"POTASSIUM DEFICIENCY AND GASTROINTESTINAL MOTILITY".

PART I. The hypochloremia produced by desoxycorticosterone and its relation to potassium deficiency.

PART II. The effect of potassium deficiency upon gastro-intestinal motility.

BY

HELEN WINTER HENRIKSON

A Thesis Presented to the Faculty of Graduate Studies and Research in fulfillment of the Requirements for the Degree of Doctor of Philosophy in Anatomy.

McGill University,

April, 1949.

TABLE OF CONTENTS

TABLE OF FIGURES AND TABLES

ACKNOWLEDGEMENTS

The work reported was initiated in the Department of Anatomy, Columbia College of Physicians, where the author served on the staff. It was supported there by grants from Dr. Philip E. Smith whom the author thanks. It was continued in the Department of Physiology, McGill University, and the author is indebted to Dr. Hebbel Hoff, formerly Professor in this department, for providing a laboratory, and ample financial assistance. The author is grateful to several professors at McGill University who, since this investigation was performed without a director of research, have given valuable advice. Dr. Hebbel Hoff, encouraged the author, particularly in the gastrointestinal studies, and gave an excellent example of experimental procedure in general. Following the preliminary work demonstrating the effect of potassium deficiency and therapy on gastrointestinal motility, he suggested the use of the X-ray technique. Dr. C.P. Leblond criticized the first part of the thesis and, both he and Dean D.L. Thomson, suggested principles of writing a thesis. Dr. B. Babkin read through in detail and discussed the second part of the thesis with me. The author thanks all these professors for their kindness in assisting. My husband, Arne Henriksen, has done all the work on preparing the figures and graphs for which I am very grateful. Miss Betty Bedard has typed this thesis, and the author appreciates her skill in reading hieroglyphics.

INTRODUCTION

INTRODUCTION

The well-known regulation of the electrolyte balance by the adrenal cortex has been attributed to its secretion of a hormone similar to, if not identical with, desoxycorticosterone. A deficiency of adrenocortical hormone lowers both serum chloride and sodium, and elevates potassium. Therapy with desoxycorticosterone rapidly restores these electrolyte levels to normal by markedly increasing the retention of sodium and chloride and reducing the renal retention of potassium. Therefore it seemed paradoxical to find that large doses of desoxycorticosterone acetate (DCA) can lower the serum chloride of both normal and adrenalectomized animals.

At the outset of this research, the problem was to determine the mechanism by which DCA produced this hypochloremia, which has been observed by several investigators. It seemed probable that it was an overdosage effect related to the loss of potassium produced by DCA, and that this steroid must increase the urine chloride excretion, contrary to the available information indicating that it produces chloride retention. These factors were investigated. Balance studies on chloride and potassium ions were performed to determine the relation between the chloride and potassium excretions in potassium deficiency. This problem of the effect of DCA and potassium deficiency on chloride balance forms the first part of this thesis.

In the balance experiments in force-fed rats, the motility of the gastrointestinal tract became seriously reduced and such a retention of intestinal contents and gas occurred that all the animals died from extreme distension. This was produced either by overdosage with DCA or by a low potassium diet, and was promptly relieved by potassium administration. These changes in the absorption of food, and the inevitable death of the animals in less than two weeks interfered with the refinement of the balance studies. The electrolyte investigation was therefore discontinued after it was possible to draw some conclusions. The major contribution of this thesis is a study of the effect of potassium deficiency on the gastrointestinal tract by the X-ray technique, and of the relief of symptoms by potassium therapy. This effect of potassium deficiency has not been observed before.

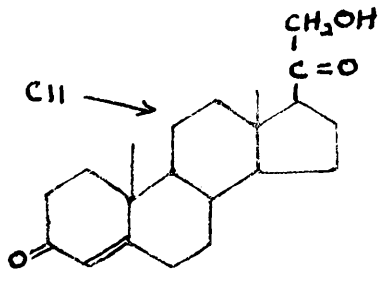
P A R T I

THE HYPOCHLOREMIA PRODUCED BY DCA AND ITS RELATION TO POTASSIUM DEFICIENCY

REVIEW OF THE LITERATURE

DCA As an Adrenal Cortical Hormone

Desoxycorticosterone was first synthetized by Steiger and Reichstein in 1937 (2a), and has been isolated from the adrenal cortex in minute amounts by Reichstein and von Euw (2b).



desoxycorticosterone

This compound is usually used as the acetate (DCA). Since the activities of DCA differed from those of other corticosteroids in some respects, there was some doubt, at the time this work was initiated, whether it could be considered a typical adrenocortical hormone.

This steroid was the most potent compound known in prolonging survival under stable conditions in adrenal insufficiency and in restoring to normal the disordered electrolyte pattern observed in this condition in man (3,4,5,6,7,8,9) and animals (10,11,12,13,14,15,16,17). In addition, overdosage symptoms, essentially the opposite to those which occur in adrenal insufficiency, could be produced in intact animals with DCA, but did not occur with other adrenal preparations tested (17a). Thus, overdosage with DCA resulted in severe depletion of potassium and im marked retention of sodium (18,19).

As a result of the potassium depletion muscular paralysis developed. As a result of the sodium retention the extracellular fluid volume expanded and edema often occurred. In the first clinical tests of DCA in cases of Addison's disease severe circulatory congestion and several fatalities resulted from the sudden increase in blood volume (5,6,20, 21), and, as has been shown since, probably also because of cardiac lesions produced by potassium depletion (22). In contrast to this, enormous doses of adrenal extracts and steroids had no deleterious effects (17a). In intact animals treated with DCA, a condition of marked polyuria and polydipsia was induced, a response not obtained with other adrenal preparations (18;17,17a). In addition, DCA was the only adrenocortical steroid to lower serum chloride (45).

In other respects DCA differed from other adrenocortical preparations. This steroid proved less effective in maintaining adrenal-deficient animals and patients unless they were kept under stable conditions, and it had no significant effect upon carbohydrate metabolism of adrenalectomized animals. (2,3,24,25,26,17). Unlike DCA, compounds with more generalized beneficial effects in adrenal insufficiency, such as corticosterone, possess an oxygen group in position 11. This confers upon the compound effectiveness in maintaining a normal carbohy-

drate metabolism in adrenal insufficiency, which is very important when the animals are subjected to stress (23).

Of interest was the hypochloremia produced by DCA. One might expect quite the reverse effect, since sodium is retained in DCA-treated animals and since hypochloremia is a symptom of adrenal insufficiency (27,28,29,30). According to the classical scheme of the behaviour of chloride in the body fluid, chloride has been considered to move in the same direction as, or to be closely associated with, the sodium ions (31,32,33). Thus the two types of hypochloremia which occur in the hyper- and hypodrenal states must differ in the mechanism by which they are induced, since sodium retention occurred in the former, while in the latter sodium loss appeared to the primary cause of the hypochloremia (29,30).

The significance of the finding that DCA induces hypochloremia is enhanced by the fact that hypochloremia has been observed in clinical cases of Cushing's Syndrome hyperadrenocorticism (34). In these cases a state of alkalosis is associated with the hypochloremia, so that the total anion content of the serum remains approximately normal. This is to be contrasted with the occurrence of acidosis in adrenal insufficiency in which both bicarbonate and chloride concentration, and therefore total anion amount are low (28,29,30). From this

it would appear that the adrenal cortex plays a role in the acid-base balance of the body. The changes in anion concentration in the serum parallel those in serum cation concentration.

At the present time the role of desoxycorticosterone as an adrenocortical hormone has been elucidated very considerably by the voluminous research on this compound and by the observation of electrolyte disturbances, similar the overdosage effects of DCA, both in rats treated with very large doses of adrenocortical extract under certain conditions, and in clinical case of Cushing's syndrome of hyperadrenocorticism. The activities of desoxycorticosterone may now be regarded as represent-ative of adrenal cortical functions in electrolyte metabolism although this steroid does not possess the activities of the whole adrenal cortex on carbohydrate metabolism. The abnormalities in electrolyte metabolism have been produced by large doses of DCA and may be regarded as due to overdosage, since, with physiological dosages, the deranged electrolyte balance of adrenal insufficiency is restored to normal. The overdosage effects are probably just distortions of the activities or the balance between activities of the normal adrenal cortex. Two to eight times the physiological dose of DCA is required in intact rats and dogs for the overdosage effects which

will be discussed in the review of the actions of DCA. This, however, depends upon the potassium and sodium content of the diet.

The Effects of DCA and of Potassium-deficiency Upon Eléctrolyte Metabolism

A discussion of the action of DCA on chloride metabolism will be presented in detail in the following section, and since the behaviour of ions are interdependent in their movements in the body, and the behaviour of any one ion cannot be fully understood without a knowledge of the behaviour of other electrolytes, the actions of DCA upon bicarbonate, potassium, sodium and water metabolism will also be considered.

There is much evidence that the hypochloremia and certain other disturbances in electrolyte metabolism produced by large doses of DCA are secondary to, or associated with, the potassium-deficiency induced. Therefore, the electrolyte disturbances produced by DCA have been compared with those occurring in animals depleted of potassium by maintenance on a low potassium diet.

(a) Effect of DCA and potassium-deficiency on chloride metabolism.

It is now established that large doses of DCA can induce hypochloremia. This has been shown in rats, mice, dogs, in five of twelve opossum (36), and in one of four cats (35). The chloremic response is influenced by the

experimental conditions including the dietary regime, especially the potassium and sodium content, the dosage and duration of treatment (35), and the species. Of all factors, the potassium intake has the most important modifying influence. Maintenance of animals on a low potassium regime per se can induce hypochloremia of comparable degree in rats (37,38,39,40), dogs (35) and man (41), and may also produce a negative chloride balance (42). When this is combined with DCA treatment, hypochloremia is more rapidly produced than when the potassium intake is normal (18,40). The resistance of the serum chloride of cats and rabbits to the action of DCA noted from the experiments of Darrow and Miller (35) and Rakoff et al (43), was probably due mainly to the high potassium or salt concentration of the diets fed these animals (fish and vegetables, respectively). Usually about 10 days of DCA injections is required for a small but significant lowering of the serum chloride concentration, in rats, although Winter (1a) found it possible to induce hypochloremia within eight hours after very large doses of DCA (6 mg) were given to young rats. In most of the investigations reported the action of DCA in reducing serum chloride was not commented upon until the recent work of Darrow (44), in which a new significance to this observation was given, as will be discussed below.

In the intact rat fed purina Miller and Darrow (45,35) found (in animals given 2 to 4 mg DCA per day over a thirty day period) that the serum chloride was reduced from a normal of 100 to about 93 meq per litre. With shorter periods of treatment (10 days) the reduction was only 3 meq. Buell and Turner noted a 10 meq decrease in serum chloride in rats absorbing only 0.3 mg of DCA per day for 7 days from a pellet (46). Hegnauer (40) noted a decrease of 14 meq in serum chloride in rats maintained in a low K diet and given DCA. Selye and Dosne (47,48) in young rats fed on purina, found the whole blood but not the serum chloride was reduced by 7 meq following 20 days of DCA injections, although the results were quite variable. These investigators concluded that the loss of chloride was mainly from the erythrocytes rather than from the serum, from their analyses on whole blood and serum. However, an analysis of the more complete data of Hegnauer (40) and unpublished observations of the author (1b), fail to confirm this view.

In the dog, DCA frequently lowers the serum chloride, by about 5 to 7 meq, (49,19.50,35,51). In these experiments the dogs were given from 10 to 25 mg of DCA per day for from two to several weeks. With doses of 2 to 4 mg of DCA, which approach the optimal physiological dosage of 1 to 2 mg (13), Mulinos et al (52) noted no significant

alteration in whole blood chloride, although disturbances in water balance occurred. On the other hand, in normal dogs receiving a high salt and potassium diet, Clinton et al (53) found that the chloride concentration was elevated by 8 meq per litre of serum when the animals were treated for five days (only). In five normal men treated with DCA and also receiving a high salt diet, Thorn et al (54) noted an increase of 5 meq in serum chloride in 4 days. In the dog (53) the plasma volume was increased at the same time so that the total chloride increase in extracellular fluid was greater than is indicated from the concentration increase. To explain the divergent observations, it is possible that the initial response may differ in its mechanism from the chronic effect of DCA. If sodium chloride intake is high the chloride ion may be associated at first with the large amounts of sodium being reabsorbed and later with the potassium being excreted in excess. The sodium retention was observed to greater than the chloride attention (54).

In the mouse treated with 1 mg of DCA per day and fed purina Winter (1a) observed a decrease of blood chloride from 78 to 66 meq per litre in 2 weeks.

In Addison's disease, of which hypochloremia is a symptom, DCA occasionally lowers serum chloride further for one or two days prior to the restoration of concentration to normal. This is due to a temporary

increase in extracellular fluid water derived from the hydrated cells and from increased renal retention of water (6,7,55).

Hypochloremia can be induced in hypochysectomized rats and thyroidectomized rats as shown by Winter (1c) and in adrenalectomized rats, as noted by Selye and Dosne, Buell and Turner and by Darrow (48,46,44). Therefore, although the absence of these glands may modify the response (1h), they are not responsible for the action of DCA.

Most investigators have found that the chloride content of skeletal or heart muscle is not statistically changed by DCA in the normal rat (35,45,56,40) and dog (19,35,50,51), other than a decrease which can be accounted for by the reduction in the chloride of the interstitial fluid of the body. That is, the chloride does not appear to be entering the cells. However, Selye and Dosne (48) observed an increase in muscle chloride in acute experiments. The water content of the muscle was not given and therefore the volume of the extracellular fluid which normally contains nearly all the chloride, is unknown. Although Ferrebee et al (19) states that muscle chloride of dogs treated with DCA was not altered, an average of the rather variable results indicates an increase (from 24.4 in the normal to 29.1 meq per kg fresh tissue) even if two extremely high values

are excluded from the injected group. His figures show this increase was prevented by potassium chloride given in the drinking water. It is more probable that this increase in chloride represents an increase in extracellular fluid of muscle than a shift of chloride into the cells. In the one cat exhibiting hypochloremia of 4 treated (35), the cardiac and skeletal muscle chloride were both increased markedly without concurrent increase in the water content of the muscle. The cardiac chloride was increased from 19 to 27, and the skeletal muscle chloride from 6 to 12 meq per 100 grams of solids. This animal was eating poorly of a high salt and potassium diet (fish) and in addition was in poor condition suffering an infection, which decrease the significance of the observation (which, incidently, was not commented upon by the author, Darrow).

Many investigators consider that skeletal muscle fibres are chloride-free (51,57,57a,58,59,50), although it is quite probable from several more recent investigations (38,60.61,62,63,64), that a small percentage of the muscle chloride is intracellular. Yannet and Darrow (61) and Darrow (62) found that approximately 1 meq (15%) of the 6 to 7 meq of chloride per 100 grams of fat-free solids in muscle is intracellular, a figure with which the data of the other investigators quoted roughly agrees.

Boyle and Conway (63), working with frog muscle soaked in solutions containing various concentrations of potassium and chloride, obtained data which indicated that, under the conditions of the experiments, the intracellular concentration of chloride is proportional to the extracellular concentration of potassium per litre of water. In cats given potassium chloride intravenously, Crismon et al (94) also obtained evidence that chloride entered muscle cells. This was associated with an increase in intracellular potassium. No studies have been performed in DCA-treated animals to determine whether chloride distribution is normal. In view of the data linking the shifts of chloride with corresponding shifts of potassium. it is improbable that intracellular chloride would increase in muscle cells which are losing potassium.

There is no evidence that chloride is lost from the kidneys but this will be discussed under the heading of sodium balance. As mentioned above Orent-Keiles and McCollum (42) noted that the chloride balance was negative throughout the whole experiment in rats fed a low-potassium diet ad libitum (no DCA) for prolonged periods.

(b) The effect of DCA and potassium-deficiency on acid-base balance.

With hypochloremia an increase in the bicarbonate level in the serum would be anticipated as a compensatory

adjustment in the presence of normal or high serum sodium concentrations (see below). In unpublished results in rats Darrow has recently observed (44Q) that if the diet is low in potassium, both DCA and adrenocortical extract may induce alkalosis, with increased serum bicarbonate and pH values. Darrow produced alkalosis in both normal and adrenalectomized rats. In adrenal insufficiency, on the other hand, there is a tendency for acidosis to occur - for the serum bicarbonate and pH to be lowered. A decrease of 0.2 pH has been observed in adrenalectomized dogs and rats (65,66). Reduction of the serum bicarbonate in adrenalectomized dogs to concentrations as low as 11 to 14 meq per litre were noted first by Loeb et al (29) and Harrop and associates (30), and have been confirmed by Muntwyler et al (65). Lesser changes developed in adrenalectomized rats (66) and in Addison's disease (28). Neither the pH or bicarbonate concentration was appreciably increased after 48 hours of injections with adrenocortical extract, which increased the chloride and sodium contentration to normal (65,30). Thorn, and Ferrebee, and their respective associates (5,9) noted a slight decrease in bicarbonate in Addison's disease which was restored to normal by treatment with DCA. The decrease in bicarbonate concentration in adrenal insufficiency is a reflection of the decrease in total base of the serum which results from the greater loss of sodium through the kidney than of chloride (28,29,30).

(c) The effect of DCA and of potassium-deficiency on potassium metabolism.

The most striking electrolyte disturbance resulting from over-dosage with DCA is the depletion of potassium from intracellular and extracellular fluids. This depletion, obviously, is more rapidly produced when the potassium intake is low (18,40,67), and may be prevented almost completely by a high intake of potassium (18,19,49). On the other hand the elevation of serum sodium and the increased water exchange are not affected by the potassium intake (19). The serum potassium can be reduced to concentrations lower than 2.5 meq per litre, and as much as 40 percent of the intracellular potassium can be lost from skeletal muscle, and smaller amounts from heart muscle. The potassium lost from the muscle is replaced by sodium. The degree of potassium depletion produced by DCA depends upon the dosage and the duration of treatment, in addition to the potassium intake.

Severe potassium depletion in the normal dog treated with large doses of DCA was first shown by Kuhlman, Ragan, Ferrebee, Loeb and Atchley (18,49,19) in 1939 to 1941. Serum potassium values, after 25 mg of DCA per daily for a month, were reduced from 4 to 2.9 - 2.2 meq per litre. A similar lowering of serum potassium has been confirmed by Muntwyler et al (51) and Harkness et al (50) and

Darrow and Miller (35) in the dog and by Hegnauer (40) in the rat, as well as lesser decreases by other investigators (45,16). Equally low potassium concentrations are produced when the potassium intake is very low (40, 35,69), and no DCA is given, as was first noted by Heppel (37). The potassium is almost certainly lost from the serum through the kidney and is replaced from the intracellular stores of this ion, since an equilibrium exists between intra- and extra-cellular potassium (70,38). The possible influence of DCA on this equilibrium has not been investigated as yet. Because of this equilibrium serum potassium may be within normal limits for some time, while the serum potassium in considerably reduced (45,35).

Ferrebee et al (19) found that the intracellular potassium of dogs treated with DCA was reduced from a normal of 76 to 48 meq per kg of fat-free fresh muscle, coincident with an increase of intracellular sodium from 6 to 32 meq per kg. Other investigators have also found that from twenty to forty percent of the muscle potassium is lost in DCA-treated animals normally fed in both rats (45,35,40) and dogs (50,51). With low potassium diets alone more than one-third of the muscle potassium may be lost after six weeks in mice and rats (71,37,42,40,72).

Hegnauer (40) found that erythrocytes behave differently than muscle cells in that DCA did not reduce the potassium concentration. On the other hand, a low -

potassium diet decreased the erythrocyte concentration of potassium. He concluded that the intracellular content in red blood cells of potassium was in equilibrium with the total base of the serum, rather than with the potassium only. Total serum base was reduced by low potassium intake but not when this was combined with DCA injections.

Most investigators consider the main action of DCA is to decrease the renal reabsorption of potassium, and thus deplete the body of this ion. However, no kidney clearance studies of potassium have been performed in DCA-treated animals to elucidate this point. It is difficult to separate the effects of DCA on sodium from that on potassium reabsorption, since both are interdependent. They are both affected by DCA and in opposite directions. There is little evidence reported for an increased potassium excretion in DCA-treated animals, other than short term experiments of from 12 hours to 5 days in duration. In one DCA-treated dog fed a normal diet Ragan et al (49) found it difficult to detect any significant changes in potassium balance, although muscle and serum values were low and the animal developed paralysis. In growing rats fed a low potassium diet but given no DCA. Orent-Keiles and McCollum found that the animals were in potassium balance for the 21 week period except during the

first week. (42) These animals were growing, and were just in balance. No calculation was made to allow for potassium to be deposited in the new tissue. Therefore, these rats were actually in negative balance with respect to their potassium requirement, and sodium filled this deficit of cation in the cells. Whether this argument may be applied to the conclusions of Ragan et al (49) cannot be decided since only one control value potassium excretion was given.

It is well-established that in short term experiments potassium excretion tends to be increased by DCA over a one-to-five-day period, although daily variations in excretion often rob the individual experiments of significance. This has been shown chiefly by Thorn and associates, but also by other investigators, for normal dogs (10,73) and man (54) and adrenal deficient dogs (10,13), rats (74) and man (3,8,73). Thorn and coworkers concluded from a similar series of experiments one-day in duration, that this action is common to many steroids-adrenal cortical, male and female sex hormones (75,76, 10,77,78), but that DCA was the most effective in this respect (10,78). In some reports the data were not presented in complete enough form for evaluation. In several reports the data is inconclusive for the normal animal except for the first day of injections, on which a negative balance did occur. In experiments in which the

urine was pooled for four or five days one is in doubt as to whether the total increase in potassium excretion occurred mainly in the first day or continued throughout treatment. In addition, the diet was usually high in salt, as for example in the experiment on the normal dog (54). The short-term experiments do not permit one to draw conclusions as to the total effect of the compounds in question. Thus, although it is established that DCA increases potassium excretion in adrenal insufficiency, this must yet be demonstrated unequivocally in the intact animal.

Loss of potassium from body fluids, whether produced by DCA or by a low potassium intake, results in severe morphological and functional abnormalities. These include extensive focal myocardial necrosis in the cardiac muscle of rats (45,35,72,80,81), mice (71), cat (35), and hogs (82), but not so far observed in dogs (35). Changes have been observed in the prominent Purkinje fibres of the bovine heart associated with electrocardiographic changes (69,79). Renal tubular hypertrophy with subsequent necrosis has been produced in rats, mice and dogs (84, 35,71,83,72). The most dramatic functional change produced by DCA was first noted by Kuhlmann et al in the dog (18). This is a muscular weakness and paralysis particularly of the hind limbs. This has been confirmed for the dog (50), one case of myasthenia treated with

large doses of DCA (85), and in clinical cases of Addison's disease (22), but has not been observed in the rat (86, 35,45). In the latter species the heart changes are most prominent. Complete paralysis was also noted in dogs maintained on a low potassium diet for 6 weeks by Ruegemer et al (87) and Smith (88), but not in rats (37).

The abnormalities in morphology and function are relieved or prevented by the administration of an adequate amount of potassium, as was shown by nearly all the investigators quoted above. The effect on the paralysed dog of potassium administration is dramatic. As described by Ruegemer et al (87) and Smith (88), an animal lying completely prostrate on the floor of the cage and able to make practically no movement, will rise from the floor and be walking around the cage within an hour after treatment. This rapid cure of paralysis by potassium resembles the action of this ion during attacks of familial periodic paralysis in man (89,90). The changes produced in the heart by a low potassium diet are arrested, but the lesions which are already present at the time of potassium therapy may heal by scarring (71). Other abnormalities noted in potassium deficiency will be discussed in the section on the gastrointestinal tract.

(d) The effect of DCA and of potassium-deficiency on sodium metabolism

In the normal animal relatively small doses of DCA have a marked effect in increasing renal retention of sodium (77,78,10,91,53,54,92). Associated with the sodium retention variable amounts of chloride and water are retained. Except for the observation of Ragan et al (49) that chronic treatment with DCA does not affect sodium and chloride balance significantly, balance studies have been only short-term experiments lasting one to five days. The subsequent effect is not therefore known. Since persistent elevation of serum sodium concentration has been observed in animals treated for two up to several weeks, (49,19,45,51) it is quite possible that sodium retention remains slightly elevated.

Since the sodium retention in DCA-treated dogs and man is usually proportionately greater than the water retention, the serum sodium concentration tends to become elevated slightly by 3 to 5 meq per litre (49,19,16,45, 51,54). An increase of 11 meq was observed by Clinton et al (53) in dogs when the salt intake was high.

The adrenal cortex has more influence on sodium than chloride balance.

In experiments lasting up to five days DCA has a greater effect in increasing sodium than in increasing

chloride balance in normal dogs (77,53) and man (54,92). The increase in sodium retention in two reports for dog (77) and man (54) by Thorn and associates was 48% and 46%, respectively, while the increase in chloride retention was 32% and 23%, respectively. Similarly, the absence of adrenal cortical hormone causes a greater loss of sodium than of chloride in dog (30) and man (93).

In adrenal insufficiency DCA is very effective in produced sodium, chloride and water retention for the duration of treatment (6,8,10).

Renal clearance of sodium and chloride is greatly increased in the dog by DCA (73) while glomerular filtration, as measured by creatinine clearance, is little affected. This experiment was probably an acute one lasting over several hours, although no details are given. It probably therefore explains the acute effect, only, of DCA on sodium and chloride retention. The effect of DCA in increasing sodium chloride retention is not specific to adrenal hormones, but also occurs to a smaller degree with androgens, estrogens and progesterone (54,77,78,10).

There is little evidence as yet to link the changes in sodium metabolism with potassium. Ferrebee et al (19) found that the administration of potassium chloride did not prevent the elevation of serum sodium in DCA-treated dogs in prolonged experiments. In potassium-deficient

rats Orent-Keiles and McCollum (42) found that the sodium balance was not significantly affected by the potassium-depletion lasting twenty-one months. There was a slight increase in the average but the values were very irregular.

The intracellular sodium content of the muscle increases both in DCA-treated rats and in those maintained on a low potassium diet. Ferrebee et al (19) and Miller and Darrow (45,38,35) found the intracellular sodium was increased in dogs and rats from a normal of approximately 1.5 - 2.5 to about 13 or 14 meq per 100 grams fat-free muscle solids. (These results have been obtained by a re-calculation of the data of these authors). The data of other investigators working on potassium-deficient and DCA-treated animals confirm this order of increase of sodium concentration, the extent of increase depending upon the potassium depletion as discussed in the section on potassium metabolism. (37,38,35,45,53,40).

A reciprocal relationship exists between intracellular sodium and potassium in muscle. This has been shown for rats treated with DCA or maintained on a potassium-deficient diet and for a variety of conditions in which potassium balance is affected (70,38,65,37,94,95,44). Darrow and Miller have been the chief investigators of this phenomenon. Thus intracellular sodium increases when potassium is depleted from the muscle through the action of DCA or from an inadequate potassium intake,

whereas intracellular sodium decreases shortly after potassium injections, and in nephrectomized and adrenalectomized animals where the serum and muscle potassium concentrations are high. Mere increase in sodium concentration in the serum without coincident loss of potassium, will not cause sodium to enter the cells (44). Therefore, the replacement of potassium by sodium is secondary to the loss of potassium. As Ferrebee et al (19) pointed out, sodium replaces but does not displace the potassium cation from the cells of DCA-treated animals. When potassium is lost from the cells, approximately one to one and a half sodium ions replace two potassium ions lost according to some investigators (38,45,50), although the data of others (19,37,40) indicate almost a one to one substitution of these ions.

According to the recent studies of Darrow (44,95), the shift of sodium into the cells such as is observed in DCA-treated or potassium-deficient animals, has an important role in compensation to certain types of alkalosis, including that from excessive amounts of adrenal hormone. It occurs probably only when potassium stores tend to be low, and represents the response of the cells themselves to compensate for a relative excess body sodium over chloride. In this way the sodium concentration of the serum is reduced. Darrow's conclusion is that in certain types of alkalosis, it is the alkalosis

itself which causes this exchange of sodium for potassium in the cells. Further fundamental work is required to establish the exact sequence of events resulting from DCA-treatment and to show the interrelation between the renal effects on sodium and potassium balance.

