslie, S.H., Johnston, B. and Ralli, E.P. J. Clin. Invest., 30; 1200; 1951.

130. Farnsworth, E.B. and Krakusin, J.S. J. Lab. & Clin. Med., 33; 1545; 1948.

131. Epstein, F.H., Lesser, G.T. and Berger, E.Y. Proc. Soc. Exper. Biol. & Med., 75; 822; 1950.

132. Relman, A.S. Bull. N. Eng. Med. Center, 13; 67; 1951.

133. Goodyer, A.V.N., Relman, A.S., Lawrason, F.D. and Epstein, F.H. J. Clin. Invest., 29; 973; 1950.

134. Siri, W.E. Isotopic Tracers and Nuclear Reactions. McGraw-Hill Book Co. Inc., 1949.

135. Bradley, S.E. N. Eng. J. Med., 240; 456; 1949.

136. Tippett, L.H.C. The Methods of Statistics. Williams & Norgate Ltd., London, 1941.

137. Eisenmenger, W.J., Blondheim, S.H., Bongiovanni, A.M. and Kunkel, H.G. J. Clin. Invest., 29; 1491; 1950.

138. Bongiovanni, A.M. and Eisenmenger, W.J. J. Clin. Endocrinol., 11; 152; 1951.

139. Locke, W., Talbot, N.B., Jones, H.S. and Worcester, J. J. Clin. Invest., 30; 325; 1951.

140. Goldblatt, H. Am. J. Med., 4; 100; 1948.

141. Trueta, J. Glasgow Med. J., 31; 217; 1950.

142. Shoor, E. and Zweifach, B.W. Am. Heart Assoc. Proc., 22nd Sc. Session, June 1949, p.7

143. Schroeder, H.A. and Olsen, N.S. J. Exper. Med., 92; 545; 1950.

144. Olsen, N.S. and Schroeder, H.A. J. Exper. Med., 92; 561; 1950.

145. Page, I.H. Bull. N.Y. Acad. Med., 13; 645; 1937.

146. Schroeder, H.A. Am. J. Med., 10; 189; 1951.

147. Perera, G.A. Bull. N.Y. Acad. Med., 26; 75; 1950.

148. Sapeika, N. Arch. Int. Med., 82; 263; 1948.

149. Selye, H. Stress. Montreal, Canada, Acta Inc., Medical Publishers, 1950.

150. Perera, G.A. and Blood, D.W. Am. J. Med., 1; 602; 1946.

151. Eisenberg, S., Buie, R. and Tobian, L. Am. J. M. Sc., 220; 287; 1950.

152. Tobian, L. J. Clin. Endocrinol., 9; 319; 1949.

153. Corcoran, A.C., Page, I.H. and Dustan, H.P. J. Lab. & Clin. Med., 36; 297; 1950.

154. Hetzel, B.S. and Hine, D.C. J. Clin. Endocrinol. & Met., 12; 296; 1952.

155. Deane, H.W. and McMasson, G. J. Clin. Endocrinol., 11; 193; 1951.

156. Pickering, G.W. and Prinzmetal, M. J. Physiol., 98; 314; 1940.

157. Sellers, A.L., Smith, S., Goodman, H.C. and Marmorston, J. Am. J. Physiol., 166; 619; 1951.

158. Selye, H. Ann. Int. Med., 29; 403; 1948.

159. Selye, H. J. Clin. Endocrinol., 6; Feb. 1946, p. 117.

160. Symposium on Cortisone and ACTH in Clinical Medicine. Proc. Staff Meet. Mayo Clinic, 25; 474; 1950.

161. Waksman, B.H. Medicine, 28; 143; 1949.

162. McCarty, M. Bull. N.Y. Acad. Med., 28; 307; 1952.

163. Weil, P. and Browne, J.S.L. Science, 90; 445; 1939.

164. Venning, E.H., Hoffman, M.M. and Browne, J.S.L. Endocrinology, 35; 49; 1944.

165. Johnson, H.T., Conn, J.W. amd Iob, V. Ann. Surg., 132; 374; 1950.

166. Welsh, C.A., Wellen, I. and Taylor Jr., H.C. J. Clin. Invest., 21; 57; 1942.

167. Ham, G.C. and Landis, E.M. J. Clin. Invest., 21; 455; 1942.

168. Dieckmann, W.J., Seski, A.G., McCartney, C.P., Smitter, R.C., Pottinger, R.E., Brunetti, R., Rynkiewicz, L.M., Allen, J. and Regester, R. Am. J. Obst. Gynec., 58; 1014; 1949.