(e) The effect of DCA and of potassium-deficiency on water metabolism.

Disturbances in water balance also occur as a result of excessive amounts of DCA. The plasma and extracellular fluid volume (thiocyanate-space) can be increased by DCA in the normal dog and man to values averaging from 11 to 17% above normal (50,53,92). In normal men Clinton and Thorn (92) found the maximum increase occurred on the fourth day and plasma volume was maintained at high levels for 17 days in one man receiving both DCA and salt supplement. Swingle et al (96), however, obtained an increase in only two of four dogs treated with DCA. In the normal organism the action of DCA has not been appreciably increased by a high intake of salt. (53,92). The adrenal deficient organism, on the other hand, is particularly susceptible to this action of DCA, especially if the salt intake is high (6,8,96). In the adrenalectomized dog Swingle et al (96) noted that the plasma volume was maintained by DCA at a level 15% above the control value in chronic (not acute) insufficiency, after the initial peak of increase had occurred.

In rats no determination of extracellular fluid volume has been performed, undoubtedly because of the obvious technical difficulties in this small animal. In muscle the chloride-space has been taken as an indication of the extracellular in this tissue, or that the concentration inside the cells is small but constant in normal and DCA-treated animals. From this type of calculation, Darrow and Miller (45,35) and Hegnauer (40) have not observed significant changes in water distribution in muscle in either DCA-treated or potassium deficient rats. Muntwyler et al (51) have also reached a similar conclusion for dogs treated for 14 days with DCA and receiving salt supplements. However, this type of experiment has not been performed in the first one or two days or even within the first week of DCA-treatment in rats, when presumably, changes would be more apt to occur. They do show that the hypochloremia which developed in the later stage of treatment was not due to dilution of the extracellular fluid.

In addition to the effect of DCA on extracellular volume, a syndrome resembling diabetes-insipidus has been induced with DCA in dogs (52,98), rats (97,10,99), and in one clinical case of myasthenia gravis (85), but not in the cat (98) or rabbit (43). This polyuria was first shown by Kuhlmann, Ragan, Ferrebee, Atchley and Loeb in

the dog (18,19,49) with very large doses of DCA (20-25 mg per day), but doses as low as 2 mg are effective in the dog (52). In the experiments of Ragan et al (49) the water exchange increased seven-fold. The polydipsia appears to be the primary disturbance (19,49,52), for it precedes the polydipsia by several days, and when water is restricted dehydration is not severe, as it is in true diabetes-insipidus of pituitary-hypothalamic origin. True diabetes-insipidus is exacerbated by DCA in rats (98,1c) and dogs (97) but not in cats (97). Two other factors differentiate this condition from true diabetes-insipidus, namely it is mainly dependent upon the salt intake, and secondly it is resistant to the action of posterior pituitary extracts (49,52,97). The polyuria appears to bear no relation to potassium disturbances, for the administration of potassium chloride to these animals may increase diuresis (18), or have no effect (19). Ferrebee et al (19) and others (52), consider the polydipsia is associated with elevated serum sodium levels. The fact that this condition of polyuria occurred after a period of initial retention of water emphasizes the deficiency of the short-term tests of hormones on fluid and electrolyte metabolism.

No water balance studies have been performed in potassium-deficient rats.

(f) Summary of the effects of DCA and potassium-deficiency on electrolyte metabolism.

Prolonged treatment with relatively large doses of DCA usually causes hypochloremia. In short term experiments DCA increases chloride retention in the intact animal, but the effect of prolonged treatment has not been studied adequately. The muscle chloride is not significantly changed, and there is no indication that chloride enters the muscle cells. Hypochloremia is also produced by maintaining animals on a low potassium diet for prolonged periods. In these animals chloride excretion increases in the first week in contrast to the retention produced by DCA.

DCA produces alkalosis in potassium-deficient rats.

DCA causes pronounced potassium depletion. Renal excretion of potassium increases in the first few days of treatment, but in longer experiments it has been difficult to detect a significant change in potassium balance. With prolonged treatment as much as 40% of the intra- and extracellular potassium may be lost. As a result of the potassium depletion severe morphological and functional changes occur in various organs. The changes produced in animals maintained on a low potassium diet are similar to those produced by DCA.

DCA increases sodium retention and produces a small increase in serum sodium concentration. Sodium enters the cells to replace the potassium lost. In DCA-treated animals neither a high nor a low potassium intake prevents the increase in serum sodium, but a high intake of potassium prevents replacement of intracellular potassium by sodium. Sodium also replaces potassium lost from cells in potassium-depleted animals.

Water retention occurs in the initial days of treatment with DCA, but subsequently a condition of polyuria develops. The retention of water is associated with increased extracellular fluid volume. No early determinations have been made in the rat. The water exchange is not dependent upon potassium depletion since addition of potassium does not alleviate the polyuria. The polyuria appears to be secondary to polydipsia which in turn is regarded as a function of the increased serum sodium produced by sodium retention. No observations have been made on water exchange in potassium-depleted animals.

From a review of the literature it appears probable that the decrease in serum chloride produced by DCA is associated with potassium depletion. Further evidence for this is provided in the next section on hyperadrenocorticism. Both urinary chloride loss and hypochloremia occur in potassium-depleted rats, receiving no DCA. There

is no evidence that DCA increases chloride excretion, but experiments have not been sufficiently prolonged to prove this point. This problem has been investigated in the experiments to be reported. The increase in extracellular fluid which occurs shortly after treatment could account for hypochloremia if the water retention were proportionately greater than the chloride retention, or if water shifted temporarily from cells to interstitial fluid because of an increase in serum sodium concentration. This dilution factor has not been examined in the intact animal but does occur occasionally in adrenalectomized animals receiving larger doses of DCA.

Electrolyte Disturbances in Hyperadrenocorticism (Cushing's Syndrome).

It is of interest to consider a type of hyperadrenocorticism, Cushing's syndrome, in connection with the overdosage effects of DCA on water and electrolyte balance. There have been at least eight cases of this syndrome reported in the literature, mainly by Kepler and associates, in which electrolyte disturbances were observed which were essentially the opposite to those associated with adrenal insufficiency. (34,102,103,104,105,106,107). Definite alkalosis occurred associated with hypochloremia, hypopotassemia and elevated or normal sodium concentration. In most instances the bicarbonate concentration ranged around 35 as compared to the normal of 27 meq per litre, but was as high as 50 meq in one case of Kepler's (104). The chloremia in the untreated patients varied from 93.9 to as low as 50 meq per litre. In the cases determined the serum potassium values were about 2.5 meq per litre. (34,106,107). The fluid balance was negative (106). and the serum and blood volume were reported low (103,104) in the four cases determined. Polyuria and sometimes polydipsia were observed in four cases (103,104). Thus all the symptoms of DCA overdosage were observed in these cases except for the fact that the blood volume was low in Cushing's syndrome. This may have been due in part to the health of the patients and particularly to the

state of diabetes which existed. The effect of DCA and of clinical hyperadrenocorticism may be contrasted in this respect also, since DCA, as noted above. has no effect on carbohydrate metabolism.

In a balance study on one patient, Willson, Power and Kepler (106) found only one period of negative chloride balance and that was following admission before any treatment with salts had been instituted or the diabetes treated. After treatment was started, the serum chloride values were slightly low (97 to 99 meq per litre), but the chloride balance was definitely positive. Cluxton et al (107) states that there is no evidence that chloride is being lost in these patients but does not present evidence. On the other hand, Kepler (104) noted that in the one case where balance studies were possible, there was evidence that chloride excretion was greater than normal. Whether this was related to the diabetes is unknown. There is evidence from electrolyte balance studies (106.107) and from analysis of muscle biopsy that considerable amounts of intracellular potassium have been lost from the muscle of these patients and have been replaced by sodium (Kepler, quoted in (44)).

The adrenocortical activity of the blood of one patient was found by Anderson et al (102) to be very high. In other cases ablation of the adrenal tumor or hyperplastic adrenal has resulted in relief from these symptoms (104.105).

The electrolyte disturbances are relieved (34,106, 107) almost completely by the addition of potassium chloride to the diet. Potassium citrate was less effective, but this salt increased the serum chloride, as well as the potassium and bicarbonate changes, in one case of Willson et al (106), although Cluxton et al (107) found potassium citrate effective only in raising the serum potassium. Ammonium chloride caused an increase in serum chloride, but only following a period of high potassium intake. Sodium chloride was completely ineffective, even when given in large doses of 10 grams a day, either in increasing chloride or in alleviating other symptoms of the condition (34,106.107). Maintenance of one patient on a low potassium diet exaccerbated the alkalosis (107).

It is believed among these investigators (106,107) that the primary defect in this condition of hyperadrenocorticism is a failure of the renal tubules to reabsorb potassium, and that the hypochloremia observed is associated with the potassium depletion. The relationship between chloride and potassium is indicated by the fact that potassium chloride and possibly potassium citrate relieves hypochloremia and hypopotassemia, whereas sodium chloride is without effect, and by the fact that symptoms are exaccerbated by maintenance of the patient on a low potassium intake.

Muscle and Serum Electrolytes as an Index of Body Electrolyte Changes.

In most of the experiments relating to potassium, chloride and sodium metabolism, conclusions have been drawn for the behaviour of these ions in the body as a whole from observations made solely on skeletal muscle and serum. Not all tissues behave alike, but, in general, they follow a general trend in so far as these electrolytes are concerned. Since it is agreed that normally 42 to 45 percent of the body by weight is skeletal muscle, in man, rats, dogs, cats, and rabbits (108,109,110), and another 20 - 22 percent is extracellular fluid, these two determinations account for roughly two-thirds of the body weight. Extracellular fluid accounts for nearly all the body sodium and chloride, except for bone and small amounts of intracellular sodium and chloride. The skeletal muscle alone contains seventy percent of the intracellular fluid of the whole body (110). Another eleven to seventeen percent of the body is bone (108) in various species, for which the exchanges with extracellular fluid are very slow; so that this excludes essentially this fraction of the body weight, leaving twenty percent unaccounted for. Twenty-five percent of the body sodium is found in bone combined with calcium as an insoluble salt to form the matrix of the bone (110). Another fraction is formed of smooth and cardiac muscle, the latter of which responds

similarly to potassium depletion to skeletal muscle, (84,42,35,72), and the former probably also resembles the other types of muscle in its electrolyte responses.

The intestines are composed of smooth muscle and connective tissue, mainly, and comprise four to six percent of the body weight in animals (108). The smooth muscle may be considered with other types of muscle. The connective tissue, according to the careful studies of Manery, Hastings and Danielson (112,113), may be classed with the extracellular fluid phase. In brain, kidney and spleen (37,56) the alterations in electrolytes produced by DCA or potassium-depletion are similar to those produced in muscle, although not as great. In the liver, on the other hand, which comprises about four percent of the body weight, the electrolyte concentrations are probably not appreciably influenced by either of these treatments, according to Heppel (37) and Darrow and Miller (35). Schweizer, however, found that DCA decreased liver potassium, as it does the concentration of this ion in other tissues (56). In this organ the potassium is more labile, and is closely associated with carbohydrate movements (114).

Thus, it may be concluded that good estimates can be provided by skeletal muscle and extracellular fluid determinations of changes in the balance of electrolytes in the body.

EXPERIMENTAL

Experimental Procedure

Experiments have been performed to study the hypochloremia produced by DCA and that produced by a low potassium dietary regime.

(a) The dosage of DCA required to produce significant hypochloremia was determined in rats force-fed a normal diet. Attempts were made to determine extracellular fluid volume in rats, in order to find out what role dilution of extracellular fluid played in the hypochloremia produced by DCA. This was unsuccessful.

(b) The acute effect on serum chloride and potassium of DCA was determined and compared with the renal excretion of these ions.

(c) The efficacy in producing hypochloremia of DCA, of a low potassium and of the combination of these, was compared in animals fed ad libitum.

(d) The efficacy of various salts of potassium, sodium and chloride in relieving DCA hypochloremia was compared.

(e) Chloride and potassium balance studies were performed in DCA-treated and potassium-deficient rats to determine whether chloride loss occurred, and how it was related to urinary potassium excretion.

Methods.

Throughout the experiments male albino rats were used. In all force-feeding experiments the animals weighed from 275 to 300 grams, initially. Animals allowed the synthetic diets ad libitum weighed on an average 150 grams initially. The body weight of rats used to test the acute effect of DCA was about 100 grams.

Diets.

Several synthetic diets were used in the course of the experiments, the composition of which is given in Table I. The diets were either used in the dry form and allowed ad libitum or were suspended in water and force-fed by stomach tube. Diets A and B contain the crystalline vitamins of B complex noted in Table I as well as an alcoholic potassium-free extract of yeast prepared according to the method of Miller (115), and mixture of many types of salts (modified from Steinbach salt mixture 32). The only difference between diets A and B is in the potassium content; all other minerals are the same. The addition to diet A of phosphate as the potassium salt was compensated for by the substitution of calcium lactate for part of the calcium phosphate. In the dry form, diets A and B contained 10.0 and 0.25 meq of potassium per 100 grams, respectively, and both contained 6.00 meq of sodium chloride. When dissolved in water, the fluid diets A and B contained

TABLE I

THE CONSTITUENTS OF THE VARIOUS SYNTHETIC DIETS

DIETS / CONSTITUENTS	A %	B %	C and D %	E grams
Sucrose	29.6	30.2	55% 56%	360
Dextrin	24.7	24.7		
casein (low ash)	21.7	21.7	25%	
Whole milk powder				600
Cellu-flour	9.9	9.9		120
Corn oil	6.4	6.4	10%	10
Wheat germ oil	1.5	1.5	1%	10
Cod liver oil	1.5	1.5	1%	10
Yeast (Pabst)			1%	100
Yeast extract *	(equivalent to 4 grams of yeast)			
Salt mixture **	2.02	2.02		40
Bone ash			2%	
NaCl	0.41	0.41	1%	
K_2HPO_4	0.82			
KCl			1% in C only	
Calcium phosphate	0	0.82		
Calcium lactate	1.48	0		
Vitamin K				100 mg.
Vitamin mixture ***	40 mg	40 mg		
Dietgrams	100 grams	100 grams	100 grams	1200
Water added for fluid diet	72 cc	72 cc		960 cc
Total volume	140 cc	140 cc	***	2000 cc

** Salt mixture for 100 grams of diet A and B

	grams
Magnesium sulfate	0.613
ferric citrate	0.133
sodium iodide	0.077
manganese sulfate	0.504
zinc sulfate	0.027
cupric sulfate	0.033
calcium phosphate	0.636

*** Vitamin mixture for 100 grams of diets A and B

Vitamin K	6 mg
Thiamin	?
Riboflavin	1.5
Pyridoxine	1
Calcium pantothenate	2.5
Nicotinic acid	1.5
Choline chloride	25.0

** Salt mixture for Diet E was Osborne and Mendel mixture which contains 22 grams of potassium phosphate and 12.5 grams of potassium chloride and 7.7 grams of sodium chloride per 100 grams of slat mixture.

* The alcohol yeast extract was prepared according to the method of Miller (115). Yeast was extracted repeatedly with water and then alcohol was added and tartaric acid which precipitates with potassium as the tartrate. The extract was partially evaporated at room temperature after preparation as agove, in order to reduce the volume of extract added.

7.06 and 0.18 meq of potassium per 100 cc of diet. In the first experiments a concentration of chloride of 7.6 meq per 100 cc of diet was used, but later this was reduced to 4.24 meq per 100 cc. This latter amount corresponds to that added to the dry diets, that is 6 meq per 100 grams of dry diet, and is more than adequate for the normal growth of rats (116). This alteration in chloride intake had no effect on the electrolyte balance. The diet fed during the control period for each animal was identical in composition to that fed during the experimental period, except for the potassium content. Diet C and D are similar diets used in preliminary experiments in which unextracted dried yeast (Pabst) is used as a source of vitamin B complex and potassium added as the chloride to the control diet. Other salts are added in the form of sodium chloride and bone ash. The content of potassium was calculated for diet C as approximately 13 meq and that of diet D as approximately 0.40 meq per 100 grams. Diet E is a normally balanced fluid diet, which contains a higher concentration of potassium from the yeast and salt mixture alone (Table I - footnote), although the potassium concentration was not determined. The chloride concentration was 13 meq per 100 cc. Animals used to test the acute effect of DCA were maintained on purina prior to the experiment.

The animals fed ad libitum were kept one or two per cage and a rough estimate of the food intake obtained by weighing the food jars daily. An estimate of the caloric intake was made from the caloric value of the constituents of the diet.

The fluid diets were in stable suspension being mixed with the aid of a "mix-master," handling for several days prior to use in experiments to adapt the animals to the factor of handling. The animals were fed at the same time each day at about an eight-and-one-half hour interval. During feeding the animals were held gently in the left hand while the tube was passed down readily into the stomach. They were placid after the first day, undisturbed by this technique of feeding. They were first adapted to the force-feeding technique by gradually increasing the daily allotment in three or four days up to full feeding, which was 25 cc per day, and following this they were maintained for at least a week before control balance studies were performed to allow for stabilization of electrolyte excretion. In several experiments throughout both control and experimental periods 18 or 20 cc of the diets A and B were fed, instead of 25 cc in order to reduce the stress upon the hypotonic gut of potassium-deficient animals and delay the onset of gastro-intestinal stasis. The control and experimental results were obtained therefore under comparable conditions.

In metabolic experiments the rats were kept in individual cages and were force-fed. The water intake (distilled water) and output was measured. Urine was collected for chloride and potassium determinations at the same time every morning after the grids and funnels were washed down with distilled water. Care was taken to prevent loss of urine by excretion during the handling of the rats, by compressing the bladder before the animals were removed from the cage.

In the balance experiments, each rat served as his own control. No fecal analyses were made. A control study of 8 to 10 days was made during the dietary A regime. The balance studies were continued in the same rats during the experimental period.

DCA was injected in crystallinesuspension in water (to permit prolonged action). Doses greater than physiological were used in order to obtain the maximal effects. From 2 to 4 mg. was injected subcutaneously per day, except in the experiment testing the effect of dosage.

Blood samples were collected during the experiment from the tail of the rat for chloride determinations, which involved error due to the chloride shift from blood cells to serum, but which gave an idea of the change produced. At the termination of the experiments the animal was anesthetized with urethane which has been found to have only minor effects on electrolytes of the serum (117), and blood was collected under oil from the abdominal aorta. Serum potassium and chloride determinations were made on this. In several instances in the force-fed animals, and in all

the animals fed ad libitum of diets A and B, blood was obtained by cardiac puncture under urethane anesthesia for both chloride and potassium determinations. This was done because it was found that serum from tail blood or blood obtained from the heart without anesthesia tended to have more irregular and higher potassium concentration. Muscle and small intestine samples were collected under anesthesia for electrolyte determinations. In the present report only the water content and a couple of muscle potassium values are reported since electrolyte studies are not completed. Tissue water was determined after dessication at 100°C until constant weight was reached.

Chemical Methods. The potassium content of the diets, urine and serum was determined by ashing the samples and by subsequent precipitation with silver-cobaltinitrite reagent at 20°C. The precipitate was washed 3 times with a washing reagent composed of alcohol, water and ether. The washed precipitate was dissolved in dilute sulfuric acid. The blue color developed by adding alcoholic ammonium thiocyanate solution to the dissolved precipitate was determined in the Evelyn colorimeter with Filter #620. A series of standards were run with each set of unknowns; and at least two determinations on each sample were made. In analysing the accuracy of the method duplicates were run through the whole chemical procedure as if they were separate samples, for a series of 80 serum and urine samples over a period of several months. The standard deviation of the differences between each mean and its duplicate values was 1.1 percent.

Chloride was determined in urine and serum by the method of Sendroy (118) and modified by Van Slyke and Hiller (119). The standard deviation of this method was 0.3 percent. Dietary chloride was determined by the usual thiocyanate titration following digestion in silver nitrate solution (Peters and Van Slyke, 120), and whole blood chloride by Patterson's micromodification (121).

An attempt to determine "thiocyanate-available" fluid volume, as an estimate of extracellular fluid volume, was made. This was based on methods used in larger animals. However, although many of the results were quite consistent about a figure of 27 volumes percent of the body weight, about one in ten values were very high in normal animals. The errors were considered to be too high to detect changes of the order of ten to fifteen percent in extracellular fluid volume.

Statistical analyses have been performed using the usual formula for standard deviation of samples within a group, and using paired samples in most cases to evaluate the difference in behaviour of animals to treatment (122). In the following formulae n refers to the number of animals in a group, n-1 being the number of degrees of freedom. $\bar{X}$ is the mean of a group, and x the individual samples, d is the difference between individual paired samples, and $\bar{X}_d$ refers to the mean of the differences.

$$\text{S.D. (standard deviation)} = \sqrt{\frac{(\overline{X} - x)^2}{n - 1}}$$

$$\text{S.E.}_{\overline{X}} \text{ (standard error of the mean)} = \sqrt{\frac{(\overline{X} - x)^2}{n\ (n-1)}}$$

$$\text{S.E.}_{\overline{X}_d} \text{ (standard deviation of differences between paired samples)} = \sqrt{\frac{\overline{X} - (x_1 - x_2)}{n\ \ (n - 1)}}$$

and "t" or Students test of significance

$$= \frac{\overline{X}_d}{\text{S.E.}_{\overline{X}_d}}$$

When the value of "t" is 3 or more, there is less than one chance in a hundred that the difference $\overline{X}_d$ is due to chance, and therefore this difference is highly significant. With a "t" value of about 2.6, there are five chances in a hundred that this difference between groups will occur by chance alone, and the difference is considered to be probably significant, or on the border between significance and insignificance.

EXPERIMENTAL RESULTS

A. The effect of various dosages of DCA upon whole blood chloride in rats.

This experiment was performed to determine whether small doses of DCA can produce hypochloremia, in order to establish whether this effect is due to overdosage.

These rats were force-fed the normally balanced diet E throughout the experiment. The potassium content of diet E is greater than that of the other normal diets used (diets A and C). Blood was collected from the tail during control and DCA periods. The results and treatment are summarized in Table II.

Table II

The effect of dosage of DCA upon whole blood chloride in rats force-fed a normal diet.

No. of animals	DCA mg/day	weeks of DCA	Whole blood chloride (meq per litre blood) Initial	S.D.*	DCA	S.D.*	"t"
10	0		77.6	1.1			
2	0.05	4	78.5	-	82.0	-	-
6	0.10	4	77.2	1.7	75.4	2.2	1.62
6	1.0	3	76.5	1.4	73.3	1.9	1.82
6	5.0	3	77.2	2.3	70.9	2.3	4.27
6	10.0	3	77.3	1.4	69.9	1.7	7.6

* Footnote: see statistical methods.

These data show that in rats force-fed, a diet of more than 1 mg of DCA per day was required to lower the whole blood chloride significantly. With doses of 5 or 10 mg. treatment for about three weeks was required to lower the serum chloride by an average of 6 to 7 meq. These doses were equally effective. The smaller dose of 1 mg. did decrease serum chloride slightly in some animals, but

this effect was not significant statistically; Therefore, it is concluded that the hypochloremia action of DCA is an overdosage effect, since the optimal physiological dose for an adrenalectomized rat is of the order of 0.1 to less than 0.5 mg. per day (17a). The physiological dose of an adrenal hormone is the dose required to maintain life in adrenalectomized animals.

A few remarks may be made regarding the water exchange. In these animals receiving a relatively high intake of sodium chloride (2.7 meq. of chloride daily) relative to their requirements, DCA increased the water exchange after a delay of one or two days. As has been observed for the dog (52) polyuria and polydipsia were parallel except during this initial period. The increased water exchange was not correlated with the hypochloremia. It occurred with doses of one mg. or more. The level of diuresis reached finally was two to two and one half times the control value of 14 to 17 cc a day. The maximum increase was as great with 1 mg as with 10 mg, but with the larger doses the increase was more rapid. The maximum effect thus was attained in approximately two to two and one half weeks with large doses, while with 1 mg dose the maximum diuresis occurred in an average of three and one half weeks.

B. The acute effect of DCA upon Serum Chloride and Potassium.

The effect of DCA on serum chloride and potassium was investigated a few hours after administration to find out

how quickly this steroid affects electrolyte concentrations and to determine whether early changes in serum chloride concentration can be correlated with renal excretion of this ion. In previous experiments of the author (1a) in young rats it has been shown that a large dose of DCA (6 mg) can lower blood chloride within four hours. The blood chloride was reduced from 75 to 70 meq per litre. In the present study this has been confirmed in one rat of body weight 105 grams given a 5 mg dose of DCA. The study is preliminary since only one control rat and one experimental animal was examined, but the results are reported since they substantiate findings in the balance studies and point to the existence of one of two mechanisms by which DCA can produce hypochloremia.

The data for these two rats are given in Table III. Urinary analyses of potassium and chloride were performed for the eight hour period of the experiment. The bladder was washed out with distilled water at the end of the period. The animals were fasted prior to the test and no food or water was allowed during the test.

The data show that within 6 to 8 hours a very large dose of DCA can reduce both serum chloride and potassium concentration to values lower than normal. The serum chloride concentration was reduced by 9 meq per litre, and the potassium concentration of the DCA-treated rat, was 0.6 meq lower than the control animal, as well as

lower than any normal rats which have been studied. The serum water was higher (93.9 grams percent) than other animals which have been observed in this colony, the range of normal being 92.2 to 93.2 grams per 100 grams of serum. As shown from the calculations below the hypochloremia could not be accounted for by the excretion of 0.1 meq of chloride. Since tissue potassium studies were not performed on the muscle, little discussion can be made of the potassium loss except to point out that the urinary excretion can only account for the decrease in the extracellular potassium concentration of 0.6 meq per litre (Extracellular fluid volume X $\frac{\text{decrease in extracellular K concentration}}{1000\ cc}$

$$= .25 \times 105 \times \frac{4.23 - 3.61}{1000\ cc \times .939} = .016 \text{ meq})$$

In calculating the total loss of chloride from the extracellular fluid which would be required to account for the hypochloremia observed, it will be first assumed that the "chloride-available" extracellular fluid of the body is not altered by DCA. The normal volume of "chloride-available" fluid is approximately twenty-five percent of the body weight (123). Therefore the total loss of chloride from this fluid (in practice, the extracellular fluid of the body) can be calculated as follows, the decrease in serum chloride being 9 meq per litre and the body weight, 105 grams:

decrease in extracellular Cl concentration x extracellular fluid volume

$$= \frac{9}{1000 \times 0.939 = 0.95} \times (0.25 \times 105) = 0.27 \text{ meq}$$

where 0.95 and 0.939 are the Donnan factor and the serum water concentration used to convert the serum chloride to extracellular (ultrafiltrate) chloride concentration. Thus, assuming that the volume of fluid in which the chloride is distributed remains constant, a loss of 0.27 meq chloride from the extracellular fluid has occurred. The loss of chloride in the urine was only 0.1 meq. Therefore this does not account for the early hypochloremia produced by DCA. Since chloride was not lost from the body, the volume in which chloride is distributed (the extracellular fluid) must have been increased. This indicates that hypochloremia can occur in the rat as a result of expansion of the extracellular fluid soon after the administration of DCA. As discussed in the review of the literature it is unlikely that chloride enters the cells as the result of DCA-treatment, while increase of the extracellular fluid has been shown to occur in dogs.

Table III

THE ACUTE EFFECT OF DCA ON SERUM CHLORIDE AND POTASSIUM CONCENTRATION

	DCA	Control - no DCA
Serum chloride meq/l		
the day prior to experiment	109.4	107.4
6 hours after DCA (tail blood)	100.2	
8 hours after DCA (arterial blood under oil)		
Serum potassium meq/l		
8 hours after DCA	3.61	4.23
Urinary potassium meq in 8 hours	0.014	-
Urinary Chloride meq in 8 hours	0.106	
Serum water grams / 100 grams	93.9	
Muscle water grams/ 100 g solid	73.3	74.5

C. The effect of DCA on serum chloride of potassium-deficient rats.

The extent to which the serum chloride can be lowered was compared in normal rats treated with DCA, in rats maintained on a low potassium diet and in rats maintained on a low potassium diet and treated with DCA. These animals were then used to test the effect of various salt solutions upon the hypochloremia. The diets were fed ad libitum.

The diets fed were diet C and D, which are normal and low potassium diets, respectively. Three groups of rats weighing between 150 and 175 grams were used. In the first diet C was fed throughout, and DCA was administered daily in a dose of 2 mg. for twenty days. In the second group the low potassium diet was fed for 35 days. In the third group a low potassium diet was fed for eight days prior and during the ten day period of DCA treatment (2 mg daily). Throughout the experiment the serum chloride was determined from tail blood after a fast of eight hours. The data are presented in Table IV.

The Effect of DCA and Low Potassium Diet on Serum Chloride

TABLE IV

		DIET	PURINA	LOW POTASSIUM		NORMAL POTASSIUM	
		DAYS ON DIET	-	8	18	35	9	20
GROUP NO.	RATS	DAYS ON DCA	-	0	10	0	9	20
I	6		106.9 * ± 1.9				99.5 ± 3.0	97.3 ± 2.1
II	6		106.1			97.2 ± 2.7		
III	16		105.8 ± 1.7	103.6 ± 2.3	92.6 ± 3.2			

Footnote: * Serum chloride concentration in meq per litre given with standard deviation.

These data show that the serum chloride was reduced 9 to 10 meq per litre by either a low potassium diet or DCA injections of 2 mg per day. DCA produced a more rapid decrease in the serum chloride than the low potassium diet. As can be noted from group III a decrease in serum chloride occurred when the rats were maintained on diet D for only eight days. This difference of 2.2 meq per litre was small but highly significant statistically ("t" was 7.3) because of pairing of samples. It occurred in all but two animals. The serum chloride was lowered 11 meq further by 10 days of DCA treatment to values which were lower than were observed in either group I or II.

These results confirm those of other investigators which have shown that serum chloride may be reduced by any of these three procedures. In addition these data permit a comparison between the effectiveness of the three treatments in producing hypochloremia, the combined treatment being as one would expect the most effective, the injection of DCA the second and a low potassium regime the third most effective means of producing hypochloremia.

D. The effect of salts on DCA-hypochloremia.

In twenty of the DCA-treated rats used in the above experiments isotonic solutions of various salts of potassium, sodium and chloride were administered to test the effect of these ions in returning the hypochloremia. This was

performed to compare the hypochloremia produced in DCA-treated rats to that observed in Cushing's Syndrome. The rats were treated with DCA for ten to twenty days and maintained on diet D, or, in a few cases, on diet C, until hypochloremia was marked. The chloride intake was normal throughout. The salt solutions were administered by stomach tube repeatedly over a sixteen hour interval each day. This was done in order to ensure that the animals received the same dose of ions. The salts administered were potassium, chloride, potassium citrate, sodium chloride and ammonium chloride. The total dose given in one and one half to two days was 0.93 to 1.8 meq of the potassium, sodium or chloride ions. Eight hours prior to blood sampling the food was withdrawn and no salt solutions were administered in this interval.

The results are summarized in Table V. These show that potassium chloride, potassium citrate and ammonium chloride increased the low serum chloride concentration partly towards normal, while sodium chloride had no effect. Four days following the salt administration in the various groups, the serum chloride had returned to the initial low levels again, since DCA-treatment was continued throughout. These experiments show that an increase of the potassium ion alone (potassium citrate), increased the serum chloride. The administration of the chloride ion in the form of sodium salt had no effect, but chloride in the form of the ammonium and potassium salts were effective in these short term experiments.

The results confirm the observations of Willson et al (106) and Cluxton et al (107) on the hypochloremia of Cushing's syndrome in regard to the effectiveness of potassium chloride and inefficacy of sodium chloride on the hypochloremia. The former investigators found ammonium chloride. following a period of potassium administration, and potassium citrate increased serum chloride. In the experiments reported in Table V which lasted for two days both of these salts also increased the serum chloride, the ammonium chloride being effective for this period even in the absence of potassium.

It was planned to run similar experiments in force-fed rats with the ions added to the diet, to remove the objection to oral gavage. The development of gastro-intestinal disturbances prevented this, except for a few tests with potassium phosphate and sodium chloride added to the diet. In these the potassium salt resulted in an increase in serum chloride, and a decrease in urinary chloride loss, while the sodium chloride, in a small dose, was ineffective. These observations will be considered with the balance studies.

Table V

EFFECT OF SALTS OF POTASSIUM, SODIUM AND CHLORIDE ON DCA-HYPOCHLOREMIA*

Treatment DCA		DCA and treatment with salts		DCA-4 days after salt treatments	
No. Rats	Cl_S**	salt	Cl_S	No. rats with increased Cl_S	Cl_S
5	93.6	KCl	99.2	4	94.5
7	93.5	K-citrate	98.6	7	93.9
4	96.3	NaCl	96.4	1	96.0
4	93.9	NH_4 Cl	99.4	3	-

* after one and one-half to two days of treatment with the various salts.

** Cl_S = serum chloride in meq/litre

E. The effect of DCA and potassium-deficiency on the potassium and chloride balances of force-fed rats

Potassium and chloride balance and serum studies have been performed in rats treated with DCA, in rats fed a low potassium diet and in rats fed a low potassium diet and treated with DCA. These experiments were performed to determine whether chloride is lost in the urine, and whether a loss, if it occurs, could account for the hypochloremia which is produced by these treatments. Potassium balance experiments were performed to determine the actual potassium loss from the body since this has not been performed before in DCA-treated rats and to correlate the hypochloremia with potassium changes. In addition, a repetition was planned of the experiments testing the effect of sodium, potassium

and chloride administration on hypochloremia, but only a few observations were made because of the gastro-intestinal changes which developed. Attempts to determine the extracellular fluid volume by an adaptation of the thiocyanate method (124,125,126,127) to the rat, were unsuccessful.

Treatment: Balance studies were performed in rats weighing an average of 290 grams. They were fed a constant amount of diet by stomach tube at regular intervals in order to eliminate variation of intake of food. The diets A and B are described in Table I and the technique of force-feeding is given in the section on method. Each animal was observed during a control period on Diet A and during the experimental period.

Unfortunately, the animal quarters were not well-regulated and variations in temperature occurred from day to day. Since these influence the sodium chloride excretion, especially, the urine was pooled for two days in most cases to help cancel variable not related to the specific treatment. Rats were first fed the full feeding of 25 cc of the diets but since disturbances in the gastro-intestinal tract developed in potassium-deficiency, it was found advisable to prolong survival by reducing the dietary allotment to either 20 or 18 cc per day by eliminating the dietary bulk, celluflour. The animals were studied until defication ceased or until retention of fluid in the gut could be detected.

At this point it is necessary to mention the gastro-intestinal disturbances which occurred in these force-fed potassium deficient rats. These prevented prolonged balance studies under these conditions and led to the death of the animals in acute distension of the whole gastro-intestinal tract. As a result of the fluid retention in the gut and possible inaccuracy it introduced at an unknown point of the balance period, the experiment was terminated after a total of 11 animals were studied and conclusions are based on these observations. Attention was then concentrated upon the abnormalities produced in the gastro-intestinal tract. In potassium-deficient rats force-fed 25 cc of synthetic diet defecation ceased as a rule in 6 to 10 days but in some animals treated with DCA no feces were excreted after 4 to 5 days. Gastro-intestinal distension developed and became of tremendous proportions.

All the untreated rats died before two weeks of potassium-depletion, and if they received DCA they usually died in about 8 to 9 days. In the distended rats fluid and gas, and later unabsorbed food, was retained in the atonic, flaccid bowel. Whether the fluid in the gut was increased prior to the cessation of defecation is not known. X-ray studies were performed in other deficient rats during the first five to seven days of deficiency which was prior to cessation of defecation. Since these did not reveal the characteristic fluid and gas accumulation or appreciable

dilatation, it is probable that the fluid loss into the gastro-intestinal tract may be neglected for the first five to six days in rats fed the full feeding of 25 days. In experiments in which survival was prolonged by feeding the animals smaller aliquots of food and eliminating cellu-flour (rats 205,204,103,105,310 and 303 of figures 1,3, and 4), longer examination of the balance of electrolytes was possible before distension occurred. The sudden increase in retention of chloride seen in rats 101 and 106 (figure 2) from the 7th-8th day and 9th-10th days, respectively, and after, was probably associated with retention in the gastro-intestinal tract since defecation had almost, but not quite ceased by the 7th and 9th day, respectively.

Growth: Force-fed animals given 25 cc of Diet A (49 calories) gained an average of 1.6 grams in weight per day after adaptation to the diet. Weight was lost initially during adaptation. When the animals were maintained on 18 or 20 cc of diet A (35 or 39 calories) the gain averaged 0.5 grams per day. The gain in weight of potassium-deficient rats was similar, averaging 1.7 grams over the first eight days in rats fed 25 cc of diet. It is possible that the retention of fluid in the body proper and/or in the gut contributed to this gain, even prior to the development of gastro-intestinal disturbances, since rats allowed food ad libitum gained little weight.

When food was allowed ad libitum to rats of initial weight of 150 grams, the daily intake of dry diet A was roughly 15 grams equivalent to 40 calories. This amount contained 1.4 meq of potassium. The average gain in weight of rats maintained on this diet for 47 days was 2.2 grams per day. Rats kept on dry diet B ate on the average 12.5 grams a day over a period of forty days, following which the intake was more irregular and the animals more wasteful of the food, so that food weighings were not continued. This was equivalent to 33 calories and contained 0.035 meq. The gain in weight of one group was 0.6 grams per day over a period of thirty days, while a second group of different strain gained 0.2 grams daily over a period of sixty days. Thus there was a difference in the rate of growth between the potassium-deficient and the control animals. This was not dependent upon the intake, which, although smaller in the deficient animals, was adequate. This indicates that the utilization by the body of the food is less efficient in potassium-deficiency than normal, a conclusion also reached by Orent-Keiles and McCollum (42). Since the animals used in the experiments reported here are adult rats, potassium deficiency was not as serious as it is in young rats and some growth was possible.

Charts: Potassium and chloride balance studies were completed on three rats given diet A and DCA, three rats given diet B, and five rats given both diet B and DCA injections. The results for each animal are represented in separate charts giving control and experimental balances for each animal (figures 1 to 4). The data have been analysed in Table VI. In the charts the chloride and potassium balances are represented, in most cases for two day intervals, for the control and experimental periods. The serum chloride values are given for the day of the balance on which they were determined. The figures on the top line are the values for the first day of the two-day period, and those on the lower line are the values for the second day. In the Table VI figures for the average daily retention of chloride and potassium during control and experimental periods are given. Since the animals were adult and the gain in weight did not differ in the two periods of each experiment, the body requirement of each ion was then assumed to be the same throughout. Therefore, the difference in the potassium and chloride balances between the control and experimental periods (column C) was calculated as the algebraic difference between retentions during the control and experimental periods. It may be mentioned again that in these balance studies the fecal excretion was excluded throughout, so that the balance figures are not absolute. In comparing the control with

the experimental period this factor presumably cancels out. The fecal potassium excretion has been observed to be very small and relatively constant in potassium-deficient and normal rats (49). In experiments preliminary to the metabolic studies reported here the author has determined chloride excretion in the feces of normal and DCA-treated force-fed rats, and found only traces of chloride excreted by this route. The amount excreted was the same in both groups of animals averaging 0.031 meq $\pm$ 0.003 per day.

Calculations: The control serum chloride concentration and the lowest serum chloride obtained towards the end of the experimental period are also presented in Table VI. From this the loss of chloride from the extracellular fluid was calculated (column F) on the assumptions that the chloride is distributed through the same volume of fluid in control and experimental period, that this "chloride-available" volume is 25 percent of the body weight (123) or 75 cc in a 300 gram rat, and that 92.5 percent of the serum is water, a figure obtained as an average of serum water determinations. Thus the total loss of chloride was calculated from the decrease in concentration in 75 cc of extracellular fluid water as follows:

$$\frac{((Cl)_a - (Cl)_b)}{1000} \quad \frac{100}{92.5} \times \frac{1}{0.95} \times 75$$

where 0.95 is the Donnan factor allowing for greater anion concentration in the interstitial fluid from which the indiffusible anion of the serum (protein) is excluded.

The loss of body potassium (column G) was calculated on the assumption that 0.25 percent of the body weight in the rat is potassium, a figure noted by several investigators (124,125,126). Thus the total body potassium in the normal rats weighing 300 grams is approximately 19 meq. The total loss of potassium in the number of days noted (column E of table 6) was calculated as a percentage of 19 meq.

Chloride balance: As can be noted from the charts (Figure 1,3, and 4), DCA increased chloride retention for about two days, significantly in five of eight rats (204,102,103,303, 301), and very slightly in a sixth (205). After the first two or three days, in all but one of the eight rats (102), a negative balance of chloride occurred, and in one case (108), on low potassium diet, this reduction to negative values occurred immediately after DCA without any initial rise or maintenance of chloride balance. The only animal of the whole group of 11 rats in which no loss of chloride occurred during DCA treatment or during potassium depletion was 102. Coincident with this observation this animal was the only one in which the serum chloride was not reduced appreciably at the end of the treatment. In two of the animals depleted of potassium by maintenance of Diet B alone (101,106), the chloride balance became negative immediately (Figure 2). The initial chloride retention which occurred in DCA-treated rats was not observed. Rat 104 on diet B

is included mainly for the potassium balance since this animal developed pneumonia from aspirated food and the loss of chloride may have been due to this on the last day (5th day). A decrease in chloride balance occurred but not until the third day. It is of interest that the addition of sodium chloride to the diet during the experimental period in rat 103 did not prevent the development of a negative chloride balance (Figure 3). Diet B with 7.6 meq percent chloride was used during the experimental period as compared with diet A with 4.24 meq percent during the control. On the other hand, the addition of potassium to the diet of rat 105 (Figure 3) immediately brought the chloride balance up to + 0.01, which was associated with a marked retention of potassium; the DCA injections were continued throughout. Two days later the DCA reduced the potassium and chloride values to slightly negative retention despite the continuation of the high potassium intake. In the case of rats 101 and 106 (Figure 2) it is very possible that retention of fluid and ions in the gastrointestinal tract occurred and that this accounts for the return of the chloride balance to normal positive as has been discussed above with the notes of the gastrointestinal changes.

Serum chloride: In several cases of DCA-treated rats a small decrease of about 2 to 5 meq per litre of serum chloride occurred during the first two (rat 102, 204, 205, 103, 105). Rats 303 and

301 were not tested until the third day when balance was already negative. This early decrease in serum chloride was associated with either an increased or normal chloride retention. At the end of the experimental period the serum chloride was reduced by an average of 12 meq below the control concentration. This average was for ten rats (excluding rat 104) and for a duration of DCA or low potassium regime lasting an average of eight days. There are not sufficient comparable data to draw conclusions as to the loss of serum chloride by the three treatments. Under the conditions of the experiment there was no appreciable difference between the rats of the three groups. The later hypochloremia was associated with the loss of chloride from the body. Thus reduction of serum chloride by DCA was produced both during the initial phase when chloride was retained or not lost, and during the phase in which chloride balance was negative. This indicates that with prolonged treatment DCA influenced the serum chloride concentration by two different mechanisms.

It is interesting that the figures for chloride loss from the urine (Table VI column E) account reasonably well for the loss of extracellular chloride (column F) in seven of nine rats which developed hypochloremia. The agreement is not so close in rats 108 and 303, the former of which behaved differently than the other rats with regard to chloride loss in urine. Among the group of nine animals the urine loss averaged 1.13

meq as compared with the extracellular fluid loss of 1.17 meq. Excluding rat 108, in which the difference between E and F is so marked, the average urine loss for 8 rats was 1.06 meq as compared with an extracellular chloride loss of 1.21 meq. The deviations were large in percent, but not in actual amount of chloride. Considering the number of assumptions and the tendency towards retention of fluid in the gut after several days of potassium deficiency, and the fact that repeated bleedings of the animals remove chloride from the body which has not been considered in the chloride loss column (E). (0.2 cc of blood was removed from the tail for a serum determination of chloride), it is surprising that the serum chloride loss is accounted for so well by urinary chloride loss. From this it is concluded that the hypochloremia produced by prolonged treatment with DCA is due to renal loss of chloride.

The addition of potassium to the diet of rat 105 (figure 3) increased serum chloride from 97.9 to 101.1 meq per litre. In another rat fed diet B alone (rat 201 not included in the balance studies), the serum chloride was increased from 81.2 to 93.4 in one day. The very low value of 81.2 was probably partly due to fluid retention in a visibly distended gut. The increase of sodium chloride content of the diet B fed rat 103 did not influence the hypochloremia produced.

Serum Chloride in rats fed ad libitum: It took longer to produce hypochloremia in rats fed diet B ad libitum. In

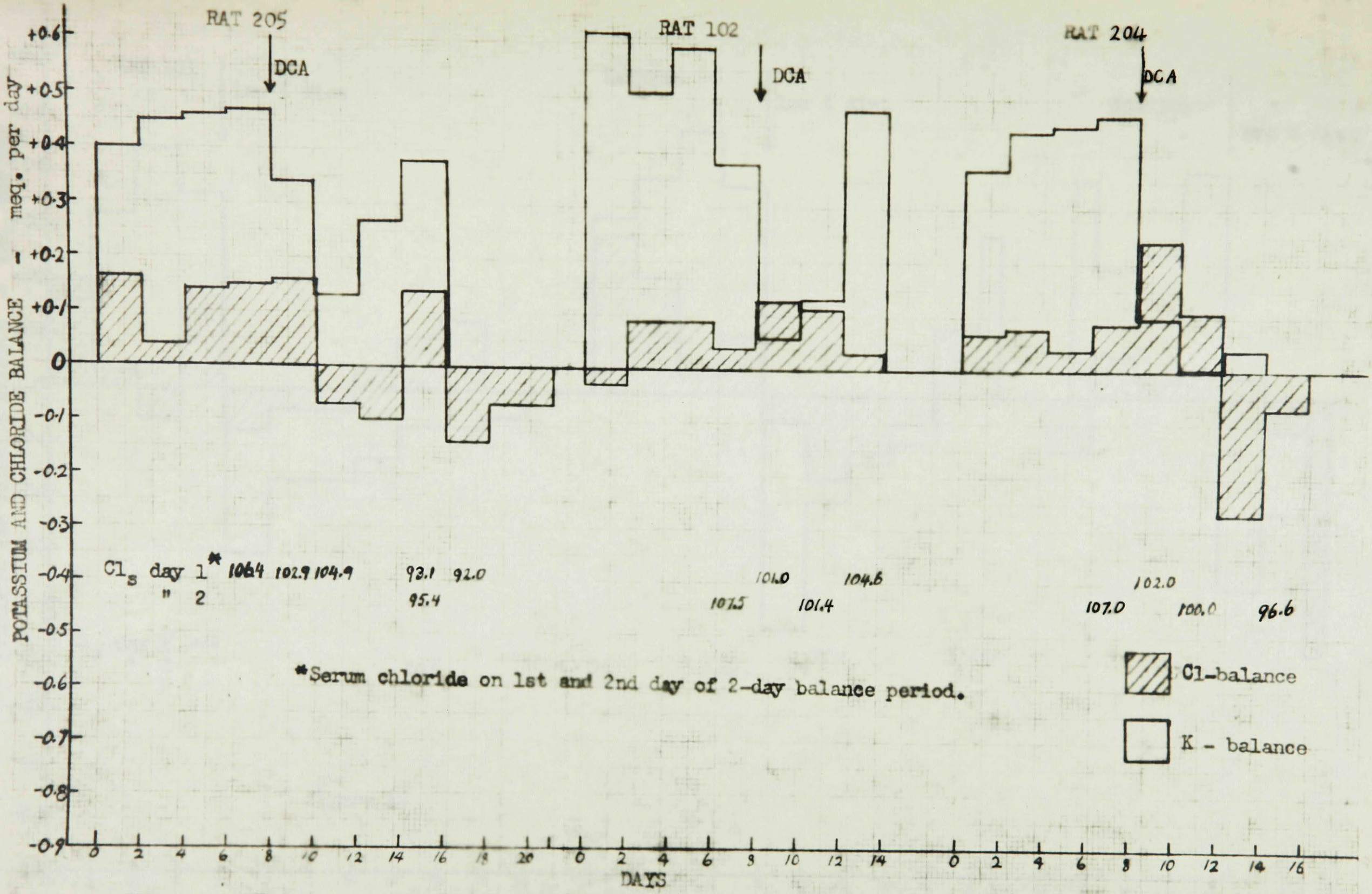


Figure 1. Potassium and chloride balance in DCA-treated rats force-fed a balanced diet.

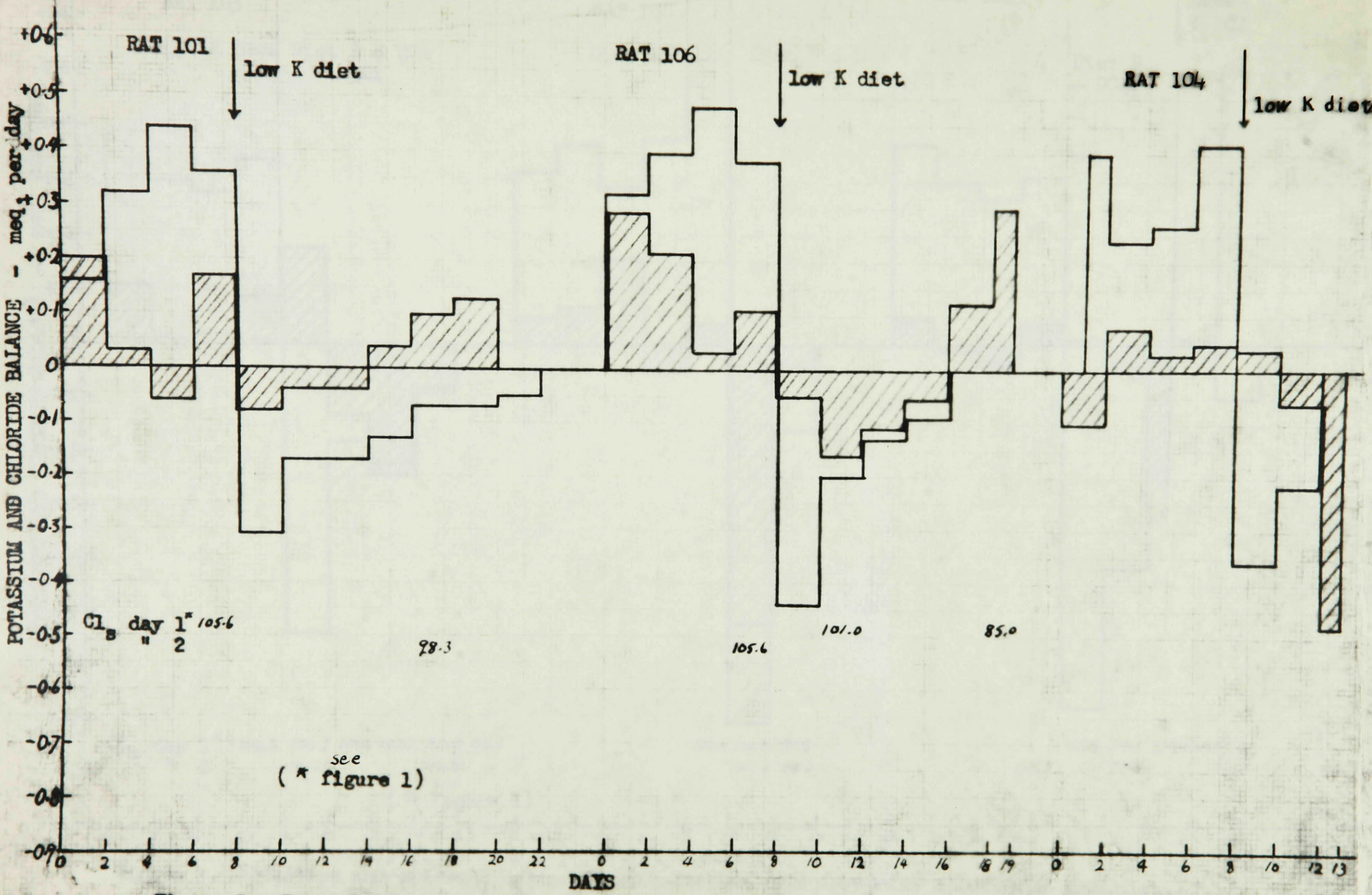


Figure 2. Potassium and chloride balance in rats force-fed a low potassium diet.

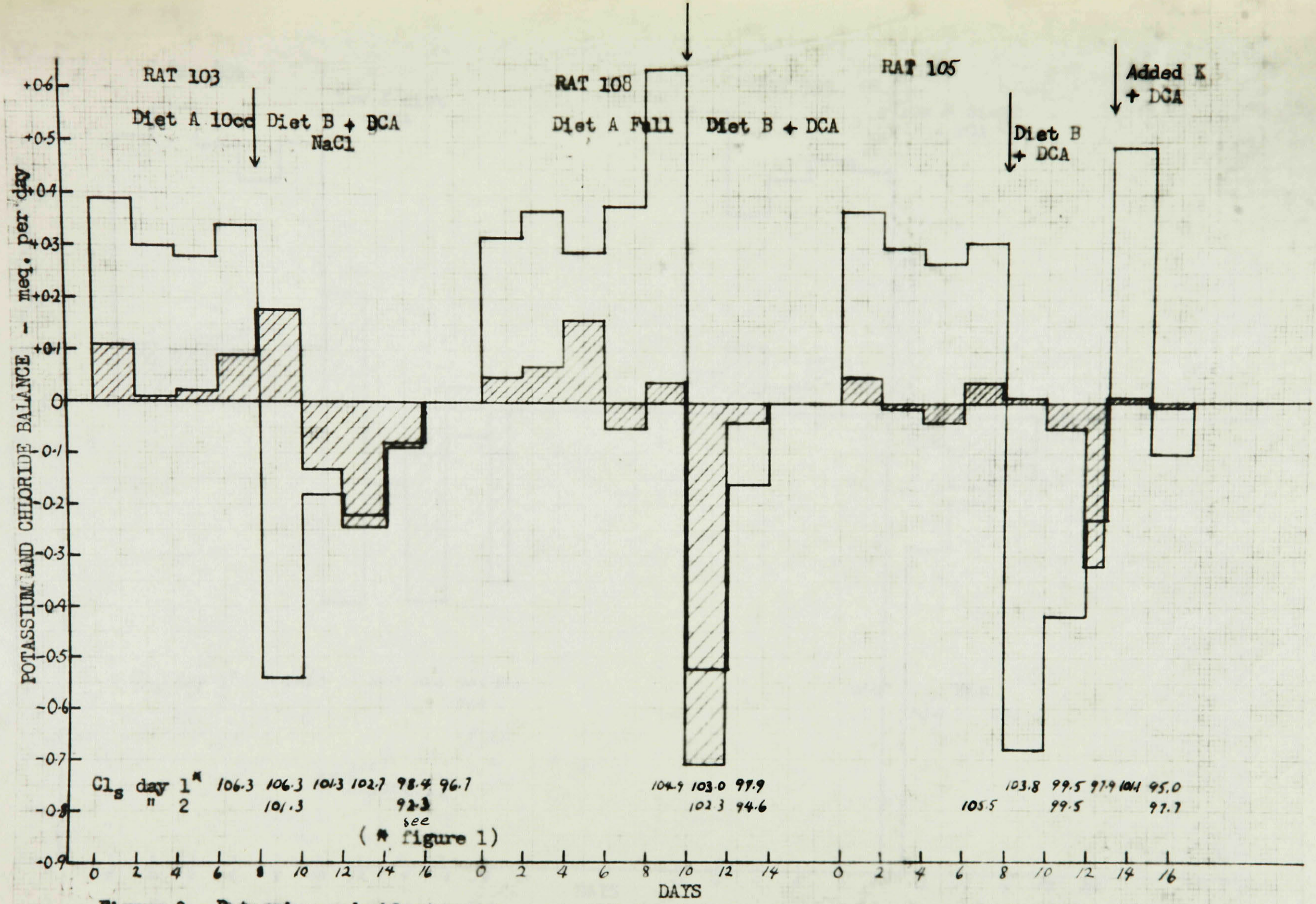


Figure 3. Potassium and chloride balance in DCA-treated rats force-fed a low potassium diet.