169. Devis, R., Devis-Vanden Eichhoudt, M. Annales d'Endocrinologie, 2; 22; 1950.

170. Parviainen, S., Soiva, K. and Vartiainen, S. Acta Obst. et Gynaec. Scand., 39; Supp. 5; 1950.

171. Chart, J.J., Shipley, E.G. and Gordon, E.S. Proc. Soc. Exper. Biol. & Med., 78; 244; 1951.

172. Ordman, B. S. African Med. J., 24; 938; 1950.

173. Smith, G.V.S. and Smith, O.W. Physiol. Rev., 28; 1; 1948.

174. Parviainen, S., Soiva, K. and Ehrnrooth, C.A. Ann. chir. et gynaec. Finniae, 39; 171; 1950.

175. Parviainen, S., Soiva, K. and Ehrnrooth, C.A. Gynaecologia, 131; 234; 1951.

176. Garrett, S.S. West. J. Surg., 58; 689; 1950.

177. Masson, G.M.C., Corcoran, A.C. and Page, I.H. J. Lab. & Clin. Med., 38; 213; 1951.

178. Masson, G.M., Corcoran, A.C. and Page, I.H. J. Lab. & Clin. Med., 38; 926 (abst); 1951.

179. Mukherjee, C.L. and Govan, A.D.T. J. Obst. & Gynaec. Brit. Emp., 58; 702; 1951.

180. Govan, A.D.T., Mukherjee, C.L., Hewitt, J. and Harper, W.F. J. Obst. & Gynaec. Brit. Emp., 58; 788; 1951.

181. Magara, M. J. Internat. Coll. Surg., 14; 215; 1950.

182. Selye, H. Brit. M. J., 1; 263; 1951.

183. White, H.L., Heinbecker, P. and Rolf, D. Am. J. Physiol., 165; 442; 1951.

184. Stack-Dunne, M. and Young, F.G. J. Endocrinol., 7; 66; 1950-51. (Suppl.)

185. Venning, E.H. and Kazmin, V. Endocrinology, 39; 131; 1946.

186. Eisenberg, S., Buie, R. and Tobian, L. Am. J. M. Sc., 220; 287; 1950.

187. Grad, B. J. Clin. Endocrinol. & Met., 12; 708; 1952.

188. Hartman, F.A., Lewis, L.A. and Thatcher, J.S. Proc. Soc. Exper. Biol. & Med., 48; 60; 1941.

189. Dorfman, R.I., Potts, A.M. and Feil, M.L. Endocrinology; 41; 464; 1947.

190. Spencer, A.C. Nature, 166; 32; 1950.

191. Simpson, S.A. and Tait, J.F. Endocrinology, 47; 308; 1950.

192. Simpson, S.A. and Tait, J.F. Endocrinology, 50; 150; 1952.

193. Marcus, F., Romanoff, L.P. and Pincus, G. Endocrinology, 50; 286; 1952.

194. Kagawa, C.M., Shipley, E.G. and Meyer, R.K. Proc. Soc. Exper. Biol. & Med., 80; 281; 1952.

195. Venning, E.H., Hoffman, M.M. and Browne, J.S.L. Endocrinology, 35; 49; 1944.

196. Daughaday, W.H., Jaffe, H. and Williams, R.H. J. Clin. Endocrinol., 8; 166; 1948.

197. Heard, R.D.H., Sobel, H. and Venning, E.H. J. Biol. Chem., 165; 699; 1946.

198. Talbot, N.B., Saltzman, A.H., Wixom, R.L. and Wolfe, J.K. J. Biol. Chem., 160; 535; 1945.

199. Colingsworth, D.R., Brunner, M.P. and Haines, W.J. J.A.C.S., 74; 2381, 1952.

200. Peterson, D.H. and Murray, H.C. J.A.C.S., 74; 1871; 1952.

201. Grossman, J., Weston, R.E., Lehman, R.A., Halperin, J.P., Ullmann, T.D. and Leiter, L. J. Clin. Invest., 30; 1208; 1951.

202. Laramore, D.C. and Grollman, A. Am. J. Physiol., 161; 278; 1950.

203. Stamler, J., Hwang, H. and Kuramoto, K. Am. J. Physiol., 165; 328; 1951.

204. Corcoran, A.C., Page, I.H., Masson, G.M.C., Taylor, R.D. and Dustan, H. Arch. Int. Med., 87; 732; 1951.

205. Bush, I.E., de Courcey, C. and Gray, C.H. J. Endocrinol., 8; xii; 1952.

206. Simpson, S.A., Tait, J.F. and Bush, I.E. Lancet, ii; 226; 1952.