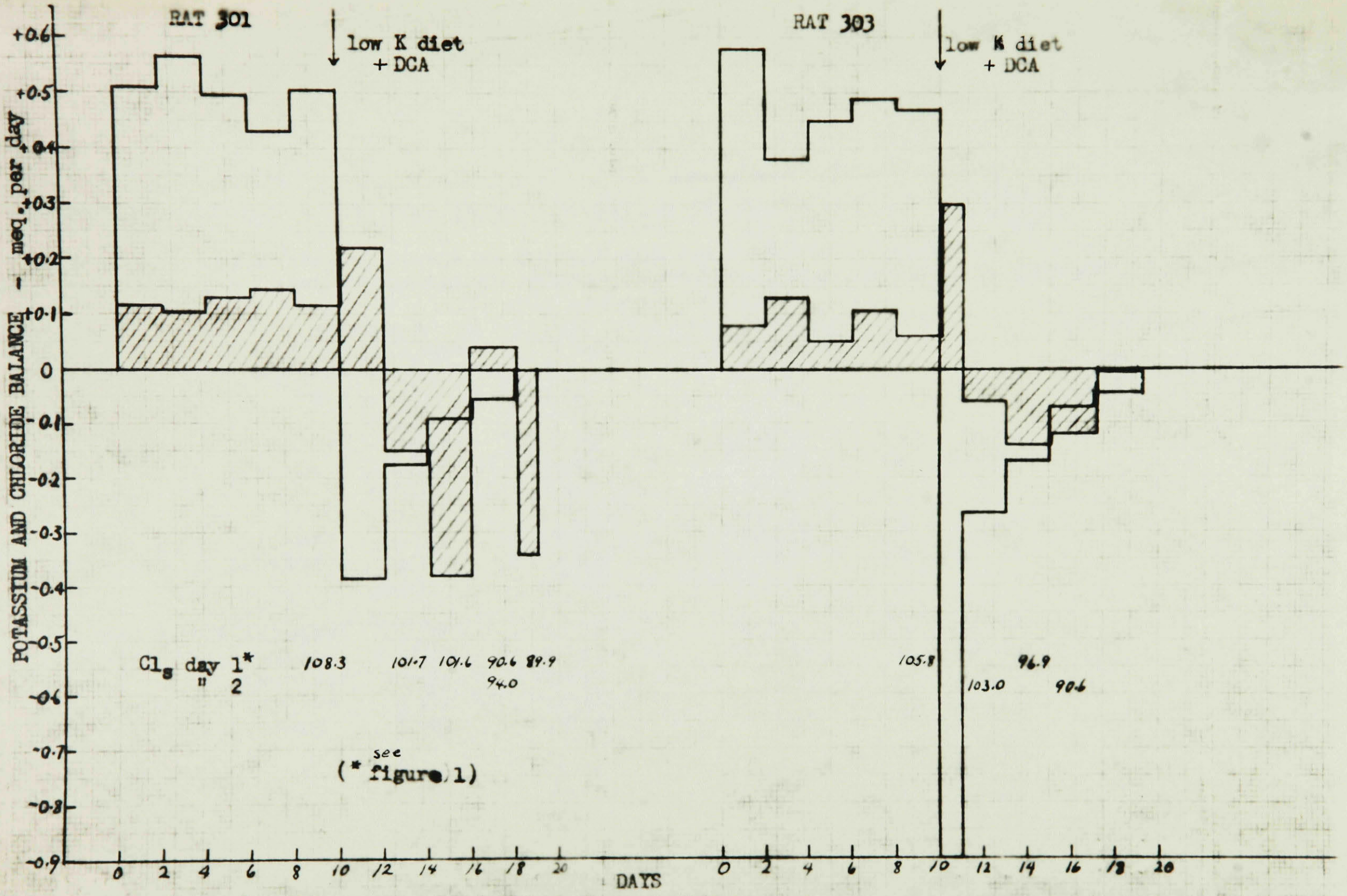


Figure 4. Potassium and chloride balance in DCA-treated rats force-fed a low-potassium diet.

TABLE VI

THE EFFECT OF DCA OR LOW POTASSIUM DIET ON CHLORIDE AND POTASSIUM BALANCE IN FORCE-FED RATS

Rat	Group	Determination	A Control (meq/day) for balance	B Experimental (meq/day for balance	C* Balance difference (B-A) meq/day	D Total loss no.days	E** meq C×D	F* Chloride lost from E.F. - meq	G* Percent body K lost
		K balance	0.43	0.05	-0.38	6	2.28		
204	DCA-K-1/2 Cl	Cl balance	0.07	+0.00	-0.07	6	0.56		11
		Serum Cl***	107.0	96.6				0.89	
205	DCA-K-1/2 Cl	K balance	0.38	0.28	-0.10	8	0.80		
		Cl balance	0.07	-0.02	-0.09	12	1.08		5
		Serum Cl	106.4	92.0				1.23	
102	DCA-K-1 Cl	K balance	0.53	0.22	-0.31	6	1.86		
		Cl balance	0.05	0.08	+0.03	6	-0.18		10
		Serum Cl	107.5	104.6					
101	low K -1 Cl	K balance	0.32	-0.14	-0.46	14	6.44		
		Cl balance	0.085	+0.00	-0.085	10	0.85		34
		Serum Cl ***	109.6	98.3				0.97	
		Serum H_2O%		90.					
104	LowK -1 Cl	K balance	0.30	-0.29	-0.61	4	2.44		
		Cl balance	0.02	(-0.10)	(-0.12)	5	(0.60)		13
		Serum Cl							
105	Low K -1 Cl	K balance	0.50	-0.21	-0.71	8	5.68		
		Cl balance	0.16						
		1st 8 days		-0.095	-0.255	8	2.04		
		all 11 days		-0.018	-0.177	11	1.92		30
		Serum Cl	105.6	85.0				1.79	
				(Added NaCl)					
103	DCA= low K 1/2 Cl and 1 Cl	K balance	0.35	-0.26	-0.61	8	4.88		
		Cl balance	0.06	-0.07	-0.13	8	1.04		26
		Serum Cl	106.3	92.3				1.18	
108	DCA-low K	K balance	0.40	-0.34	-0.74	4	2.96		
		Cl balance	0.05	-0.37	-0.42	4	1.68		16
		Serum Cl	104.9	94.6				0.88	
105	DCA-Low K	K balance	0.32	-0.48	-0.80	5	4.0		
		Cl balance		-0.08	-0.09	5	0.45		21
		Serum Cl		97.9				0.65	
301	DCA-low K	K balance	0.50	-0.28	-0.78	8	6.24		
		Cl balance	0.12	-0.076	-0.196	9	1.75		33
		Serum Cl	109.3	89.9				1.66	
303	DCA-low K	K balance	0.49	-0.22	-0.71	9	6.39		
		Cl balance	0.065	-0.03	-0.095	9	0.86		34
		Serum Cl	105.85	90.6				1.30	

* See text for method of calculating figures in columns F and G
Balance difference, column C, is derived on assumption that this is the algebraic difference of B-A

** Chloride lost in shed blood for Cl determinations was not included in the balance calculations. Only 0.2 cc blood approximately was drawn for each determination

*** Serum Cl in column A and B is in meq per litre of serum

these animals fed diet B for 39 days and given no DCA, the serum chloride was reduced by an average of 8.1 meq.

Potassium balance: The charts show that the potassium balance of rats on diet B was markedly negative throughout the regime of low potassium diet in both DCA-injected and non-injected animals. (Figure 2,3,4). In the DCA-treated rats on diet A, (Figure 1) there was sharp decrease in the potassium excretion, but actual negative balances (excluding fecal excretion) were not obtained. Following the initial decrease there was a gradual return towards the normal limits of retention. The total loss of potassium in the number of days noted (column E of table 6) was calculated as a percentage of the total body potassium of a normal rat weighing 300 grams and is given in column G. Approximately one-third of the total potassium was lost in eight days in rats force-fed the low potassium diet B. regardless of whether DCA was administered, although the number of observations are too small to permit the conclusion that DCA does not increase enhance potassium excretion in rats maintained on a very low potassium intake. The initial potassium loss seemed greated in the DCA-treated group, as can be noted from comparing figure 2 with figures 3 and 4. After much potassium was lost during the first four to sic days, presumably there was less potassium available for excretion and the potassium loss fell off sharply. The loss of body potassium was much less in the group receiving DCA and diet A, being of the order of 10 percent in 6 to 8 days.

Tissue potassium: The potassium concentration was determined for the muscle and intestines of 2 potassium-deficient rats, one force-fed and the other (rat 139) severely deficient from eating diet B ad libitum. The muscle potassium was 29.1 and 30.2 meq/100 grams fat-free solids, and intestinal potassium was 30.6 and 31.7 meq/100 grams fat-free solids. Two normal animals had concentrations in muscle and intestine of 44 meq and 42 meq, respectively. For the muscle and intestine in these animals the loss of potassium was 33 to 25 percent, respectively.

Serum potassium: Serum potassium concentrations for both experiments with force-fed rats and with rats allowed unrestricted amounts of food are given in table 7. These will be referred to again in the section on the gastrointestinal tract. In rats fed ad libitum the serum potassium was decreased by a low potassium intake from a normal of 4.87 meq per litre (diet A) to an average of 3.0 meq per litre in 21 to 35 days. More prolonged maintenance on diet B (57 days) lowered the potassium concentration to 2.73 meq per litre. Rats given DCA and eating the normal diet A showed a similar reduction in serum potassium. In one animal treated with DCA and given diet B ad libitum, serum potassium was very low, 1.91 meq per litre. In all groups of the force-fed rats potassium was lost from the serum more rapidly. After an average of eight days of diet B with, or without DCA, the serum potassium was 2.42 as compared with a normal of 4.8 meq. In one rat given DCA alone the serum potassium concentration was similar, 2.65 meq per litre.

TABLE VII

SERUM POTASSIUM CONCENTRATION IN POTASSIUM-DEFICIENT RATS

Diet	fed	days	no. rats	DCA	serum K meq/l	S.D.** meq/l
B	ad lib	35	7	0	3.01	0.5
B	ad lib*	21	5	0	3.06	
		57	9	0	2.73	0.37
B	ad lib*	33	1	+	1.91	
A	ad lib*	46	3	+	2.90	
A	ad lib	38	5	0	4.87	0.35
B	force-fed*	9	6	±	2.42	0.27
A	force-fed	7	1	+	2.65	
A	force-fed	9	4	0	4.79	0.26
E	force-fed*	15	1	0	5.25	

* Rats of these groups were used in the gastrointestinal experiments of Part B in the X-ray studies.

** Standard deviation.

The administration of potassium chloride of rat 383 promptly increased the serum potassium in a day from 2.45 to 5.81 meq per litre, and in rat 138 from 3.0 to 5.57 meq in 2 days.

Moribund potassium-deficient animals were found to have higher serum potassium than did those in good condition. Thus values in force-fed rats of 5.81,5.74, 3.75, and 3.80 meq per litre were observed in animals moribund from gastro-intestinal distension, and values of 3.45,5.45, and 5.1 were found in moribund animals fed ad libitum. The animal (#5) whose terminal potassium was 5.1 meq will be referred to in section B. This rat was in good condition after 30 days of diet B regime, and had a serum potassium of 2.33. It was then force-fed one day which resulted in death, the blood being taken prior to death.

Water balance: In these experiments in which the sodium chloride concentration of the diets A and B was much lower than in other diets (purina or Diet E), DCA did not affect the water exchange.

Tissue water: The water content of muscle and intestine of potassium-deficient and normal rats did not differ significantly, being 75.4 and 81.8 grams per cent for normal muscle and intestine, respectively, and 75.0 and 82.1 grams percent for these tissues in potassium deficient and DCA-treated rats.

DISCUSSION

Serum chloride: The experiments on intact rats have shown that physiological doses of DCA do not lower the serum chloride, and that, therefore, the hypochloremic effect of DCA is due to overdosage. This was anticipated since the doses of DCA used by other investigators to produce electrolyte disorders, including hypochloremia (35,47,40,19), have been relatively large. Doses of more than 1 mg of DCA were required to produce significant lowering of the serum chloride in the animals tested, which were force-fed diet E, a normal diet with relatively high potassium content. In other experiments a 2 mg dose was consistently effective in producing hypochloremia. In rats the physiological dose is less than 0.5 mg of DCA for various assays in adrenalectomized young animals (17a).

The hypochloremia produced by DCA was related to the potassium intake, which has been shown before as discussed in the review of the literature. A low potassium-intake per se (0.035 to 0.045 meq per day) produced a small but statistically significant decrease of 2.2 meq per litre in the serum chloride in 8 days, and a decrease of 8 to 9 meq in 35 to 39 days. When DCA was administered to rats force-fed a diet higher in potassium than the others used, Diet E, it required 5 mg and three weeks treatment to produce significant lowering of the blood chloride (by 7 meq per litre). With the other normal diets containing less potassium and fed ad libitum a 2 mg dose of DCA lowered serum chloride in 10 days

by 7 meq. Thus the DCA produced hypochloremia more rapidly than the low potassium dietary regime. When both these procedures were combined, a greater reduction of serum chloride occurred. With 10 days of DCA injections and a total of 18 days of the low potassium intake, serum chloride was reduced by 11 to 15 meq per litre. In force-fed rats on diets A or B, both serum chloride and serum potassium were reduced to very low levels in five to ten days, regardless of whether depletion was produced by DCA or a low potassium diet. It is possible that the rats fed ad libitum can respond with appropriate electrolyte adjustments more readily than force-fed animals since food is not suddenly forced into the animal.

Further evidence of the relationship between the potassium intake and hypochloremia was obtained by treating hypochloremic animals with potassium salts. Potassium chloride, or potassium administered as a non-chloride salt, elevated the serum chloride and increased chloride retention by the kidney sharply. The latter effect was associated with a large positive retention of potassium in the one animal examined. Sodium chloride was without effect in either respect, in elevating serum chloride or preventing chloride loss from the urine. Ammonium chloride given by gavage repeatedly increased the serum chloride despite a low potassium intake for the short duration of the experiment, two days. These results parallel the findings of Willsnn et al (106) and Cluxton et al (107) in clinical cases of hyperadrenocorticism. In these

it was shown that potassium chloride, or potassium citrate, according to Willson et al, restore the serum chloride towards normal values, while massive doses of sodium chloride are without effect. Willson et al obtained an effect with ammonium chloride after a period of high potassium intake, while the findings in the rat indicated that ammonium chloride was effective in the absence of potassium. This point should be investigated further.

Chloride balance: The data from balance experiments show for the first time that the action of DCA upon chloride excretion and balance has two phases. The initial effect observed was an increase in chloride retention in most animals. This initial chloride retention has been repeatedly observed by other investigators, and as discussed in the review of the literature is probably related to the sodium retention which occurs at this time. The second effect of DCA which occurred shortly after the retention, was to increase the chloride excretion so that chloride balance was negative. This dual action of DCA occurred both in animals fed a normal and in those fed a low potassium ration. In force-fed rats [illegible] low potassium diet but given no DCA, chloride balance became negative immediately, without the initial phase of retention. Chloride loss in potassium deficiency has also been observed by Orent-Keiles and McCollum (42) for rats fed ad libitum.

The chloride loss from extracellular fluid could be accounted for by the decrease in chloride balance. The figures were derived on two assumptions, one, that the "chloride-available" fluid, in practice the extracellular fluid, represented twenty-five of the body weight in both periods, and two, that the decrease in balance was the algebraic difference between the experimental and control retentions. That is, it was assumed that, since gain in body weight was the same in the two periods, the requirements for normal balance remained the same. This assumption was also made for the potassium balance figures discussed below.

Potassium balance: The urinary loss of potassium in rats force-fed a low potassium diet for an average of 9 days was considerably more than that produced by DCA in the presence of a normal potassium intake. In rats force-fed a low potassium diet,with or without DCA injections, approximately one-third of the calculated body potassium was lost through urinary excretion (under the assumption mentioned above). This percentage loss of the body potassium was similar to the loss of skeletal muscle and intestinal potassium observed in potassium-deficient rats, one force-fed and another fed ad libitum for a much longer period of 89 days. Other investigators have observed a loss of approximately one-third of the muscle potassium by maintenance of several species of animals on a low potassium diet of injecting with DCA, for several weeks.

(19,45,35,40,37,42,71). This shows again that loss of potassium, in addition to that of chloride, occurred more rapidly with force-feeding than with normal feeding. In rats treated with DCA only, for seven days, the potassium loss was only ten percent of the body potassium, as compared with one-third for the animals maintained on the potassium-deficient diet with or without DCA injections. The serum potassium was approximately as low, however, in the one animal examined, 2.65 meq/litre.

Factors influencing chloride metabolism.

Among the three groups of rats used in the balance experiments there was a very rough correlation between the reduction of chloride and potassium balance per day. However, the several variables involved, including in particular the duration of treatment and the use of both DCA and a low potassium diet to deplete potassium preclude the drawing of conclusions from the data obtained to date. The correlation between the balances are presented in figure 7. This would be an interesting relation to establish in view of the considerable data accumulating showing the relation between chloride and potassium shifts in the body fluids (63,94,128,44,95).

Further data reported on chloride metabolism indicated that DCA can decrease serum chloride during the first two days of treatment by/second mechanism which is not related to chloride excretion. In acute experiments it has been shown that DCA

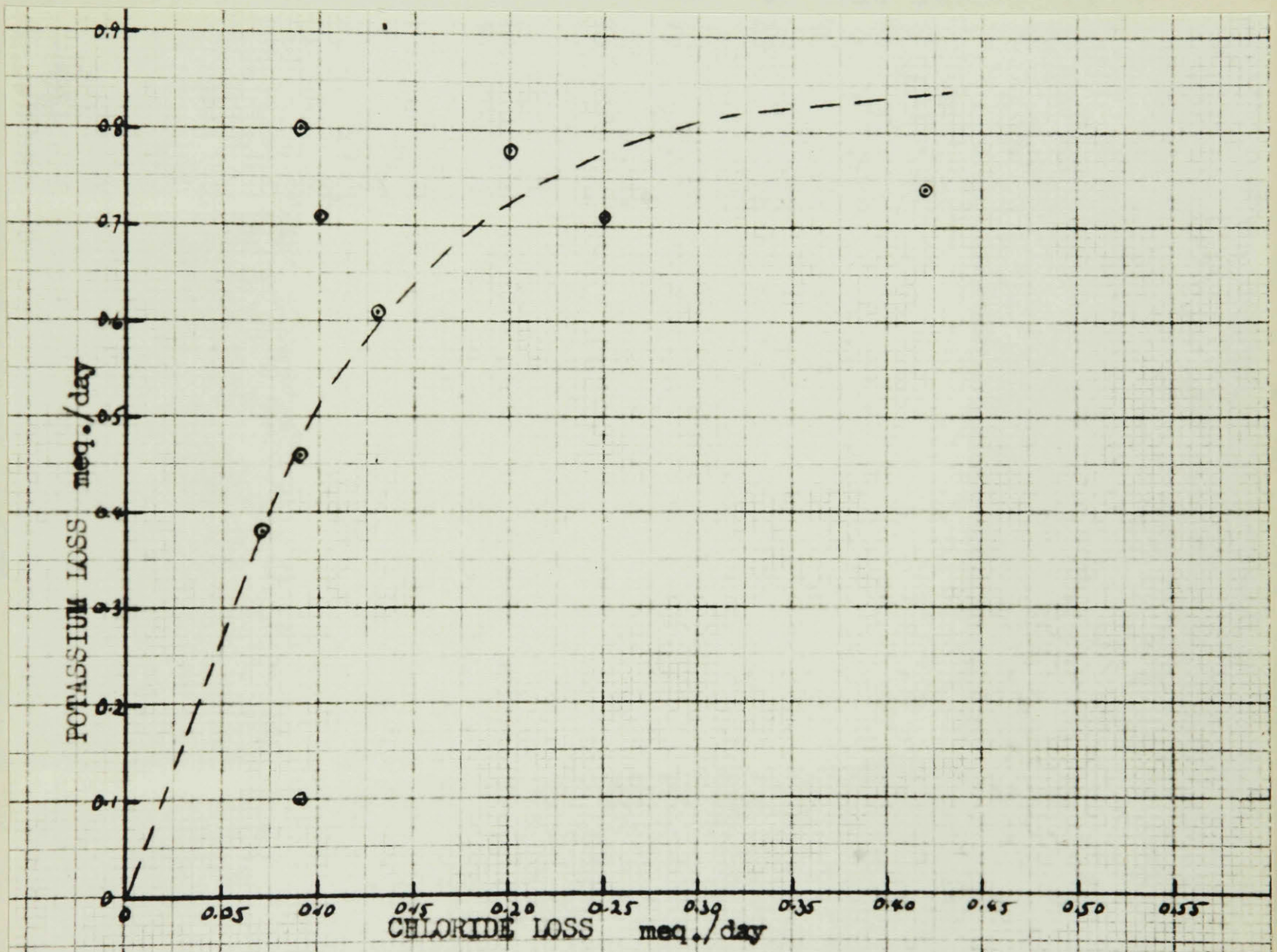


Figure 5. The relation between chloride and potassium loss in potassium-depleted rats.

in large doses produces hypochloremia within a few hours after administration. In previous experiments a decrease of 5 meq of blood chloride was obtained (1a), and in the one rat tested in this report, a decrease of 9 meq was obtained in six hours after DCA administration to a small rat. In this animal the hypochloremia could not be accounted for by the urinary chloride excretion. In addition, in the DCA-treated rats used in the balance studies, serum chloride decreased from 2 to 5 meq per litre during the first two days of treatment. Therefore, the hypochloremia produced in the initial stage of treatment was associated with chloride retention. Thus this early hypochloremia differed from that produced by prolonged treatment with DCA. The latter has been shown to be associated with potassium loss.

The early hypochloremia which has been produced under these two conditions may be related to an expansion of the extracellular fluid volume, but since it was not found feasible to determine extracellular fluid volume in these small animals, the arguments supporting this view rest mainly on inference. It has been shown by several investigators that DCA increases serum and extracellular fluid volume in dogs and man (50,53, 90). Rats have not been examined. It has been discussed in the literature review that it is very unlikely that chloride shifts into the cells under the action of either DCA or potassium depletion. This report has demonstrated the urinary

chloride loss is not responsible for the early hypochloremia. Therefore dilution of the extracellular fluid dissolving chloride probably occurred. One experimental fact was obtained in the acute experiment which supports the view that serum dilution, and therefore, presumably extracellular fluid expansion, occurred. This was the observation that the serum water increased to 93.9 grams percent as compared with the normal range of 92.2 to 93.2 and the normal average of 92.5 grams percent.

Where would this water come from to increase extracellular fluid volume? In the acute experiment in which no water was permitted it could only come from a shift of water from the cells themselves. That DCA and adrenal extracts can exert such an effect is indicated from studies in adrenal insufficiency in which the cells are hydrated (8,55). Whether this effect occurs with very large doses of DCA in the normal animal permitting an initial shift of intracellular water to the extracellular phase remains to be determined, but it would not be an anomalous effect in view of the observations in adrenal deficiency.

To explain the early decrease in serum chloride which occurred in the force-fed rats treated with DCA, a retention of water in excess of chloride could account for this decrease, aside from the possible shift of fluid discussed above. It has been shown in dogs treated with DCA (52) that water retention occurs in the first few days from both a reduced output

and an increased intake. Evidence, from the rats treated with DCA and fed diet E, of an initial lack of parallelism between output and intake of water was indicated, but the volumes involved are so small for the rat that a conclusion was not drawn. Whether the DCA produces hypochloremia in rats eating ad libitum was not determined. It is possible that the forcing of food and the water dissolving this food bidaily brought out maladjustments in electrolyte and water distribution which would not occur in a rat eating normally.

With regard to the acute experiment a theoretical discussion of the magnitude of increase in extracellular fluid volume which would be required to lower the serum chloride by 9 meq, as observed, will be presented.

Taking the difference in serum water percent between that in the DCA rat and the highest value observed in a normal rat (93.9 - 93.2) which is 0.7 percent increase in serum water, this indicates that roughly the serum proteins have been diluted by sufficient water to decrease the concentration from 6.8 to 6.1 grams percent. Assuming that the total protein content circulating in the serum has not been increased by body stores during this interval, the serum volume would have to be increased by ten percent to cause such a dilution. At least some of this increase of volume of serum would be reflected in an increase in the interstitial fluid volume which is in equilibrium with the serum. If the total extracellular fluid

volume were increased to the same extent of ten percent as the serum volume, which is roughly so in the dog (50), treated for longer periods, the serum chloride would be decreased from 109 to 99 meq per litre. This compares favorably with the observed reduction to 101 meq of chloride per litre. In addition, 0.1 meq of chloride was excreted in the urine in the interval of the experiment. However, this excretion of chloride was within the range of normal for this interval of 8 hours, as has been determined in many previous experiments performed on fasted and thirsted normal and DCA-treated rats by the author. Thus it seems possible that hypochloremia may result from extracellular dilution produced as an early effect of DCA. Since the limits of experimental error in the thiocyanate method of determination of extracellular fluid volume approach the difference between the experimental and control animals (50) even in the dog, some other method must be used to investigate the volume changes. The inulin method as used by Boyle et al (64) might prove satisfactory.

Water exchange: Increased water exchange was observed in rats treated with 1 mg of DCA or more and fed diet E, which has a relatively high concentration of sodium, but this did not occur in those fed lower but more than adequate amounts of salt in the diet (diet A or B). The increased water exchange was not correlated with the action in causing hypochloremia, since the diuresis rose to as high levels with one as with ten

mg of DCA, while significant reduction in blood chloride did not occur with the one mg dose.

In moribund animals, or in those exhibiting a marked distension of the abdomen, it was observed that serum potassium became elevated. This was probably due to anoxia or possibly to adrenalin secretion, both of which have been shown to increase the serum potassium concentration (129,130,131,132).

SUMMARY

The purpose of the experiments reported has been to investigate the mechanism by which DCA produces hypochloremia. Experiments were conducted to determine whether DCA hypochloremia is an overdosage effect, how rapidly it can be produced and how it is affected by the potassium intake. Balance studies have been performed in rats force-fed a constant dietary intake to determine whether chloride is lost in the urine of DCA-treated rats, how this compares with the potassium depletion produced and how the potassium and chloride changes of DCA-treated rats compare with those produced by pure potassium deficiency produced by a low potassium intake. In addition, the relative efficacy of potassium and sodium salts on hypochloremia and chloride balance were compared.

It has been shown that (a) DCA hypochloremia is an overdosage effect since a 1 mg dose given daily for a month was uneffective in lowering serum chloride, (b) hypochloremia can be produced within six hours after the administration of a large dose, and that (c) the effectiveness of DCA in reducing serum chloride is increased by maintaining rats of a low potassium diet. The latter observation confirms that of other investigators. DCA was more effective than a low potassium diet in decreasing serum chloride in rats fed ad libitum. A decrease of an average of 14 meq can be produced within 18 days by the combination of DCA treatment with a low potassium intake.

In force-fed animals both serum potassium and serum chloride changes were produced much more rapidly by either DCA treatment or a low potassium diet, than in rats fed ad libitum.

In balance studies on rats force-fed a constant dietary intake, it has been shown for the first time that DCA has two effects on chloride excretion, whether the potassium intake is normal or low. The first effect is retention of chloride lasting one to two days, and second effect, occurring shortly thereafter, is a loss of chloride so that chloride balance is negative. Pure potassium deficiency produces only the latter effect, a negative chloride balance.

Balance experiments have shown that the decrease of serum and extracellular fluids is accounted for by urinary chloride loss. During the initial phase of chloride retention produced by DCA a small decrease of serum chloride usually occurs. Both this initial hypochloremia in force-fed DCA-treated rats and the hypochloremia produced in the acute experiment could not be accounted for by loss of chloride in the urine. The possibility that these are due to dilution of the extracellular fluid has been considered in the discussion. An increase in serum water occurred in the rat examined in the acute experiment.

Balance studies have shown that one-third of the body potassium may be lost in approximately nine days or even less, in rats force-fed on a low potassium diet, whether receiving DCA injections or not. DCA produced a sharp decrease in

potassium balance in animals receiving the control diet, but balance was gradually restored towards normal, and the net loss in 7 days was only 10 percent of the body potassium.

A very rough relation between the decreases in chloride balance and potassium balance per day was obtained for the data in the balance experiments. This requires further confirmation.

The administration of potassium as the chloride or a non-chloride salt, increased the serum chloride of DCA treated animals, and restored chloride balance to positive values. Sodium chloride was ineffective in these respects.

CONCLUSIONS

It is concluded that the hypochloremia produced by DCA is an overdosage effect, and results from the potassium depletion produced by this adrenocortical steroid.

It is concluded also that DCA has two effects on chloride metabolism, The initial effect is to decrease, and the second, to increase chloride excretion. The hypochloremia produced by prolonged treatment can be accounted for by the chloride loss in the urine, while the early hypochloremia observed under certain conditions results from the rearrangement of the chloride stores in the body.

P A R T I I

THE EFFECT OF POTASSIUM DEFICIENCY UPON GASTROINTESTINAL MOTILITY

REVIEW OF THE LITERATURE

In this review the influence of the potassium concentration of the fluid environment has been considered in relation to the structure and movements of the gastrointestinal tract. Experiments performed in isolated strips and those performed in the intact animal have been discussed and compared. A brief outline of the normal structure and movements of the digestive tract insofar as these relate to the problem under discussion will be presented prior to the consideration of potassium effects upon these properties.

The Movements of the Gastrointestinal Tract

The structural arrangement of the walls of the gastrointestinal tract is similar throughout, from the oesophagus to the rectum (133), and from species to species among the mammals (134). There are four coats in the walls of the gut, which, from the lumen to the outside surface are the mucosa, the submucosa, the muscularis propria and serosa. The mucosa consists of the epithelial layer and glands, the connective tissue lamina propria, and the muscularis mucosae. In the submucosa and associated closely with the muscularis mucosae is the intrinsic submucosal nerve plexus of Meissner. The muscularis propria is composed of an inner circular and outer longitudinal coat of muscle to which is added, in the case of the stomach, an oblique layer inside the circular coat. The intrinsic nerve plexus, the myenteric plexus of Auerbach lies between the two layers of muscle in the muscularis propria.

The serosa or connective tissue membrane covers the organs, and contains additional nerves and ganglia.

The intrinsic nerve plexuses of the submucosa and muscularis consist of an intricate network of ganglia and nerve processes. The vagus, and less possibly the splanchnic nerves, make connections with these, (135,136,137,138) but the plexuses, can regulate, or even initiate movements of the smooth muscle of the digestive tract independently of the extrinsic innervation. The number of ganglia in these two plexuses vary roughly in parallel in the different parts of the gut (139, 140). The number of ganglia is greatest in the pylorus and colon. Smaller numbers are found in the small intestine, approximately one half to two thirds as many, but there is no significant difference throughout the small intestine in the concentration of ganglia. There are relatively few in the fundus and upper part of the body of the stomach in which peristalsis is feeble.

The extrinsic nervous supply to the alimentary tract is provided by vagal and splanchnic sympathetic nerve fibres. Usually, stimulation of the vagal fibres is excitatory to the movements and tone, while the splanchnic stimulation has the reverse effect of inhibition (14). However, the responses obtained depend greatly upon the condition of the stomach and intestines at the time, and upon the experimental methods (137,138). In several species, at least, digestive functions may continue

fairly adequately without extrinsic innervation, but in the normal animal the extrinsic nerves permit rapid adaptation of the gut to changes in digestive and environmental conditions (143,142,137,138).

There are many types of movements which have been described for the various parts of the bowel, but in this discussion only the more important types will be considered, since these only were of importance in the X-ray studies to be reported. These are tonus, rhythmic segmentation and rhythmic pendulum movements, and peristalsis.

Much of the knowledge of the tonus changes and movements of the gut has been derived from the classical work of Cannon (143 to 155), who was the first to study the gastrointestinal tract by roentgenological technique following a radio-opaque meal (144a). Since then Alvarez and associates have made valuable contributions to the study of the normal movements of the gut, which have been reviewed lately by Alvarez (138). The subject of smooth muscle has been reviewed by Evans (156) in 1926 and more recently by Fischer (158) in 1944 and also by McSwiney in relation to the stomach (137).

(a) Tonus.

As in all organs encased in smooth muscle, the gastrointestinal tract exhibits the property of continual tonus. Gastrointestinal tonus varies greatly in strength according to the requirements for digestive processes, and the nervous and chemical stimuli affecting the gut. Tonus may be considered

as the resistance of the smooth muscle to extension. It is a property characteristic of, and inherent in, smooth muscle. Tonus is regarded now as a partially inhibited or very slow relaxation of smooth muscle (158,159), rather than as a partial contraction of the muscle as Cannon and Lyman (154) believed. It is not considered to be qualitatively different from other contractions of smooth muscle (156,159,157). The relaxation may be increased in speed or completeness by various agents, and the effect is termed a decrease in tonus (159,160). The chief stimulus for tonic contraction is tension, which, in a hollow organ such as the digestive organs, is produced by an increase in internal pressure. This stimulus is also the natural stimulus for rhythmical movements. Within limits an increase in internal pressure increases the tonus, but if the distension is too great the muscle is fatiqued and fails to respond with increased tone (150). Thus the resistance to extension exists no longer and the organ as a whole becomes dilated. The tonus is increased by acetyl choline and decreased by adrenalin, as a rule (150,152,161,162). It may vary independently of the rhythmical movements.

According to Cannon (150,151,153) a certain degree of tonus is essential in order for rhythmical movements to occur in the gastrointestinal tract. He found that an atonic bowel does not respond with rhytmical movements to the normal stimulus of distension.

Since the principal movements are most clearly seen in the small intestine, we will start the discussion with the small intestine.

(b) Rhythmic Movements in Small Intestine.

The rhythmic segmentations occur in the small intestine. They are series of narrow bands of circular constriction, pulsating frequently and at relatively constant rate for a particular species (145,146.153.163). In the rat the frequency is more than forty and in man it is about ten per minute. These closely-spaced, biting constrictions are local contractions stimulated by the presence of food or material in the lumen within a length of intestine and which divide and redivide the contents and mix them with juices. These movements are considered to be myogenic in origin, since they can occur in the absence of all nerve cells (164,165,166,167) and since smooth nuscle which normally contains no nerve cells show similar rhythmicity (168.169.170). They are not prevented by repeated circular transections of the gut up to the submucosa (153). Pendular movements are a swaying type of contraction in which the contraction of the longitudinal muscle is more prominent and in which longer lengths of bowel contract as a unit. This shifts the food to and fro along the intestinal mucosa. Alvarez (138) considers the length of bowel contracts as a unit because of reflexes set up in the intrinsic nerve plexuses by the distension of food.

(c) Intestinal peristalsis.

Intestinal peristalsis is a different type of wave than the rhythmic segmentations (145,153,171.138). These waves travel down the gut and propel food actively in the caudad direction. They begin at an area of high irritability or activity, more often at the pyloric sphincter than in other segments, and travel down the intestine for variable distances (145,138). Another feature differentiating these contractions from segmentations is that the length of bowel alternately contracting and relaxing is much greater. In the cat, for example, four or five cm. of intestine contract at a time as the waves of contraction move down the intestine (153). Unlike the segmenting movements, peristalsis is prevented by transections up to the submucosa at short intervals along the gut (153). This indicates that peristalsis requires the plexus of Auerbach (the Myenteric) for conduction of impulses, while the segmenting movements do not.

(d) Gastric movements.

The fundus and the upper part of the corpus of the stomach produce only tonic contractions (144,172,173). By gradually increasing its tone as digestion proceeds and food leaves the stomach, this part of the stomach "feeds" the food material to the lower part, the motor part of the stomach. In this part vigorous rhythmic movements called peristalsis churn the food and mix it with the juices (144). The peristaltic waves are circular waves of contraction which originate as shallow waves in the middle of the corpus and travel down the stomach wall to the pylorus, becoming more and more powerful as they progress downwards. These rhythmical movements occur at

regular intervals. As first observed by Cannon (144) in the cat, they occurred every ten seconds and took thirty-six seconds to pass down the pylorus. Thus several constrictions were present at any one time. The vigor of contraction depends upon the food material present. Contractions are very powerful when solid particles are present, and much less so when only a bismuth or barium and water suspension is present in the stomach (144, 147,138). The movements of the stomach vary according to the species and to the shape and structure of the stomach (137). In the rat the stomach is clearly divided into a thin-walled membranous cardiac half and the lower thick-walled half with much stronger muscle layers.

(e) Movements of the large intestine.

The cecum may exhibit strong contractions particularly in herbivors in which this organ is an important organ of digestion for cellulose. Both peristaltic and antiperistaltic waves have been observed by Cannon (145) and Elliott and Barclay (174).

In the colon, (145,174,175) small waves of contraction similar to the rhythmic segmentation in the small intestine, are noted, and in the proximal part of the colon antiperistalsis occurs, particularly in herbivors, if the contents are soft (174). In addition, peristaltic waves travel down the colon. These are broader and deeper contractions than the shallow waves mentioned above. As the fecal material passes down to the terminal colon it becomes separated into individual masses, that is, it becomes segmented. Periodically mass movements in the colon and defecation occur, but these vary from species to species. In the rat defecation occurs throughout the day, and there are no periods for mass movements and defecation.

The Effect of Changes in Potassium Concentration on the Gastro-intestinal Tract.

The main type of experimentation with potassium on gastro-intestinal motility has been *in vitro* studies on isolated strips. These are suspended in physiological saline solutions and the concentration of potassium varied, or the ratio of calcium to potassium changed. There has been little work reported on the influence of potassium on the bowel in the intact organism. These have included reports as to the effect of potassium deficiency on the gross appearance and histological structure, and experiments in which body potassium was increased by injections of adrenalectomy. No study has been made of the effect of potassium deficiency upon the motility of the digestive tract in the intact animal.

(a) The effect of potassium deficiency on the morphological appearance of the gastrointestinal tract.

There are conflicting reports regarding the effect of potassium deficiency in the intact animal upon the gross and microscopic appearance of the gastrointestinal tract. In most of the experiments the low potassium diet contained 0.01% potassium. The most carefully carried out experiments have been those of Follis, Orent-Keiles, McCollum (42,72) and of Kornberg and Endicott (176) in the young rat. The former investigators found animals fed a low potassium diet survived indefinitely and did not develop intestinal abnormalities. Their diet was supplemented by liver extract and oil-soluble vitamins, but

not by crystalline vitamins. Kornberg and Endicott, on the other hand, found that animals of the same size (weanlings) fed a diet of the same potassium content (0.01%) died in an average of 22 days and developed marked changes in the gastro-intestinal tract. In these experiments pathological changes were noted in the intestines only. The gross changes confirm the observations of Schrader et al (177) which are discussed below. Both large and small intestine were dilated with thin walls or in some cases, walls swollen, pearly white and thick in appearance. The involvement was often segmental, with congestion in occasional segments. Intussusceptions were numerous. Grossly hydrothorax and ascites were noted and there was evidence of edema in many tissues. This may, however, have been due to heart failure since Liebow et al (71) have observed congestive heart failure in some potassium-deficient mice and lesions in the myacardic consistently occur (72,35). Microscopically the intestinal walls showed dilatation or edema, particularly in the submucosa. The smooth muscle fibres of the muscularis propria were swollen, occasionally hyalinized and staining intensely oxyphylic. Atrophy of the lymphoid follicles was also noted.

The diet used by Kornberg and Endicott contained no natural source of vitamins but was supplemented with all the crystalline vitamins of the B complex known to date, and larger amounts of these vitamins had no effect on the various pathological changes produced by the low potassium diet. The vitamins added were thiamine, riboflavin, nicotinic acid, pyridoxine, panthothenate,

biotin, inositol, para-amino-benzoic acid, choline, folic acid, and vitamins A, D, E, and K. Their rats on the control diet grew as well as did those of Orent-Keiles and McCollum. In addition, pair-fed control rats maintained on the same amount of food as eaten by the potassium-deficient rats, did not develop any pathological changes in the various organs examined. Follis (80) later found that their rats died an average of 25 days when the diet contained only 0.001% potassium, although he considered the difference in survival may have been due to the substitution of crystalline vitamins (in smaller amounts than used by Kornberg and Endicott) for liver extract. He mentioned no observations on the gastrointestinal tract.

The first report of intestinal changes produced by a low potassium diet was by Schrader et al in 1937 (177). These investigators found that when young rats were fed a low potassium diet unsupplemented with vitamin B complex except for thiamine, marked changes in the digestive tract developed. The animals died in an average of 23 days. At this time abdominal distension was marked and ascites severe. The gastrointestinal tract was enlarged and atonic. Changes were particularly severe in the ileum and jejunum but in a few animals the whole tract was affected. The ileum presented a beaded appearance with alternating thickened and collapsed areas. Intussusceptions were numerous. However, these changes were prevented by the addition of a Fuller's-earth adsorbate of a yeast, and the animals were not pair-fed. The investigators concluded that the changes

observed were due to vitamin B deficiency. The content of potassium of the adsorbate was not given so it is not known whether this was a factor in the curative effect of the yeast. Heppel and Schmidt (178) in 1938 noted flaccidity and dilatation in potassium-deficient animals receiving extracts of rice bran and liver in the diet as a source of vitamin B complex. The rats gained little weight but survived at least for the seven week period reported upon. However, further experimentation by Heppel (84) failed to confirm his previous observations on the gastrointestinal tract. He considered that this may have been due to the use of a more potent batch of extracts as a source of vitamin B, and that therefore the former changes noted (178) were probably due to vitamin deficiency. In mice no changes in the microscopic appearance of the intestine were observed in potassium deficiency by Liebow et al (71) in 1941. In these animals potassium-deficiency was severe enough to produce loss of twenty-five percent of the cardiac potassium and severe cardiac lesions. Skinner and McHargue in 1945 (179) performed experiments on rats fed a ration containing 0.005% potassium, supplemented by nearly all the known crystalline vitamins of the B complex. Their observations indicated that potassium deficiency probably produced intestinal changes in motility although their statement regarding these is merely the following: "In fact, because of accumulation of fluid in the intestinal tract, the animals usually weighed a few grams more at death

than when placed on the experimental ration". The rats died between the twentieth and fortieth days. In all their experimentation with low potassium diets (0.01% potassium) and DCA treatment on adult rats, Darrow and Miller (45,35.39,67) have never mentioned changes in the gastrointestinal tract.

Robertson and Doyle in 1935 (180) noted intestinal stasis on a low mineral diet in young rats. Additions of Vitamin B extracts did not relieve the stasis. Addition of calcium and sodium had little effect, but the addition of both potassium and calcium prevented stasis. In their preliminary tests addition to the diet of potassium carbonate only gave variable results. The two measures of stasis were the delay in excretion of carmine and the difference in total weights of the intestines in normal and deficient rats, which was presumed to be due to increased contents. It is possible that in these experiments the intestinal stasis may be associated with potassium deficiency, although administered potassium may not be able to act in the absence of calcium or, less possibly, of sodium.

Various investigators have examined skeletal and smooth muscle of potassium-deficient rats and mice, and have found, on the whole, the changes in histological structure relatively slight or non-existent (45,72,81). The changes in the smooth muscle of the gut observed by Kornberg and Endicott were mentioned above. In skeletal muscle of potassium-deficient mice Liebow et al (71) observed no microscopic changes but noted that following recovery on potassium diet occasional muscle fibres

showed degenerative changes. The significance of this is not known. As mentioned in the section on electrolyte metabolism changes are invariably produced in the cardiac muscle. Electrocardiographic changes and degenerative changes of the Purkinje fibres of the bovine heart have also been noted (69,79).

Thus there is evidence that maintenance of rats on a low potassium diet can induce macroscopic and microscopic changes in the gastrointestinal tract which are indicative of decreased tone, although in the majority of experiments these were not found. It is possible that there are differences in various strains of rats, which render some more susceptible to potassium deficiency insofar as gastrointestinal disturbances and mortality is concerned (42,79,176).

The potassium intake of rats maintained on 0.01% potassium diet eating 5 to 8 grams is 0.5 to 0.8 mg, and on a 0.005% diet it is half of this. These are very small amounts to deal with. Miller (181) found that for optimal growth in young male rats 15 mg of potassium was required daily, while females required half this amount. The observation on young male rats has been confirmed by Kornberg and Endicott (176) who found a diet containing 0.17% potassium satisfied the minimum optimal requirements for this ion. Adult male rats required only 2 mg of potassium per day (181) and thus potassium deficiency is not as severe in these animals which are not growing as rapidly as young rats.

(b) The effect of injected potassium on the motility of the gastrointestinal tract in the intact frog.

In the conditions in which body potassium is increased there is evidence that the small intestine becomes more contracted, or that the tone of the stomach increases. Kupaloff (182) injected potassium intravenously into a frog and measured the movements of the stomach by a balloon inserted into the stomach of the intact animal. The administration of relatively large doses of potassium chloride, 0.01 to 0.2 meq resulted in an initial rise of tone, but soon thereafter the amplitude and rate of contractions became inhibited and the duration of relaxation increased. A crude estimate of the frog body potassium on the assumption that 0.25% of the body weight is potassium, as it is in the rat, indicates that the total body potassium is no more than 0.15 meq, so that the above doses were enormous. This observation of an initial stimulation followed by inhibition falls in line with observations made of the effect of potassium on other excitable tissues. This will be referred to in the section on <u>in vitro</u> studies.

(c) The effect of adrenalectomy on the movements of the gastrointestinal tract.

In the adrenalectomized rabbit and cat, Vogt (183) and Fowler and Cleghorn (184) have observed that the gut became markedly constricted or spastic in the terminal stage of insufficiency. These changes were not found in other than moribund animals. The constricted state of the gut was not

due to starvation, which was tested in normal control cats (183). The cause of this spasticity is not known, although it was implied that the changes are due to electrolyte and water metabolic changes which occur in terminal adrenal insufficiency, rather than to a specific effect on the adrenal cortex upon the gut. What role the absence of the adrenal medulla played in these adrenalectomized animals has not been considered, but, since the condition developed only in the crisis due to adrenocortical insufficiency, adrenalin probably played a neglible role. Vogt found that neither treatment with cortin of excised strips of intestine, nor of one rabbit with cortin in vivo, prevented the spastic state of the intestine. Fowler and Cleghorn treated one cat with sodium chloride with resulting slight relief of the contracted state, and restoration of the usual response of relaxation to splanchnic nerve stimulation. In adrenalectomized and adrenalin dogs Clarke and Cleghorn (185) found an increase in the concentration of potassium in small intestine, but their results on the rat cannot be considered as indicative of any change at all in potassium concentration, although the potassium content of other tissues increased. The intestinal potassium of adrenalectomized cats and rabbits has not been determined.

A decrease in blood volume produced by vitamin B deficiency was responsible for gastricatomy observed in the dog according to Rose et al (186,187). Therefore, much more work is needed before it can be concluded by a decrease in blood volume per se in adrenal insufficiency produces spasticity of the gut.

(d) In vitro experiments with potassium.

The response of strips of isolated stomach and intestine to changes in ionic concentration are similar to those of other smooth muscle preparations. The presence of the intrinsic nervous elements probably contributes to the complexity of results obtained with ionic studies and complicates the interpretation of results. In this review mammalian smooth muscle and gut of vertebrates only, mainly of mammals, will be considered.

The effect of potassium depends upon the amount added (187,188,189), on the duration of treatment (190,191), and also upon the initial potassium concentration of the control both (189,192). The effect of a particular concentration of potassium depends upon whether that concentration is an increase or a decrease from the control concentration of potassium (192).

The main effect of adding potassium is to increase the tone of gut or smooth muscle preparations, or to produce a strong contraction in non-contracting preparation (192a,193, 192,189,194,191). This may then be followed by an inhibition (191). The effect on the rhythmical movements is less consistent or neglible. The frequency of rhythmic contractions is dependent more upon the ratio of potassium to calcium, concentration, than on the potassium concentration alone. This ratio also affects the tone of the muscle. In general, the tone is increased by an increase in the ratio of potassium to calcium, while the rate of rhythmic contractions is decreased (195,196,

189,196). Treatment with large doses of potassium may produce inhibition of tone, and treatment for prolonged periods with a relatively high concentration of potassium produces progressive inhibition or paralysis of the movements and tones (190).

With concentrations of potassium below the normal Tyrrode's solution effects are more irregular than with concentrations above the normal. Thus, starting at the normal concentration, a decrease as well as an increase in potassium concentration may increase the tone, or produce a contraction (193,192,198, 199,200). There is considerable variability in the responses of different smooth muscle preparations to the same change in environmental conditions. The fact that many processes involving surface membranes, including contractile and excitability responses, are dependent upon the ratio of potassium to calcium complicates the interpretation of the <u>in vitro</u> studies performed with potassium concentrations less than normal, in particular. In the complete absence of potassium contractions become feebler progressively and eventually cease altogether (201).

The effect of potassium added to isolated gut and smooth muscle preparations is consistent with its effect on other muscle and on nervous tissue. The initial effect is one increasing excitability and contractility, while with prolonged treatment excitability and contractility gradually decrease, and eventually, with high concentration paralysis or nerve-block occurs. Very small doses may prolong excitatory effect,

while very large doses may cause paralysis without the initial excitatory effect. In all these experiments with ion osmotic concentrations must be controlled. These dual effects on excitability have been shown in vertebrate tissues of many types, including smooth muscle, as discussed above, skeletal muscle (202 to 207), nerve cells and fibres (208,209,210), and for transmission in the central nervous system (192a, 211, 212,213), at neuromuscular junctions (214) and in ganglia (215, 216). Even in mammalian heart muscle a slight increase in rate of contraction sometimes occurs with a small increase in concentration for a short time (217,218), which is followed by the usual decrease in rats (219) and excitability (220). Hegnauer (221), working with frog skeletal muscle, showed that the optimal concentration of potassium for maximum irritability was about 20-23 mg percent, the irritability falling off rapidly on either side of this range. These concentrations are double the normal for the frog.

There has been a great deal of research on the problem of potassium and its relation to acetyl choline, which is involved in transmission in the nervous system, both peripherally and centrally. It has been shown that potassium liberates acetyl choline, in vitro, at cholinergic nerve endings (216,222), but that it also liberates adrenalin-like substance at adrenergic nerve endings (223). In tissue respiration studies it has been shown that the acetyl choline synthesis of brain tissue is increased by small doses of potassium, but inhibited by

large amounts of this ion (224). Both acetyl choline (225,159) and potassium (226,191), however, exert an effect on smooth muscle of the gut which has been functionally freed of nervous elements. This indicates that these substances can both act on the muscle cell itself, in addition to acting upon nervous elements. Much further work will have to be done to elucidate this problem of the action of potassium on rhythmic movements and tone in active tissues.

Potassium probably exerts its physiological effects in at least two ways, one upon the cell membrane and permeability, and the other, within the cells themselves. In all probability the addition of potassium <u>in vitro</u> has an immediate effect on the surface and a delayed effect following diffusion into the cell. This concept was first proposed by Eichholtz and Starling (227) to explain the dual effects of potassium upon the kidney tubular cells.

In summary, the effect of potassium <u>in vitro</u>, and when injected in large doses into intact frogs is mainly to cause increased tone of the gastrointestinal strip. This effect depends upon the duration of treatment and the concentration, and may be followed by an inhibition on the contractility of the muscle. The effects on the rhythmical movements are dependent more upon the ratio of potassium to calcium. In view of the fact that the observations have been more acute effects it is difficult to apply these results to a chronic experiment performed in the intact animal.

In view of the difficulties expressed in these paragraphs it is impossible to apply analogies from experiments in isolated preparations of gut to observations on the intestinal tract performed in the intact animal in potassium deficiency, except to anticipate that an initial stimulatory effect of potassium at least would be obtained in potassium-deficient animals. As noted above variable effects have been produced in isolated gut and smooth nuscle preparations following reduction of the potassium. In isolated preparations the reduction of potassium is an acute effect, and the results on tone and movements irregular, while in the intact animal potassium is gradually lost and replaced by sodium. The deficiency of potassium, whether as a primary effect or a secondary, causes changes in cardiovascular system, and possibly influences the nervous elements of the gastrointestinal tract.

(e) The release of potassium from active tissues.

There is much evidence that potassium is released from contracting tissues in excess of thosewhich are non-contracting. This has been shown for the stomach isolated and contracting in potassium-free Tyrrode solution by Cicardo (228,229). The loss of potassium is especially marked when contractions are rhythmical as was shown by Fenn (230). Miller and Darrow, however, found that voluntary contractions of skeletal muscle, unlike those resulting from electrical stimulation mainly in vitro, did not alter the potassium concentration of the muscle (45). Presumably the exchange with the blood potassium

in these intact rats was rapid enough to prevent the decrease in muscle concentration, since the evidence appears strong that loss of potassium does occur during activity of many types of excitable tissues, including muscular, nervous and glandular. This release of potassium during activity has been shown by Heppel (231) to occur even in potassium-deficient rats. Potassium released during contraction is restored quickly following recovery. This whole subject of the release of potassium during activity of excitable tissues has been very completely reviewed by Fenn (59,114), who, with his associates, has contributed much information on this problem. These data indicate the importance of the potassium ion during various types of cellular activities.

(f) Summary of the literature review.

There are conflicting reports of the effect of potassium deficiency on the gastrointestinal tract. A few investigators have found that marked changes in the gross and microscopic appearance occur in the digestive organs which are indicative of a decreased tone. There has been no demonstration of the effect of potassium deficiency on the function of the gastrointestinal tract. Experiments showing the paralysis of skeletal muscle which is produced by potassium deficiency of DCA (Part I-Historical Review) suggest that smooth muscle of organs such as those of digestion may show similar defects in contraction. The site of action here could be on either muscle, or nervous elements or on both.

The importance of potassium in the activities of excitable tissues has been shown mainly from experiments performed in vitro. Inference cannot be applied to the intact animal from these experiments to explain the effect of potassium deficiency on the gastrointestinal tract and of replacement therapy with potassium.

EXPERIMENTAL

The experiments reported in this section have shown the effect in the intact animal of prolonged potassium deficiency on the motility of the gastrointestinal tract and the effect of subsequent therapy with potassium. Experiments were conducted both in rats force-fed the synthetic diet and in animals eating in the normal manner, ad libitum. Since DCA also produces potassium depletion, the effect of this steroid was also investigated.

Experimental Procedure and Methods.

The effect of potassium deficiency upon the gastro-intestinal molility has been studied in rats by means of X-ray observations following barium sulfate and by inspection of the gut. Determinations on potassium balance have been given in Part I but will be referred to in this section. An X-ray test was performed on each normal animal prior to the experiment and animals showing abnormal gastrointestinal behaviour were eliminated. The diets used are the same as used in Part I (table I). The series of rats were divided into the following groups for X-ray studies:

(1). Force-fed rats

(a) Diet A- controls

(b) Diet B- low potassium

(c) Diet B plus DCA

(d) Treatment of some of the animals of (b) and (c) with potassium

(e) Controls of the force-feeding technique:

(i). Control on handling - sham-tubing of rats

(ii). Control of gastric distension - administration of water bidaily by stomach tube.

(iii). Control on the diet - force-feeding diet E of completely different composition to that of diet A and B.

(2) Rats allowed food ad libitum

(a) Diet A - controls

(b) Diet A plus DCA

(c) Diet B

(d) Diet B plus DCA

(e) Treatment of some of the animals of (c) and (d) with potassium.

In the course of the studies more than 85 rats have been observed of which 70 were examined by X-ray.

Most of the details of the experimental procedure and methods have been presented in Part I. The initial body weight of the rats (males) averaged 150 grams for experiments in which food was allowed ad libitum, and 275-300 grams for the force-feeding experiments.

Prior to use in any of the experiments the rats were handled several times daily for several days to accustom them to handling to remove the factor of excitement. They were placid animals even initially. No sudden movements were made in relation to the rats, and they were not handled with gloves nor wrapped in a towel during feeding.

In all force-fed rats 25 cc of diet A or B containing cellu-flour was administered except for preliminary observations made on a few rats used in the electrolyte studies. These received smaller amounts of a cellu-flour free diet in order to prolong survival. The rats were accustomed to the

handling so that the tubing did not disturb them. This is important for studies of the gastrointestinal tract, since excitement inhibits gastrointestinal motility, [illegible] an effect mediated through the sympathetic nervous supply or due to adrenalin release (148). The intake of potassium for force-fed animals was 1.68 meq and 0.04 meq per day of diets A and B (fluid), respectively, and for animals fed ad libitum the potassium intake was 1.40 and 0.035 meq of dry diets A and B, respectively. Serum potassium determinations were performed at intervals throughout the experiments. The results were presented in Table 7 in Part I. Since these values showed little correlation with the degree of dysfunction produced in the gastrointestinal tract, but were low in all animals exhibiting moderate symptoms, these will not be considered in detail. No balance studies were performed in animals eating freely. For force-fed rats the results of studies reported in part I may be applied to the present series of rats (see Table 6, diet B and Diet B plus DCA). An average of one-third of the calculated body potassium was lost in about eight days through renal excretion.

In animals in which the gut was inspected for gross changes the abdomen was opened during urethane anethesia, which has been shown little effect on motility (272). In force-fed rats this was done in a few animals soon after defecation had ceased and in all moribund animals, as well as in several animals prior to this state. In rats fed ad libitum in which changes

in gastrointestinal function were not usually clinically visible, fourteen animals were examined in the above manner when it was anticipated that changes had occurred and in two or three animals in which the gastrointestinal distension was visible from the distension of the abdomen it caused. Pictures of the severe changes which occurred from potassium deficiency in a force-fed rat and in one fed ad libitum, are compared with a picture of the gut of a normal animal in figure 6.

X-ray studies were performed in rats following a fast of twelve to fifteen hours. A suspension of 20 grams of barium sulfate in 100 cc of water was administered by stomach tube in a volume of 12 cc for all but the rats weighing 150 grams or less. These received 10 cc. The rats were taped by the four legs to a board for X-ray examination which was done when the animals were quiet and relaxed. A small portable X-ray machine was used. Frequent fluoresopic examinations were made and films taken at appropriate intervals. Routinely the rats were inspected at least at one, three and five hours by X-ray, and sometimes at twenty-four hours, and usually more frequently by fluorescope, but examination was continued over the first 12 to 14 hours when deficiency changes were appreciable as well as at 24 hours. The feces were collected for most of rats after the first tests, and X-rayed with the rat to provide a crude estimate of the barium excretion. Estimates of barium retained or excreted from X-ray plates can only be regarded as a crude index of quantities, but are definitely of value in grading response.

In rats in which no barium is detected in the gut by X-ray, chemically detectible amounts may be present (233), but these are not important for the purposes of the experiments presented in this report.

The factors of handling the rats and tubing them bidaily and of sudden distension of the volume of fluid diet administered by stomach tube were examined in two groups of rats. In the first group of 4 rats the tube was passed down to the stomach twice daily but nothing was introduced. In the second group of 4 rats 12.6 cc of water was given by stomach tube twice each day, although water does not stay in the stomach long. In addition, a normally balanced synthetic diet (diet E, Table 1) of completely different composition was force-fed to another group of five rats to compare the gastrointestinal response to the force-feeding of this diet with that to the feeding of diet A. This diet E had a higher caloric content approximately 73 calories per 25 cc aliquot, and consisted mainly of whole milk powder and sucrose. The butter fat content was 8.7 grams per 100 cc, and the fat added as oil was 1.5 percent (a total of 10.2 percent by weight) excluding that in yeast.

In diet A fats were added as oils forming 6.6% of the fluid diet. Diet E is one used by Ingle as a medium balanced diet.

In force-fed animals most of the X-ray tests were performed one to two days following cessation of defecation.

In animals eating freely, X-ray tests were made at intervals to establish roughly when the symptoms developed. Unfortunately an X-ray machine was not immediately available so that a few tests were made prior to the twentieth day of deficiency. However, at this time changes were slight. Unless necessary not more than one barium test meal was given in a week, and, if this was done at least four days elapsed between tests to permit the refilling of the gut emptied during the fasting and from the barium administration. If results indicated that development of abnormalities was very slow in a given rat, longer intervals of two to three weeks elapsed between tests. In animals in which the effect of potassium was to be tested, the low potassium diet was continued after the X-ray examination for a few days prior to treatment with potassium, so that recovery from the barium test would be obtained.

Experimental Observations

The experimental observations on the effect of potassium on the gastrointestinal tract have been presented according to the plan which is presented below. In the distinguishing between the observations made in rats force-fed and in those fed ad libitum, some repetition has been involved. It is necessary to separate the results of the rats according to the method of feeding because the results differ greatly quantitatively, although not qualitatively.

Plan:

(a) Preliminary observations from gross examination of potassium-deficient rats.

(b) X-rays of normal and control rats.

(c) X-rays of force-fed rats. The effect of potassium deficiency and acute and chronic therapy with potassium.

(d) X-rays of rats fed ad libitum. The effect of potassium deficiency, of acute and chronic therapy with potassium, and of restoration to purina diet.

(e) Observations on potassium of body fluids.

(a) Preliminary observations from gross examination of potassium-deficient rats.

Force-fed potassium-deficient rats. In preliminary experiments observations were made on the clinical condition and from the inspection of the gastrointestinal tract of potassium-deficient rats. In force-fed rats every potassium-deficient animal developed a typical sequence of gastrointestinal disturbances which led to death in all untreated rats. Death resulted primarily, at least from this dysfunction. The animals fed 25 cc of diet a day appeared normal for about five to ten days, but, soon thereafter, changes occurred which indicated that the gut was incapable of handling the food administered. Defecation ceased from the fifth to eleventh day, usually occurring one or two days or so earlier in the DCA-treated animals fed diet A or B than in the rats fed diet B alone. In all these animals a large hard mass was palpable in the cecum. Distension of the abdomen quickly developed as the gut became distended with gas, fluid and later with unabsorbed food. Respiratory difficulties were associated with the distension. The animals became moribund in about three days after defecation ceased and died before the fourth day. Upon inspection of the gut under urethane anesthesia no contractions were detected in the gut both prior to severe distension and in moribund animals. The severe changes which occurred in all potassium-deficient rats may be observed from figure 6. This picture was obtained from a urethane anesthetized rat in which gross distension was present but which was expected to live about 12 to 18 hours longer. The walls of

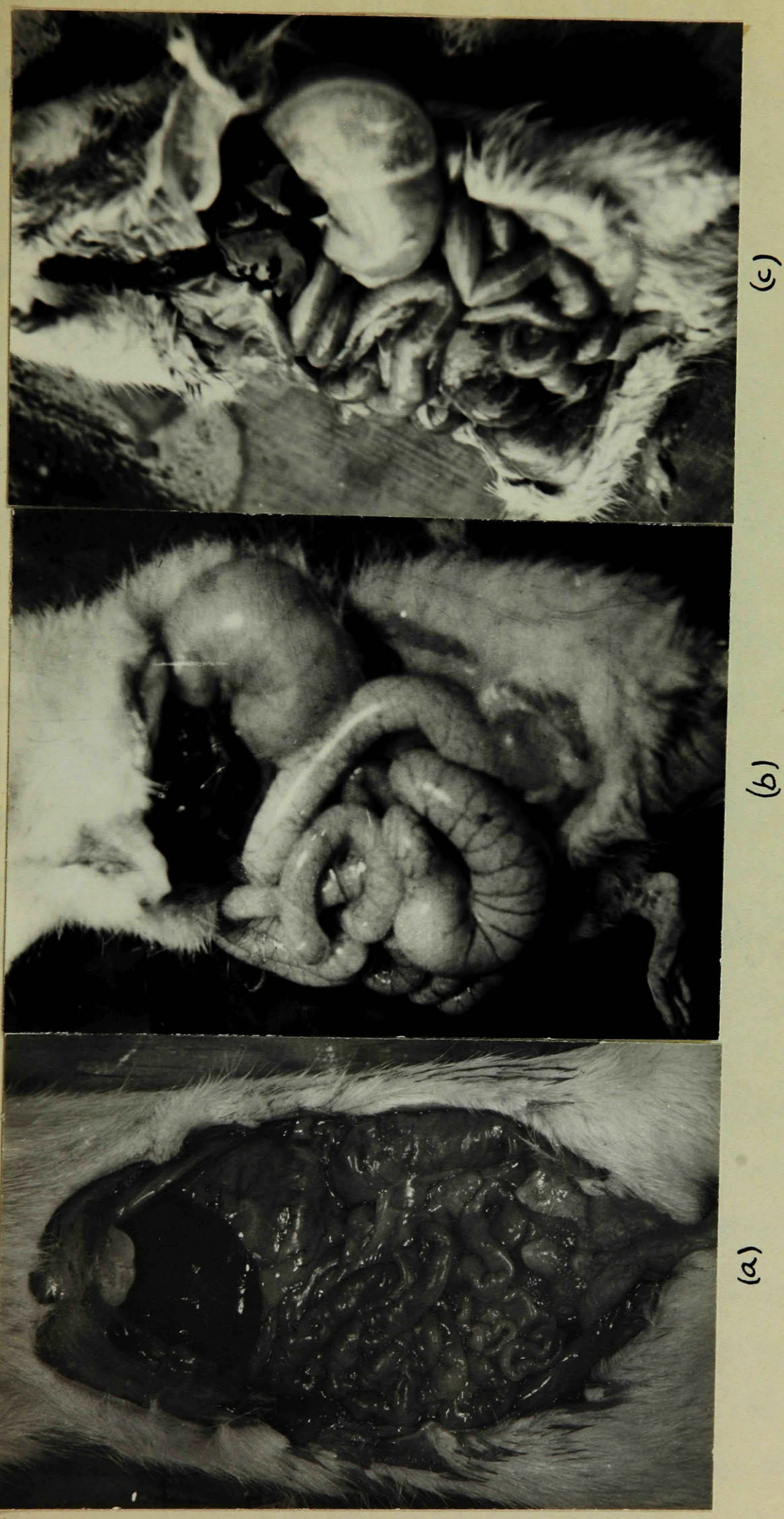


Figure 6. Photographs of the gastrointestinal tract in rats: (a) normal (b) potassium-deficient force-fed and (c) potassium-deficient fed ad libitum.

the gut were thin in their greatly distended condition and the intestines, both large and small, were in heavy coils which looked like a balloon. This state of extreme flaccidity distension and amotility was seen in all segments of the gut from stomach to rectum. The cecum was enormous with retained fecal material and gas. Engorgement of intestinal and abdominal blood vessels, particularly the veins, was observed in these animals as well as in potassium-deficient rats fed ad libitum, but is not readily seen in the picture. This vascular stasis was probably due in part to imparied venous return resulting from the pressure exerted on the abdominal blood vessels and will be discussed below.

By eliminating the bulk (cellu-flour) or reducing the dietary allotment to 18 or 20 cc, the onset of the symptoms were delayed a few days, but eventually identical disturbances in motility occurred. Thus while defecation usually ceased in about six days in rats injected with DCA, this function ceased in 9 to 12 days when cellu-flour was omitted and the food intake was reduced. In several animals feeding was discontinued when the symptoms obviously interferred with electrolyte studies. This did not alleviate the condition appreciably, but did prevent further enhancement of distension. However, upon refeeding the animals, the symptoms soon led to death. Defecation could not be stimulated by enemas or by mecholyl and prostigmin.

The effect of potassium upon the clinical condition and gut was dramatic in these animals. This was tested in four rats in severe condition in which the abdomen was so distended that the animal lay on its back, breathing with difficulty. A total dose of 1.55 to 2.0 meq of potassium was given by subcutaneous and injection by mouth. The following morning, fifteen hours after the treatment, each cage was filled with many feces and each animal appeared normal and active. From 16 to 30 grams of body weight were lost overnight in these animals as a result of the excretion of retained fluid and fecal material. The administration of potassium in adequate amounts to two DCA rats (1.95 meq per day) prevented the development of symptoms for the period of 17 days over which they were examined.

Rats fed ad libitum: In rats eating ad libitum potassium deficiency caused gradual and less drastic changes. Although all potassium-deficient force-fed animals died from gastro-intestinal disturbances, relatively few rats fed ad libitum died from potassium deficiency. It could not be concluded that these deaths were due to gastrointestinal symptoms per se except probably for the rat in figure 6. Flaccidity of the stomach and particularly of the small intestine was noted in all rats examined. Distension occurred in the small intestines as well as other segments of the bowel were noted but was not as marked as in force-fed rats. In a few rats the cecum reached enormous proportions and was distended chiefly with gas.

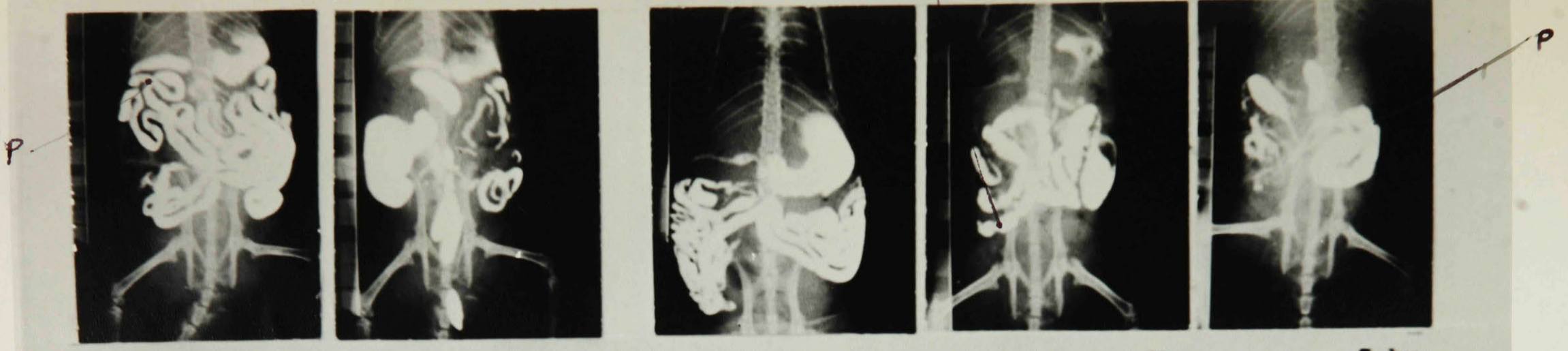
In several animals examined which were in fair condition, the stomach and small intestine were filled with clear fluid, which may be compared to the observations of Skinner and McHargue (179) quoted in the introduction. In figure 6 a picture of the gut of an anesthetized moribund rat the day following a barium test is shown. The changes are severe particularly for the stomach, which is dilated with gas and retained barium, and for the gas-distended small intestines, which are amotile. Since the main symptoms of gastrointestinal dysfunction were detected by the X-ray examination and confirmed in the severe stages by gross examination the details will be considered below. The engorgement of the blood vessels has been noted above for force-fed animals. This occurred in the rats eating freely when the gut was definitely hypomotile even when distension was only moderate.

After the severe stage of potassium deficiency had been reached the animals ate less regularly and were more wasteful of food and therefore measurements were not continued of food intake. They were not in poor condition. It is possible that the more severely deficient animals ate less in accordance with the reduced capacity of the gut to fulfil its functions, since usually little food was discovered in the stomach and small intestines on non-fasted rats when they reached this stage.

Potassium-deficient animals of the "ad libitum" group in good clinical condition could not tolerate the stress of

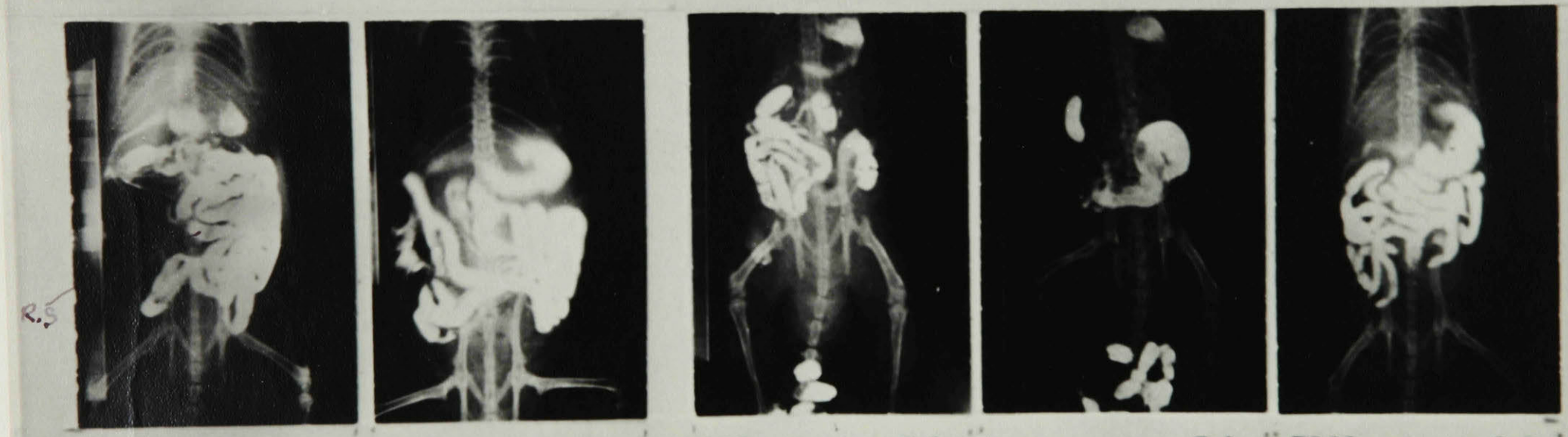
force-feeding even of small amounts of diet. Three animals which had been maintained on diet B ad libitum for 30 days and were eating well, were then force-fed two feedings of diet B of 7 cc each in one day. Although the animals had been healthy in appearance, soon after the first feeding the animals became visibly ill. They all died twenty-four to thirty-six hours later showing the typical picture of acute gastrointestinal distension which has been described for force-fed rats. Much of the food was retained in the stomach but the gut was distended with gas and fluid. From data presented below, only moderate reduction of motility and little decrease in tone occur after thirty days of maintenance of diet B ad libitum, but evidently the digestive tract cannot adapt to the stress of force-feeding. Three normal rats unadapted to the technique of force-feeding tolerated two feedings in a day of 12.5 cc each without visible effect.

The serum potassium for rat #5, of the deficient group, was 2.33 meq/litre prior to force-feeding and 5.1 meq/litre when moribund. The tendency for serum potassium concentration to rise in stress in the rats has been discussed in Part I.



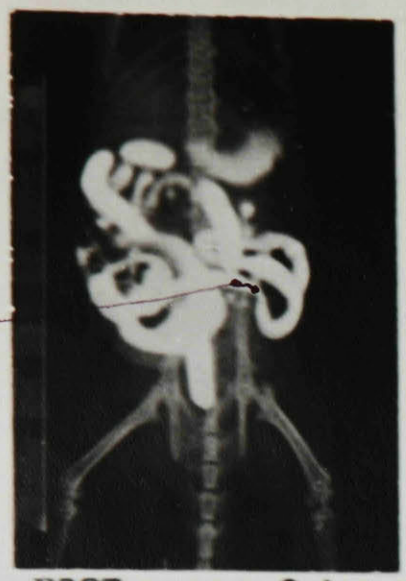
R397 1 hr 3 hr
a)Purina control b) Cont'd

R398 1 hr 3hr 5 hr
c)Purina control d) Cont'd e) Cont'd.



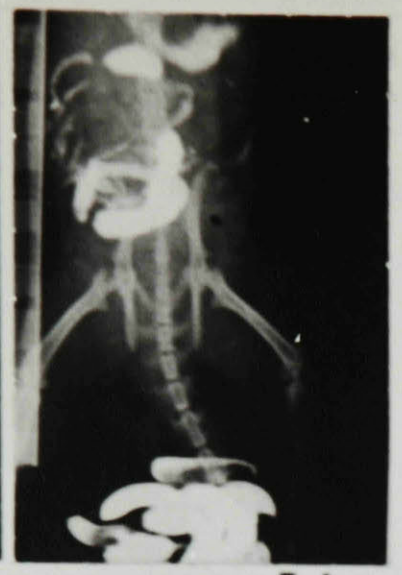
R353 1 hr. R353 1 hr R101 3 hr 5 hr R103 1 hr
f)Purina control g)Water by tube Purina h)Normal K-61 dy i) Cont'd J)Normal K-61 dy

Figure 7. X-rays of the gastrointestinal tract of rats fed purina or the normal control diet A.



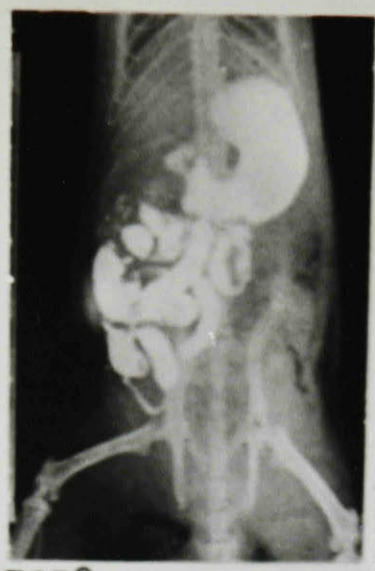
R397 3 hr

a) Force-fed normal diet A



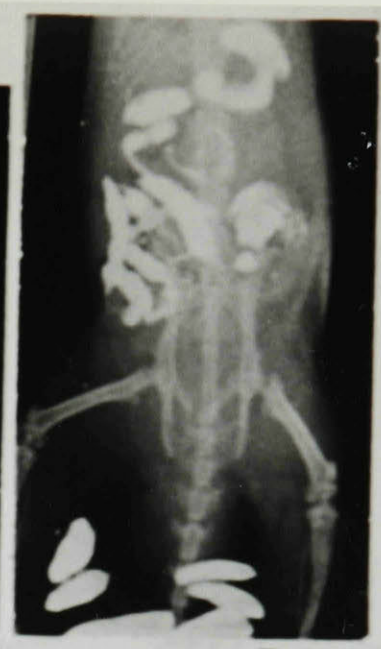
5 hr

b) Cont'd



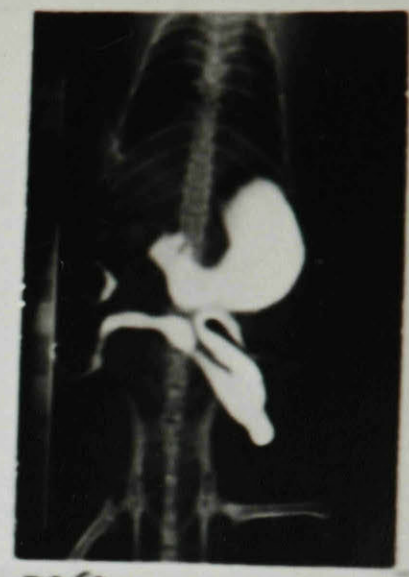
R358 1 hr

c) Force-fed normal diet A



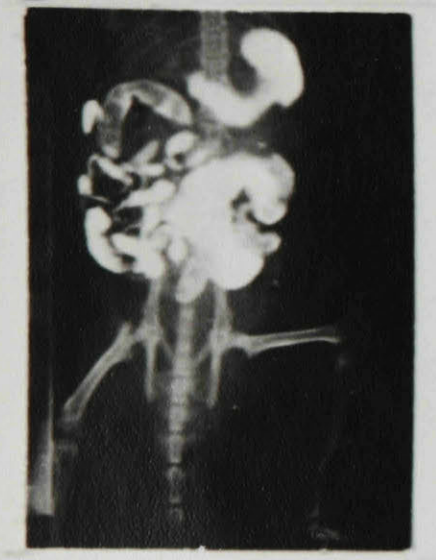
5 hr

d) Cont'd



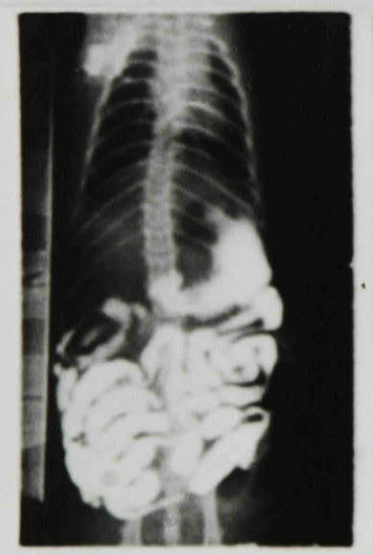
R361 1 hr

e) Force-fed diet E



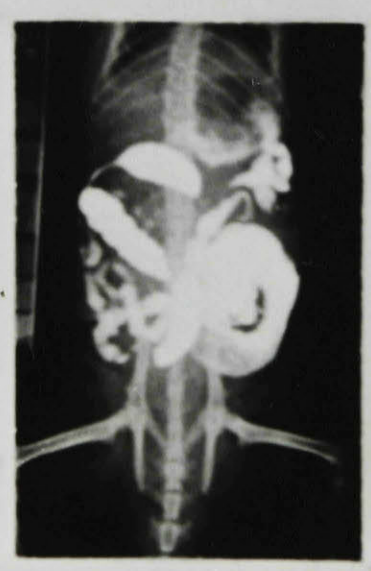
5 hr

f) R361 Cont'd



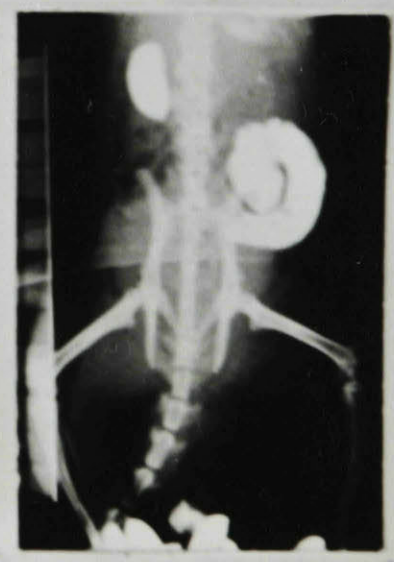
R358 1 hr

g) sham-tubed purina



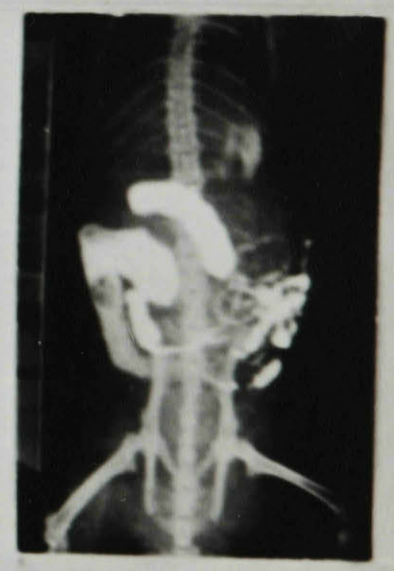
3 hr

h) Cont'd



5 hr

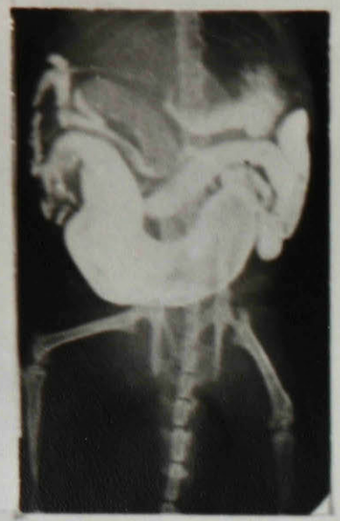
i) Cont'd



R353 5 hr

j) Water by tube purina

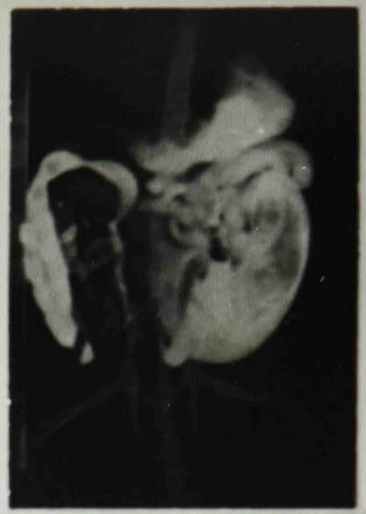
Figure 8. X-rays of the gastrointestinal tract of rats force-fed the normal control diet A and normal diet E, and of rats maintained on purina and sham-force-fed.



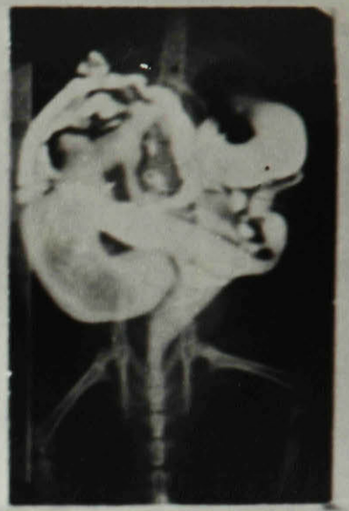
R387 24 hr.
a) 1st Ba
DCA-low K-8 dy

½ hr
b) 2nd Ba

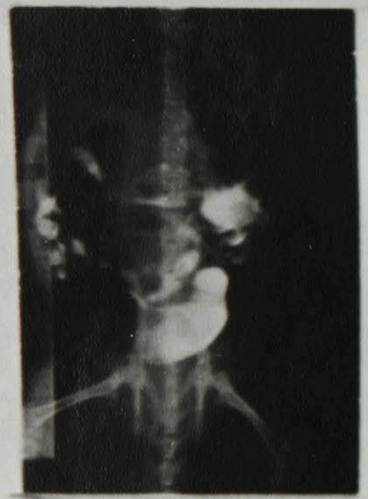
4 hr
c) Cont'd



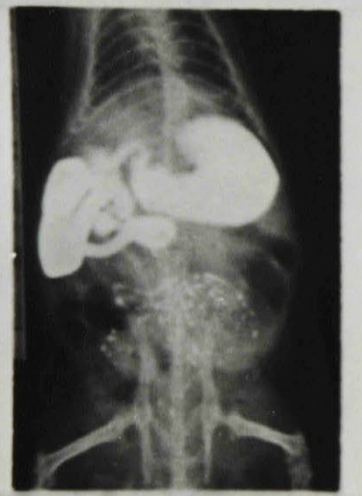
6 hr.
d) KCl effect



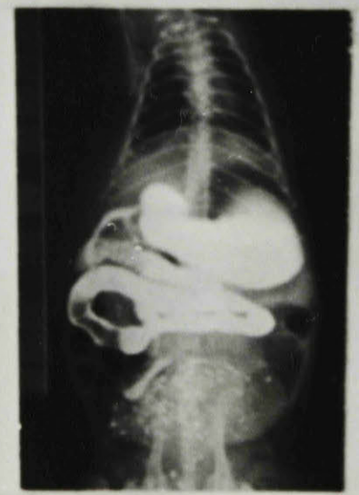
14 hr.
e) Cont'd.



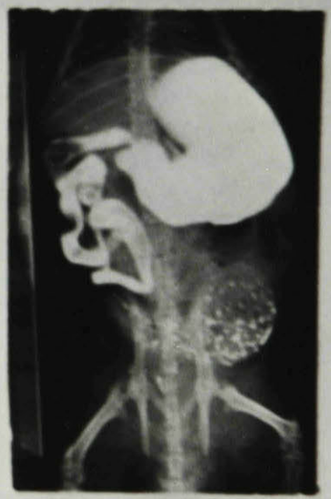
24 hr.
f) R387 Cont'd



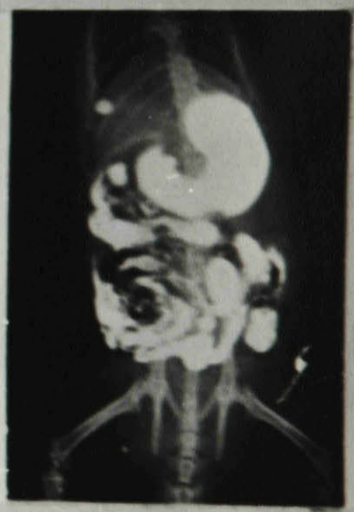
R390 5 hr.
g) Low K-DCA



6 hr.
h) KCl effect

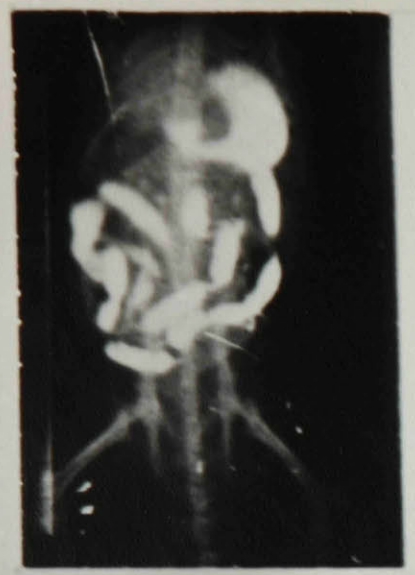


R383 1 hr.
i) Low K-DCA-8dy

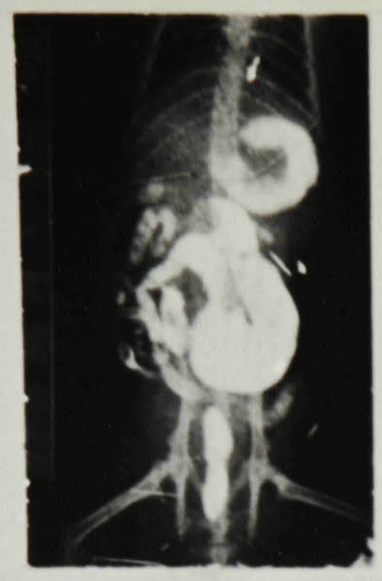


R355 6 hr.
j) Low K 9 dy.

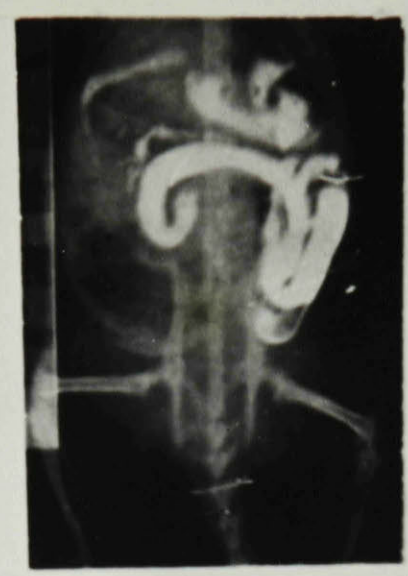
Figure 9. X-rays of the gastrointestinal tract in force-fed rats: The effect of potassium-deficiency and DCA and of acute therapy with KCl.



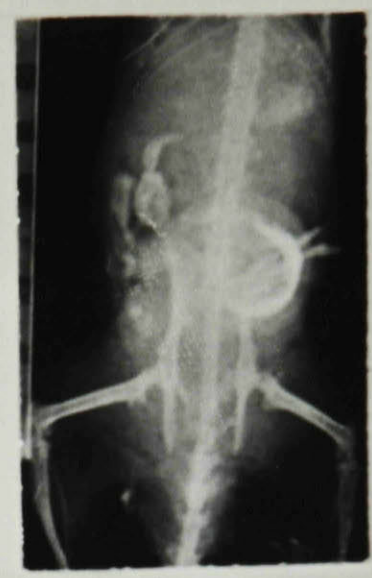
R354 2 hr
a) Low K-DCA 6dy



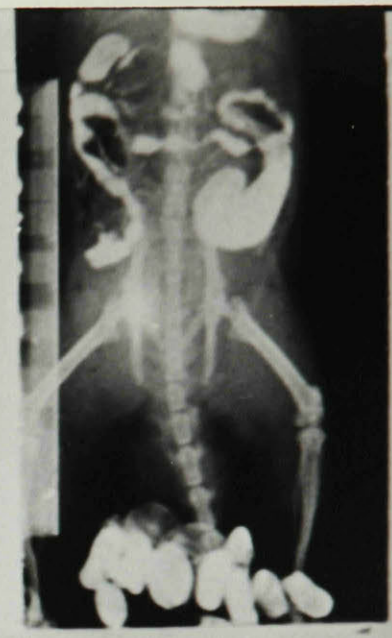
5 hr
b) Cont'd



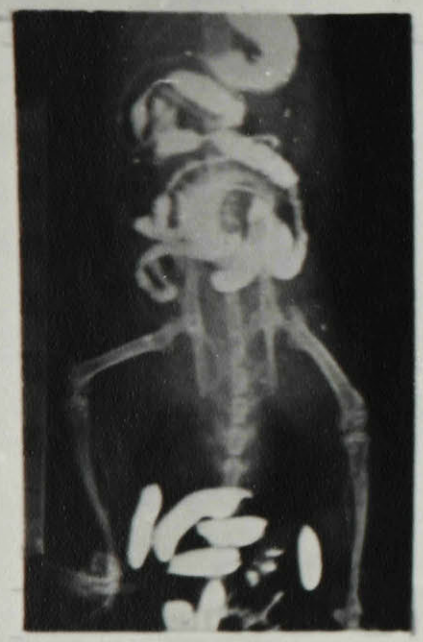
6 hr
c)Low K-DCA 9½ dy



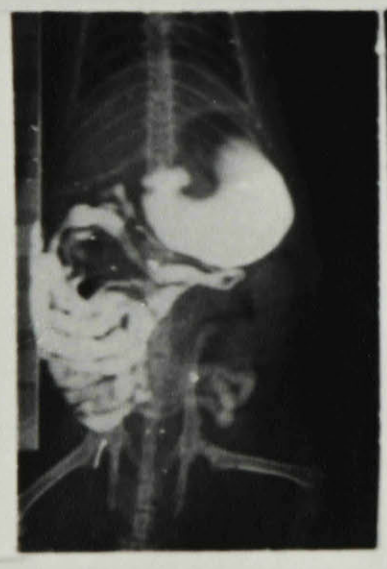
24 hr
d) KCl effect



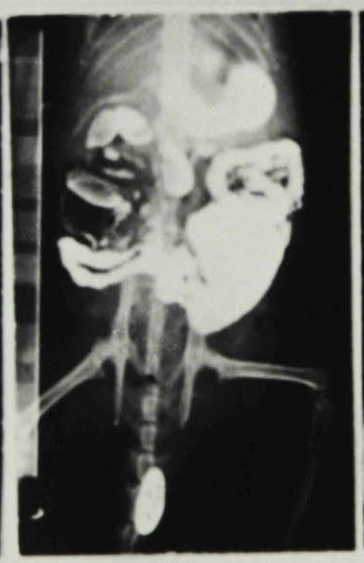
5 hr
e) Normal K ad lib 45 dy.



R355 5 hr
f)Recovery when force-fed normal K-3dy.



R383 1 hr
g)Recovery when force-fed normal K-12 dy.

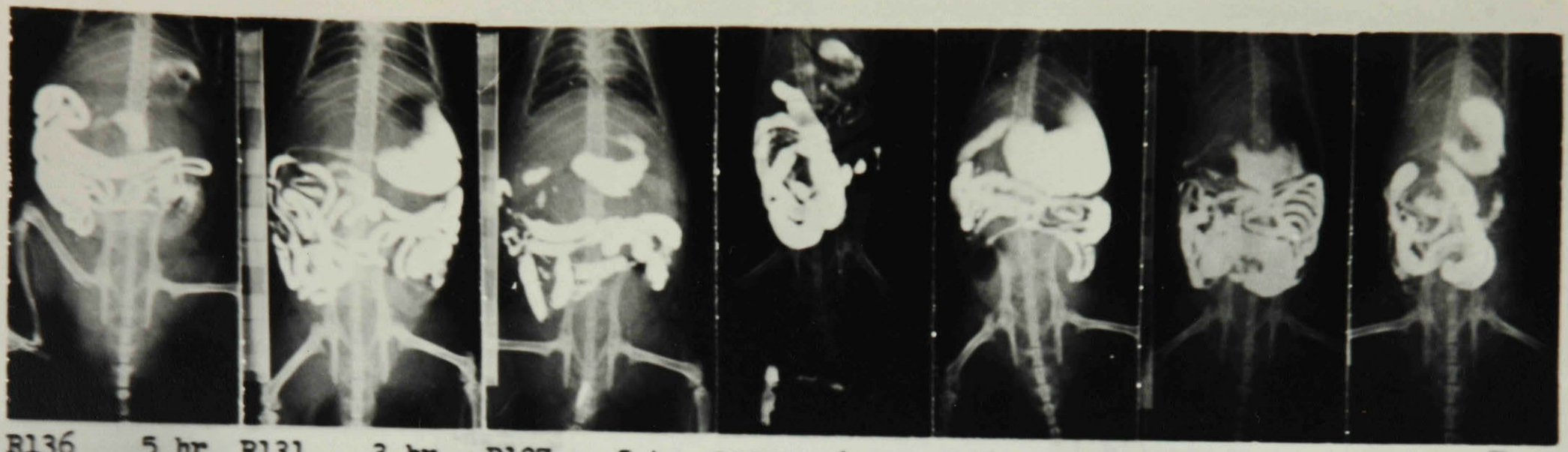


5 hr
h) Cont'd.

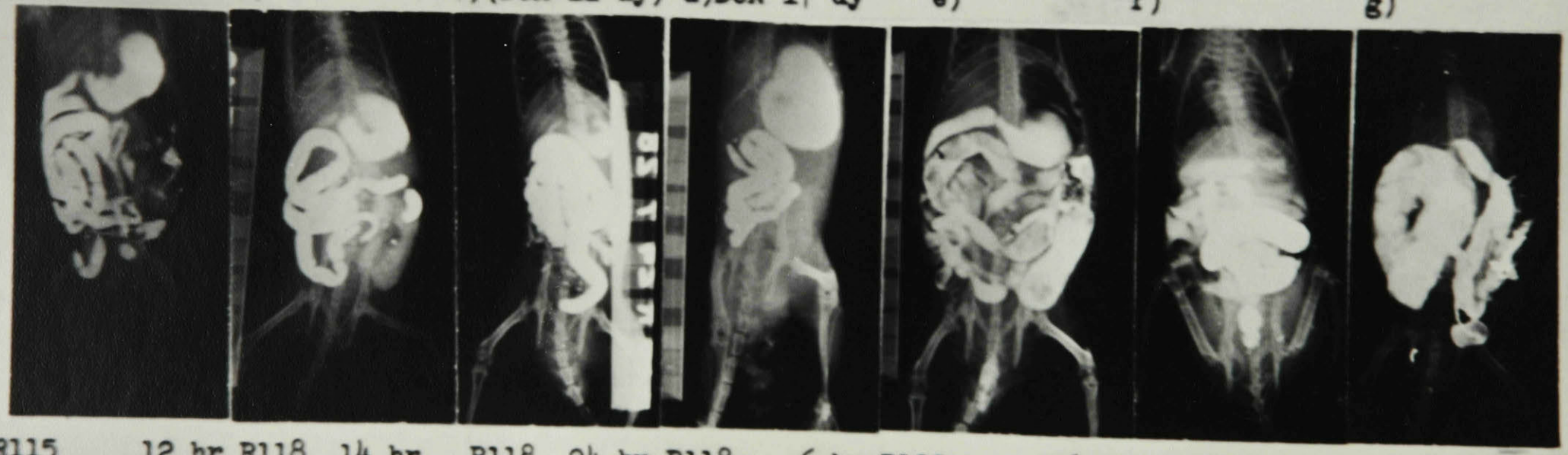
9 hr.
i) Cont'd.

R394 5 hr.
j)Recovery when force-fed normal K-4 dy.

Figure 10. X-rays of the gastrointestinal tract in force-fed rats; Effect of potassium-deficiency, and the effect of force-feeding the normal control diet.



R136 5 hr Low K 30 dy a)
R131 3 hr Low K 56 dy b)
R107 5 hr Low K 22 dy c)(DCA 11 dy)
R117 6 hr Normal K d)DCA 17 dy
R136 1 hr Low K 64 dy e)
R138 12 hr Low K 69 dy f)
R138 26 hr Low K 69 dy g)



R115 12 hr Normal K h)DCA 39 dy
R118 14 hr. Low K 65 dy i)
R118 24 hr Low K 65 dy j)
R118 6 hr Low K 99 dy k)
R132 26 hr Low K 84 dy l)
R106 20 hr Low K 33 dy m)(DCA 22dy)
R110 8 hr Low K 46 dy n)(DCA 33 dy)

Figure 11. X-rays of the gastrointestinal tract in rats fed ad libitum: The effect of a low potassium diet and/or DCA.

(b) Gastrointestinal X-rays of normal and control rats.

Explanation of figures: In the X-ray figures 7 to 12, the magnification of the pictures is the same in all cases. The rats of figures 7 to 10, except of rats 101 and 103, were larger than those of 11 and 12. The rat number is given, as e.g. R138, the hour after barium administration of the X-ray plate, and the treatment, e.g. low K (diet A), normal K (diet B), DCA (4 mg per day for force-feeding and 2 mg for ad libitum experiments). If DCA is in brackets this indicates that treatment with DCA was given with diet B for the time indicated.

Normal: Examples of the normal pattern of the digestive canal and the transit of barium in fasted rats maintained on purina are presented in figures 7 and 8. Within five minutes after barium administration the first half of the small intestine, at least, was outlined with barium. The immediate transit of barium is not shown for these control rats but was the same as that for rat 133 following recovery (Figure 12h, 2 minute X-ray). For about twenty minutes the barium regurgitated up to the oesophagus, often to the level of the pharynx, at regular intervals about seven to ten times a minute. This was not related to respiratory movements. Within a half-hour to an hour, from one third to two thirds of the barium (figures 7a,c,g, and 8g) and occasionally almost all the barium (7f) was evacuated from the stomach. Normally gastric evacuation was complete by from three to five hours. (figures 7e,8i,j).

Barium entered the cecum about two hours after administration and usually appeared in the feces by the end of the third hour (figure 7b,d). An estimated half or more of the barium was excreted in the feces by the end of five hours. The remainder was retained in the cecum and colon, or occasionally traces were present in terminal loops of the small intestine. The stomach was emptied (figure 7e and 8 and i). A small fraction of the barium was retained in the colon in fasted rats at the end of twenty-four house. In the stomach of the rats examined in this study peristaltic contractionswere not vigorous, probably because only barium sulfate and water were administered. The stomach was normally deeply constricted approximately in the centre (figures 7 and 8), and the contractions which were picked upon on the X-ray plate occurred below this. The strongest contractions occurred in the pyloric canal. It may be noted here, with regard to the place at which the stomach was constricted, that when the stomach was abnormally dilated the constriction occurred at a lower point than the centre of the stomach. This is illustrated in figure 8e, for example. The thin-walled upper part of the stomach provides less resistance to distension, that is has weaker tone, and dilates more readily thereby retaining the larger mass of the retained material. If the tonus is lower than normal, this dilatation of the upper part is more marked. Following the administration of the large volume of barium suspension, the stomach normally became distended (figure 10h), but as a

result of the increased tonus, the stomach contracted in volume within twenty minutes.

In the small intestine the barium was normally in a continuous stream within a long loop of segment of the bowel (figures 7a,c,f,g, and 8g). Rhythmic segmentations (r.s. in the figures) were seen fluoresconically, pulsating at the rate of approximately 40 a minute. The food material was tossed rapidly to and fro between adjacent segmenting constrictions. There was considerable variability in the strength of these contractions from time to time in the same rat, but they occurred almost continuously in the normal rat in one segment or another and often throughout. Very strong contractions were observed for rat 398 at the three hour X-ray (7d) whereas a more quiescent stage was picked up by X-ray at one-hour (figure 7c). The more usual appearance of these segmenting contractions can be noted in figures 7f,10g, and h,. Occasional peristaltic waves were noted which may be seen in the X-ray as longer constrictions (figures 7 and 8, designated as p), and occasionally by thinning of the barium shadow caused by the rapid spreading of the barium over the mucous membrane (figure 7a).

In the colon small shallow waves were noted traversing the surface (figure 8i,j), as well as deeper and wider contractions (7e designated as p.) In the distal colon the barium containing fecal material became separated into individual masses distending the colon lumen (7b,e, and 8i,j). This

breaking up of the column of fecal material occurred much higher up, even in the proximal colon, than occurs normally in other species such as man and dogs.

Controls on Diet A: Before discussing the effect of potassium deficiency upon the motility of the digestive tract the influence of the control synthetic diet A will be considered. After rats were fed diet A ad libitum for two months very slight abnormalities developed in the small intestinal pattern, but these did not influence significantly the rate at which barium was evacuated from the stomach or the rate excretion. As discussed later the factors of handling and the various experimental procedures may influence motility. In force-feeding experiments lasting from 9 to 22 days, there was a tendency for the column of barium to be separated into individual masses in the small intestine and a slight delay in gastric emptying time occurred, but the rate of excretion of barium in the feces was not much less than normal. The changes noted in both cases were very slight in comparison with and differed from those observed in potassium deficiency.

It may be mentioned here that the breaking upon of the normally continuous column of barium into separate masses or clumps which may be of different length or size, has been termed "abnormal segmentation of barium" by the radiologists. Barium is present in a small segment of bowel and separated from the next small mass of barium by empty bowel. The length of the so-called segmented barium masses i.e. divided barium, varies according to the condition of the gut.

Upon inspection of some animals (potassium-deficient or abnormal discarded animals) it was found that, when this "abnormal segmentation of barium" was marked, the masses were hardened in the intestine by the absorption of water and the length of intestine between masses was contracted somewhat and empty of contents. It must be emphasized therefore that in the future pages we will be talking of "abnormal segmentation of barium" and also of rhythmical segmentations, i.e. the rhythmical movements described by physiologists.

In figure 7h,i.j X-rays for the contls fed <u>diet A ad libitum</u> are shown for rats 101 and 103 maintained on this regime for two months. The small intestine was within normal behaviour in R101, but evidence of slight "abnormal segmentation of barium" was seen in rat 103. The transit through the gut was normal in both animals (the five hour X-ray of 103 was very similar to that of 101). A very slight trace remained in the upper part of the stomach of rat 101 at the end of five hours, probably due to adherence of traces of barium on the mucosa. This is not enough to classify the gastric evacuation as prolonged. But it does suggest the possibility that mucus secretion of this part of the stomach may be affected by the treatment or handling, not only in the case of this animal, but in other animals in which traces of barium remain long after the rest has been evacuated. The adherence of traces of barium to the walls was confirmed in several animals by inspecting the stomach directly.

X-rays for two force-fed diet A rats are given in figure 8a to d, rat 397 representing the average behaviour of the gut of this group fed diet A, and rat 358 showing the most extreme changes produced. In the latter animal feeding purina did not have much effect in restoring motility towards normal as it did in most animals, so that some secondary factor probably contributed to the symptoms produced during force-feeding. (The effect of restoring animals to purina following the synthetic dietary regime will be discussed with data on potassium-deficiency). In the force-fed controls from a trace to approximately twenty percent of the barium was retained in the stomach at the end of five hours, whereas normally the stomach is cleared by this time. Barium was present in the cecum in two hours, and from one third of the barium to normal amounts of one half or more were excreted in five hours. Thus the transit was only slightly prolonged. The tone of the stomach was often reduced which resulted in greater or more prolonged dilatation of this organ after the barium was administered (figure 8c). The main abnormalities were in the small intestine in which "abnormal segmentation" sometimes occurred (figure 8c but not 8a) and other irregularities of pattern not diagnosed. The wispy outlines of bowel noted in some force-fed animals indicated the presence of small amounts of gas in bowel constricted more than normally, which, added to the finding of abnormal segmentation in some rats, suggested irregularity in tonus. The changes in the large intestine were not appreciable.

Sham force-fed rats: The changes in gastrointestinal motility produced in force-fed rats were not due to the actual tubing and probably not to sudden gastric distension with fluid since experiments in which these factors were tested showed that the motility of the gut was normal in behaviour. In rat 358 the tube was passed down into the stomach bidaily without introducing anything, and in 353 water was introduced by stomach tube bidaily. From figures (8g,h,i) for rat 358, and figures (7g,8i) for rat 353, it can be observed that the transit of barium through the gut and the small intestinal pattern were normal for both groups.

Diet E: It was possible that the small changes produced by force-feeding may have been due to the diet A fed. Therefore, a different diet (E) was administered for 16 days. The changes produced by this diet were much more marked, and were within the range produced in force-fed animals in mild potassium deficiency (figure 8e,f). The transit of barium from the stomach and through the gut was prolonged. Approximately one quarter to one third of the barium was in the stomach at 5 hours and gastric evacuation was not complete until 9 to 11 hours. Barium passed into the cecum and colon between the third and fifth hour, but few feces containing barium were excreted before seven hours after administration. In most of the five animals examined approximately half had been excreted by nine to eleven hours. The effects appeared to be due in considerable part to spasm of the pyloric

sphincter. Most of the barium meal was retained for more than an hour in the dilated stomach. However, the barium reaching the small intestine appeared to move slowly also. The pattern of the small intestine was abnormal. "Abnormal segmentation of barium" was noted, and in some X-rays irregularities in the tone occurred, but dilatation was not a prominent feature. It seems improbable that these changes were due to vitamin deficiency in view of the fact that extract equivalent to five grams of yeast were added per 100 cc of diet. The potassium content was higher than that for diet A. It is possible that the higher content of fat in diet E as compared to that in fluid diet A contributed to this difference on the digestive tract, especially since the fat of diet E is mainly in the form of animal butter fat, while that of diet A is mainly in the form of vegetable oils. It is well known that fats delay gastric evacuation time. With the force-feeding of animals the distension produced in the stomach bidaily might have a chronic effect on the tone of the stomach and on the pyloric sphincter, which could be detected with the barium suspension in the absence of food. In addition, the osmotic pressure of diet E was greater than that of diet A, since it contained sucrose and lactose, rather than dextrin and sucrose. Substances with high osmotic concentration delay gastric evacuation, but whether the difference between diet A and E was physiologically significant is one undetermined point.

To summarize, the normal animal maintained on purina exhibits the following characteristics in barium tests prior to experimental use: (a) complete gastric evacuation in 3 to 5 hours, (b) continuity of the barium fluid column in the small bowel and regularity tonus, (c) excretion of an estimated half or so of the barium in 5 hours. The rats fed Diet A ad libitum showed slight changes in intestinal pattern. Those force-fed this diet showed somewhat more changes in the small intestine and slight prolongation of gastric evacuation time.

(c) Gastrointestinal X-ray studies on potassium deficiency in force-fed rats.

The development of deficiency effects: Examination of the gastrointestinal motility was made following a fifteen hour fast. In the potassium-deficient rats a rough idea of the sequence in which gastrointestinal disturbances developed was obtained from examination of different rats at various times after the potassium depleting regime was initiated. It was not possible to trace the development of changes in a single animal, because three or four days, at least, must elapse between barium tests, and because these tests per se affect the development of the symptoms. Animals were examined during the following stages and were categorized as indicated: (a) Early changes - in rats two to four days prior to cessation of defecation, (b) Moderate changes - in rats examined about the time which defecation ceased, (c) Severe changes - in rats examined about one and one half to two days after defecation ceased and when abdominal distension was visible, but not extreme, and (d) Extreme severe changes - in rats examined about three days after defecation had ceased when abdominal distension was extreme and the animals were not expected to survive for a day. Most of the observations were made on rats of the category (c). Symptoms observed in DCA-treated and non-injected rats force-fed diet B were the same and the observations will not therefore be grouped separately.

In discussing the sequence of events leading to severe gastrointestinal dysfunction the data considered has been that presented in figures 9 and 10, as well as that obtained in X-rays not included because of the bulk of the material, and that obtained from detailed fluorescopic notes. The X-rays in figures 9 and 10 will be considered in more detail following the discussion, in relation the improvement produced by acute or chronic administration of potassium.

Early changes: The X-ray examination of four rats prior to the detection of clinical symptoms showed that only slight changes occurred, (in comparison ~~to~~ with the controls force-fed diet A), in the first five to six days of deficiency in these force-fed rats. Three of the animals did not differ appreciably from the force-fed controls of figure 8a to d except that "abnormal segmentation of barium" in the small intestine was more evident. The changes were more evident in rat 354 (figures 10a,b). The chief abnormalities which occurred in this animal were slightly prolonged gastric evacuation time, "abnormal segmentation of barium" in the small intestine but little or no evidence of distension, and slightly prolonged transit of barium. Since a few days later rat 354 (figure 10c) and one of the others (figure 6b) developed severe changes, the gastrointestinal disturbances are therefore sudden in onset rather than gradual.

Moderate changes: About the time defecation ceased the chief changes observed were in the stomach and the rate of

transit of barium through the whole tract. Animal 355 (figure 9j; see 9ṙ, also) illustrates changes observed at this time. The stomach was markedly hypotonic and spasm of the pylorus probably occurred. The tone of the small intestine was not appreciably reduced, but abnormalities in the pattern of the small intestinal contours were noted. Evidently the whole tract was markedly hypomotile, because barium remained in stasis for many hours, up to the 12th hours, despite the early distribution of the barium evacuated from the stomach throughout the small and large intestine.

Severe changes: (Figure 9a.b,c,g,i,10c). In these animals the dilatation of the stomach in the first one or two hours was not more marked than occurred in the animals at the time of cessation of defecation, but it persisted longer. The chief difference between the observations in these animals exhibiting clinical distension and those prior to this stage was in the dilatation of the small and large intestine and the weakness of the rhythmic segmentations. For up to eight to twelve hours most of the barium was retained in the dilated stomach and the rest was a few dilated loops of small intestine, in continuous columns rather than segmented. This was typical of changes which occurred in these and more severely affected animals. In the group examined about two days after defecation ceased, barium remained in stasis until the tenth to twelfth hour and usually did not reach the cecum until after this time. Variable amounts of gas and fluid were present. The distension

by gas of the gut tended to increase during the X-ray examination. Usually no barium was excreted in twenty-four hours and gastric retention of barium still persisted. The rest of the barium was found in dilated loops of small intestine and large intestine and especially in the enlarged cecum which was characteristic of severe changes in the force-fed rats (figure 9a).

Extremely severe changes: Unfortunately no X-rays are available to demonstrate the severest effect such as occurred in the rat illustrated in figure 6b. However, detailed fluoroscopic notes were made on other animals exhibiting similar abdominal distension. In three rats fed diet B for seven days and injected with 4 mg of DCA, an examination of the animal by X-ray prior to the barium administration showed the accumulation of a large amount of fluid (partly unabsorbed food) and gas in the stomach and intestines, and demonstrated the marked abdominal distension. Great dilation of the stomach was seen after the barium meal in all examinations during the first 9 to 12 hours. The gastric contents were mixed with gas in large amounts. The gastric distension at five hours was as great or greater than that at one hour in figure 9i, and persisted almost unchanged until the tenth hour at least. Probably not as much as fifty percent of the barium was eliminated from the stomach at this time and no barium had approached the cecum. Thus the barium remained in stasis from the fifth to tenth or twelfth hour in a much enlarged stomach and a few coils of

small intestine containing large amounts of gas. At twenty-four hours changes were more severe than is observed in figure 9a. About one third of the barium was retained in the stomach.

The changes in the stomach may be elaborated upon. Once "moderate" disturbances in the motility of the gastrointestinal tract were detected, about the time defecation ceased, the tone of the stomach was greatly reduced. In addition, the prolonged retention of fluid and barium in the stomach, not only in the force-fed rats but in depleted rats fed ad libitum, may have been due to spasm of the pyloric sphincter. Thus in figures 9b and c, it is evident that some increase in gastric tonus has occurred from the reduced size of this organ when not much barium had been evacuated, but this increase in tonus has been able to evacuate but little barium and fluid from the stomach. Normally in man and animals fluid is readily eliminated from the stomach with only weak peristaltic contractions (138). As seen from the normal rats of figure 7 and 8 and 12h when barium is administered as an aqueous suspension it is rapidly eliminated from the stomach without strong peristaltic movements.

Potassium deficiency and acute therapy with potassium:

A typical example of the effect of severe potassium deficiency in a force-fed rat and the stimulatory effect of potassium is shown by rat 387. (figure 9a to f). In this animal the clinical symptoms as discussed for the category

"severe changes". Twenty-four hours after the first barium meal (figure 9a) no excretion had occurred. The stomach was distended with gas and contained barium. Most of the barium was retained in the distended cecum and ileum, which was flaccid. Faint contours of the colon show this segment to be dilated. A second dose of barium was then given. The half-hour and four-hour X-rays show that little movement of the old barium has occurred and that the new barium mainly in the stomach. By four hours about one quarter had been released into the upper loops of small bowel which, though dilated, showed some feeble segmenting contractions. At four hours 3 meq of potassium was injected subcutaneously. From fluorescopic examination little change occurred for more than one hour, but by the end of two hours active rhythmic segmenting contractions were observed continually pulsating and active peristalsis was noted (figure 9a). As shown from the six hour X-ray taken at this time, the cecum had shifted completely from one side of the abdomen to the other: it was smaller and its contours changed. The cecum continued to show this mobility, shifting back and forth from side to side at least every couple of hours, and much of the old barium was soon released from the ileum into the cecum. Contractions increased in vigour in the small bowel and although distension was still present, it was much reduced. Barium was excreted between the twelfth and fourteenth hour (eight to ten hours after potassium administration) in the form of diarrhea. The movements of the gut are well-shown in

figure 9e. As seen from the twenty-four hour X-ray taken the following morning (figure 9f) most of the total amount of barium had been excreted, although some gas was still present in the tract and a trace of barium was in the stomach. This compares favourably with the barium excretion in a day by normal rats. Thus an animal which had not excreted anything for nearly four days cleared the alimentary canal almost entirely of two barium meals and a large amount of fluid, fecal matter and gas within 20 hours after the administration of potassium. Similar relief of gastrointestinal hypotonicity and hypomotility was obtained shortly after potassium administration in rat 383, in which the deficiency symptoms were comparable to those of rat 387. The one hour X-ray only is given (figure 9i). Several hours prior to the test the potassium concentration was 2.45 meq per litre. A total of 5 meq of potassium was administered during that day. The following morning the serum potassium concentration was 5.81 meq per litre. The gastrointestinal clearance of barium was more complete than for rat 387. Rat 390 was in poor condition after 7 days of potassium deficiency (Diet B and DCA) although the abdominal distension was not more marked than for the above rats. As in all of these animals, nearly all the barium was retained in the stomach by the end of five hours (figure 9g). Ten cc of hypertonic potassium chloride (310 meq/litre) was administered subcutaneously after the five-hour X-ray. The rapid effect of potassium therapy in initiating rhythmic segmentation was readily seen within a half hour.

The six-hour X-ray (figure 9h) shows the segmenting movements and peristalsis. Barium had passed down the small intestine noticeably in an hour, whereas the barium had been in stasis prior to this. This animal died a few hours later. Death was in part due to the effects on the lung of hypertonic potassium solution given to an animal in poor condition. Upon autopsy it was seen that barium had passed down only a few inches of the small intestine at which point a constriction in the wall of the gut was noted. In later experiments isotonic potassium chloride solution was administered rather than the hypertonic solution.

In rat 354 the early symptoms are contrasted with the advanced changes produced three and one half days later. The earlier X-rays (figure 10a,b) show that a few feces were excreted at five hours. Most of the barium had reached the cecum, and about a quarter was in the stomach. At no time did the barium contours indicate that the stomach was appreciably more enlarged than normal force-fed controls. "Abnormal segmentation of barium" in the small bowel was quite marked (figure 10a), but movements were quite active. There was some dilatation of the proximal colon and possibly of the cecum. Three-and one-half days later distension of the abdomen suddenly developed. Gas was present in the stomach and intestine and therefore the quantity of barium retained in the stomach was masked and could not be estimated. The rest of the barium at the end of six hours was in a couple of flaccid and dilated loops of small

intestine (figure 10c). A total of 2.2 meq of potassium was injected. Between the 13th and 18th hour after barium most of this substance had been excreted from the gut in the form of diarrhea. Contractions in the ileum, cecum and colon were seen to be very vigorous. The twenty-four hour X-ray (figure 10d) shows practically no barium remained in the gut which is an improvement over the normal. This animal was thenceforth maintained ad libitum on diet A for a month and a half.

Potassium deficiency and relief of effects with a normal diet: The "moderate" degree of deficiency changes occurred in rat 355 which was force-fed diet B but was given no DCA (figure 9j). (For control X-rays on diet A compare figure 8b). The initial gastric dilatation and retention of barium at one hour was as marked as for rat 383 (figure 9i), but the subsequent X-rays showed that there was not appreciable intestinal dilation in rat 355 until about the twelth hours, whereas in the other animals of figures 9 this was a prominent feature. At least one half to two thirds of the barium was still in the stomach at six hours. The rest of the barium was dispersed though the gut and one feces was excreted. Segmenting movements were fairly active. Irregularities in contour in the intestine may be due to small gas accumulations and/or irregularities in tone. The six-hour X-ray might indicate that only the gastric changes were very marked. However, the barium remained in almost stasis until the twelfth hour at least, slight dilatation developed in the small bowel, and no further feces were excreted.

Movements were weaker. This demonstrates the hypomotility which occurred throughout the tract, and points to the necessity for repeated examination to follow barium transit.

This animal was force-fed diet A, that is, a normal potassium diet. (The low potassium diet was continued to permit recovery from the test, and symptoms became more marked in this interval).

After recovery from the test (three days on diet B continued), the rats were <u>force-fed diet A,</u> containing a normal amount of potassium. The improvement in motility after three days is evident from the six-hour X-ray (figure 10f), especially since symptoms progressed during the continued feeding of diet B after the test (above). In six hours, one quarter to one third of the barium was excreted and less than one quarter was in the stomach. Movements in the small and large bowel were active. Abnormalities which persisted after this short treatment of three days were in the small intestine mainly. More of the barium appeared to be retained there than normally. However it is difficult to make out with certainty which loops were colon and which small bowel.

Rat 394 showed similar deficiency changes to 354, ℵ was treated with 1.8 meq of potassium by injection and then restored to the control diet A fed by stomach tube for 4 days. It can be seen that gastric evacuation of barium was almost normal at five hours (figure 10). Several feces has been excreted, but most of the barium was present in the small and large intestine.

Some dilatation of the loops of small intestine occurring between areas of almost spastic contraction indicated the unevenness of tone in the loops. Most of the barium was present in the colon ready for excretion. By the tenth hour only a quarter or so remained in the large intestine. The rest of the alimentary canal was completely cleared.

For rat 383 restored to diet A for two weeks, the one hour X-ray (figure 10g) may be contrasted with that obtained during deficiency (figure 9i). This shows the great improvement in gastric evacuation and motility of the small bowel. Gastric peristalsis appeared to be active but the tone of this organ was not normal. Approximately one quarter was retained at the end of five hours, but only very slight traces were present by seven to nine hours (figure 10i). The small intestine was completely normal (figure 10g,h) as shown by the active rhythmic segmentations and peristalsis and the continuity of the barium column in loops of bowel of normal tone. Excretion rate was prolonged. This appeared to be due mainly to delay in the cecum to rectum as can be seen in figure 10h. By nine hours about one half to two thirds of the barium was excreted and the rest was in the large intestine.

From the data of these and other rats relieved of potassium deficiency by force-feeding diet A, it was concluded that very marked improvement occurred within a few days in tone and movements of the stomach and small intestine, and in the

evacuation rate of the barium, but that recovery to the normal motility observed in force-fed rats (figure 8a to d) prior to deficiency could not be obtained while the diet was force-fed. The transit time was prolonged, and gastric evacuation time was slower. Contractions in the small intestine were active, but "segmentation of the barium" in the small intestine was more marked, and tone was often not normal or was uneven. Improvement, almost to normal, usually occurred when the diet A was fed ad libitum as discussed below.

The improvement in the gastrointestinal tract following potassium deficiency was much greater when the animals were allowed diet A ad libitum, than when they were when force-fed diet A. This is shown for rat 354 (figure 10e) six weeks after the termination of potassium deficiency regime (figure 10c). Only slight abnormalities persisted in the gastrointestinal tract of this animal, although in other animals recovery was not as complete. In the case of rat 354 these consisted mainly of a delayed gastric evacuation time (figure 10e) so that about ten percent of the barium was retained at five hours, slight hypomotility of the stomach, and slight irregularity in the small intestinal pattern. In most animals "abnormal segmentation of barium" occurred with variable severity from animal to animal, larger amounts of barium were found in the small intestine at the end of five hours, and often the barium excretion was subnormal.

When rat 354 was placed on purina diet for two weeks the transit of barium was normal, although certain features of the small intestinal pattern differed from the pre-experimental controls of figure 7. This will be discussed with rats fed ad libitum. The stomach was normal in rat 354 and approximqtely normal in the other animals treated similarly.

(d) Gastrointestinal X-ray studies on potassium deficiency in rats allowed food ad libitum.

The development of potassium effects: In rats fed ad libitum it was more difficult to trace the sequence in which changes occurred in gastrointestinal motility than it was in force-fed rats. For, as shown by X-ray studies, the rate at which gastrointestinal dysfunction developed varied considerably from animal to animal, the organ or organs of the digestive tract first affected or most severely affected varied from animal to animal, and the changes produced in the small intestines were extremely complex. However, in all the animals observed changes of one type or another occurred in the small intestine. Usually decreased tone and motility of the stomach was associated with this, but in a considerable proportion of animals the stomach motility was not affected until intestinal disturbances were moderately advanced. One animal showed typical severe intestinal changes after 99 days on potassium deficient diet, but the stomach was completely evacuated of barium in 6 hours. In several animals the changes in the cecum were advanced far beyond the abnormalities in stomach and intestine. It was necessary to consider the response of the whole tract over a period of at least 12 hours once the symptoms were moderately advanced. For, in several cases, the five hour X-ray presented a completely different appearance to that in subsequent X-rays at 10 to 14 hours. Certain of these X-rays were difficult to interpret without direct

inspection of the gut. However, there were two effects common to nearly all the animals,-one, the small intestine passed through a series of changes which were indicative of first a decrease in motility and then a gradual and progressive decrease in tone, and two, symptoms were produced in the severest stage which can be considered typical of the effect of potassium depletion in rats fed ad libitum. The final stage of deficiency was extreme hypotonicity and hypomotility of the stomach and small intestine, and similar but more variable effects on the cecum and colon.

From repeated examination of the twenty-eight rats used in ad libitum experiments, it has been possible to classify the data in the order in which symptoms developed in the majority of animals. The animals have been grouped together regardless of whether treated with DCA or not, since the changes were similar. The symptoms have been categorized into two groups on the basis, mainly, of the changes in the small intestine and the delay in transit of barium through the alimentary canal. The development of abnormalities is illustrated in figure 11. The X-rays are arranged according to the estimated severity of changes as judged from the gastrointestinal movements and barium in transit during the test day.

In the <u>early</u> stages definite hypomotility of the small intestine was observed, but the tone was normal, or possibly even increased. Later "abnormal segmentation" of the barium column in the small bowel occurred and then tone decreased.

The transit of barium through the digestive tract was decreased considerably in most cases. In some, no barium-feces were excreted in five hours, whereas the normal animal excretes about half the barium in this time. The stomach was affected in about half the animals, in which an estimated ten percent to one third was present at five hours. Gastric dilatation was not pronounced in these animals. Examples illustrating these early changes are presented in figure 11a,b,c. In rat 136 few intestinal contractions were seen in three X-rays taken over a five hour period. The motility was therefore reduced in the small intestine. The barium was spread through very narrow, ribbon-like loops of intestine in a continuous column, with one considerably dilated loop. Whether this pattern of the small intestine was indicative of increased tone at this stage, has not been ascertained as yet. It occurred in the majority of the animals examined in the early stages. It could also be due to incomplete filling of the loops because of reduced motility of the mucosa since normally this aids in spreading the material over the surface. Little barium was passed into the large intestine in five hours.

In rat 131, the changes in the small intestine are indicative of hypomotility but not hypotonicity. The contours of the bowel are smooth. In addition, this X-ray suggests the beginning of abnormal segmentation of barium, since the barium is not in a continuous column. Gastric retention was greater than normal both at three hours (figure b) and at five hours.

In rat 107, which was maintained on diet B for 22 days, and given DCA (2 mg per day) during the last 11 days, definite changes in the small intestine were present. "Abnormal segmentation of barium" occurred but the tone was not appreciably affected in the loops which were filled with barium. Gastric retention of barium was noted at five hours. In addition, in this animal and four others the stomach was observed to be completely inverted. This was not related to the severity of the changes. This change is indicated in the five-hour X-ray of figure c, but was observed more clearly at three hours.

Gradually the tone decreased and the barium, still abnormally segmented, was seen to fill sausage-shaped loops of intestine. The appearance was similar to that of figure c, except that the loops were more dilated. These were regarded as a transition between the early changes and the later changes described in group (b).

(b) Following the above changes, the tone of the small intestine gradually became greatly reduced in nearly all the animals. In addition the motility progressively decreased. As a result the barium remained in stasis for some time, the duration of stasis being a measure of the deficiency effects. In most animals the stomach was greatly reduced in tone and there was evidence of pylorospasm, as discussed in the force-fed rats. The distension of this organ and the small amounts released in the first one to three hours was often comparable to the observations for force-fed potassium-deficient rats. This

is illustrated by rat 136 (figure e) which, from later X-rays on this same day, was shown to have deficiency symptoms less severe than rat 138 (figure f and g). More severe gastric effects developed than this in the stomach as the symptoms in the digestive tract progressed (figure k).

As mentioned above the tone of the small bowel progressively decreased in most animals. In a few animals this decrease did not occur for the duration of observation such as for rat 138 (figure f), but if the transit through the whole tract was greatly reduced symptoms were considered to be of this group (figure g). In nearly all animals the barium was no longer abnormally segmented (i.e. broken up), but was present in a continuous column in the small intestine which exhibited feeble or practically no rhythmic segmentations and variable degrees of dilatation.

Animals of this category have been grouped roughly into subdivisions to present an idea of the sequence in which these symptoms developed, and, as discussed below, the average time in which the various changes occurred. Moderate changes included symptoms of moderate hypotonicity and hypomotility, where the barium remained in stasis for about two to four hours, but in which several feces were excreted between the eight and twelfth hour. An example of this is rat 117, treated with DCA (figure d). This particular animal showed no gastric retention at six hours. Definite dilatation of the small bowel can be noted, but some rhythmic segmentations

are detectible. Several feces were excreted before nine hours and only about one quarter to one third of the barium was retained in the gut at the end of twenty-four hours.

Moderately severe changes included those in which stasis existed for a longer period in the gut, and in which barium reached the cecum in twelve hours. In this group no barium was excreted until after twelve hours, and a large proportion of the barium was retained in the gastrointestinal tract at the end of twenty-four hours. These are illustrated by rat 138 (figure f and g), in which the reduction in tone of the bowel was not as marked as in other animals, but the barium was in stasis, had reached the cecum by 12 hours, and was retained in all parts of the digestive canal at 26 hours.

Severe changes included those in which barium existed in stasis in the stomach and upper part of the bowel for many hours, up to more than 14 hours in some, in which no barium entered the cecum until after 12 to 14 hours, and in which little or no barium was excreted at the end of twenty-four hours. In all these animals marked hypotonicity and hypomotility existed in the small bowel, and usually in the stomach. Lesser changes occurred in the cecum and colon.

These changes are illustrated in figures 11h,i,j,k,l,m and 12a. The small intestine in many of these animals presented what might be termed an "unwound" appearance (rat 118 figure 11i) which was the most characteristic appearance of the severest stage produced by potassium depletion for the

duration of these experiments. No movements at all were detected fluoroscopically. It seemed as if the barium was consolidated in these loops. All parts of the small and large intestine of this rat 118 were distended at 24 hours (figure j). No feces were excreted. In another animal studied in parallel with 118, no feces were excreted for 72 hours after barium administration. The gastric symptoms of rat 118 became more severe with continuance of the low potassium regime (figure k).

Gas distension was less pronounced in the animals fed ad libitum, as compared with the force-fed animals. However, in several animals considerable amounts of gas accumulated throughout the gastrointestinal tract and distended the organs (figures 11 1,m, and 12a). The changes observed in rat 132 (figure 11 1) were almost as severe as those produced in force-fed rats examined two days after defecation ceased.

In about eight rats the cecum was greatly distended with gas. Once the barium entered this organ, it was retained there for long periods even up to two days. In rat 110, treated with DCA and maintained on diet B, the stomach was normal (figure 11n). The small bowel was distended with gas but showed some rhythmical movements. At eight hours nearly all the barium was in the distended cecum which almost filled the abdomen of this small animal. At 26 hours the cecum was similar in appearance and contained all the barium. Cecal distension occurred more often in the DCA-treated animals (on diet A or B) than in the others fed diet B alone.

From a series of tests in 15 rats maintained on a low potassium diet and consuming about 0.03 meq of potassium daily the following estimates were obtained of the times at which the various types of gastrointestinal changes occurred. In all animals examined some abnormalities occurred but since few animals were examined before 20 to 25 days, the exact time of onset of the early changes was not determined. In rats on diet B the average times in which the symptoms became "moderate", "moderately severe" and "severe" (according to the above classification) were 41 days, 51 days and 62 days, respectively. Thus the tone of the small intestine was decreased more slowly (this distinguishing the early from these later changes), but, once it did decrease, symptoms became severe rapidly, in an average of 21 days. If rats on diet B were injected with DCA the symptoms developed more rapidly. Severe changes were noted in three of four animals maintained on a low potassium diet for 33 days and given 2 mg of DCA per day for the last 22 of these days. If the rats treated with DCA were maintained on diet A, there were indications that symptoms developed more rapidly, being on the average, moderately severe after 35 days of treatment. The small number of DCA-treated rats examined however, prevents a direct comparison of these figures with those of the larger group of animals maintained on diet B alone.

The changes produced by DCA appear very similar to those produced by a low potassium diet with the possible exception of the cecal changes. However, it is possible that DCA acts

in some other way in addition to its effect in depleting the body potassium. The administration of potassium produced striking improvement in one animal moribund from DCA and a low potassium intake.

Potassium deficiency and relief of effects with the normal diet A or purina.

The acute effect of potassium is demonstrated by rat 112. The gastrointestinal tract after 28 days of deficiency (DCA diet B) was hypotonic and completely amotile in the nine-hour X-ray (figure 12a) and the animal was cold and moribund. The 1.5 meq of potassium administered at 12 hours produced striking effects. The following morning (at 24 hours) the animal was warm and active. About one half to two thirds of the barium was excreted (figure 12b), and the rest was in the large intestine, mainly in the terminal colon. This is to be contrasted with the twenty-four hour retention of rat 106 (figure 11m) which was similarly depleted of potassium, but showed symptoms less marked at 9 hours than those of rat 112. The acute effect of potassium in other animals was similar to that in the force-fed animals and need not be discussed further.

Of the animals placed on the normal diet A to recover from potassium deficiency, about half received 1.5 meq additional potassium by mouth and injection during the first few days only (rat 138). This did not appear to affect the improvement appreciably (except probably for rat 138)(figure 12c).

In rat 138 the initial stimulatory effect of potassium added to the diet was very pronounced. This animal had been maintained on a low potassium diet for 76 days, and the symptoms on the 69th day were moderately severe (figure 11f,g). The animal was placed on diet A and given 1.5 meq of potassium by injection and stomach tube for two days (total intake of potassium per day 3.1 meq, which is not high for rats). The barium test the following morning showed that the motility of the whole digestive tract was increased above normal. Barium left the stomach rapidly, appeared in the cecum in less than one hour, and in the feces before one and a half hours. At three hours (figure 12c) little barium remained in the tract, whereas normally only several feces are excreted in this time. Tone and rhythmical movements appeared normal, insofar as they could be judged. The feces were well-formed and of normal consistency, showing that there was no sign of diarrhea. It is quite possible, however, that the initial stimulatory effect in this animal may have been due to excessive amounts of potassium, in addition to the restoration of the body fluids to normal concentration. The serum potassium was 5.57 meq/litre which is somewhat higher than normal.

Soon after this test, regression in the improvement occurred as it did in the other rats. However, this was not appreciably alleviated by placing rat 138 on purina diet, as it was in other animals. This animal was examined several

times during the next six months and the evacuation rate became worse. Possible factors which may have contributed to this progressive regression are chronic infection, or, less likely, ageing.

Rats 125 and 133 show the typical improvement which occurred in the majority of animals after potassium deficiency was relieved with a normal intake of this ion (diet A). On the 67th and 84th day, respectively, the abnormalities in the gastrointestinal tract of these animals were comparable to those of figure 11i and h. When these rats were fed the normal diet ad libitum for five days (rat 125 figure 12d) and nine days (rat 133 figure 12h,i), the condition of the alimentary tract was almost normal. In rat 125 only a small amount of barium remained in the stomach at five hours and the tone and contractions of the small intestines were normally active. In this time the excretion of barium was normal, and the cecum, which had been enlarged during deficiency, was normal in size. The abnormalities seen were a reduced tone of the upper part of the stomach at three hours relative to the normal controls at this time and retention of a trace in the stomach at five hours, as well as of more than normal amounts in the actively contracting small intestine. The small intestinal pattern was almost normal. By eight hours gastric evacuation was complete and the barium retained was in the large intestine and cecum. On the 12th day of recovery some abnormalities in the motility and tone were noted which showed that the

initial effect of potassium regressed somewhat. The main changes were reduced gastric tone and a prolonged gastric evacuation time and some "abnormal segmentation of barium" in the small bowel. Nearly all the barium except a small amount in the stomach (10 to 20 percent) was excreted by five hours (figure 12e). This animal was placed on purina for two weeks. X-rays (12f,g) show that the movements of the gut and transit of barium were approximately normal. However, the pattern in the small intestine was not the same as that of controls prior to use in experiments. Again the abnormalities appeared to be due mainly to "abnormal segmentation of barium".

Rat 133 shows similar effects. In two minutes (figure 12h) many loops of small bowel were filled with barium and exhibiting rapid and vigorous rhythmic segmenting contractions, (the loops are superimposed in the X-ray), and the stomach was normally distended. At one hour rhythmic segmentation and peristalsis were evident and the barium was in a continuous column in the small bowel, as is normal (figure 12i). A slight abnormality in the small intestine pattern was detected at three hours (not shown). At five hours the X-ray was comparable to normal except for a trace of barium in the stomach. Again, with more prolonged feeding on diet A regression in the barium pattern of the digestive canal occurred, the chief changes being prolonged gastric evacuation time, definitely "abnormal segmentation of barium" in the small bowel, an apparent reduction in the volume of barium suspension dispersed through the

intestine, and in addition a reduced rate of excretion. This is shown in figure 12k. Improvement occurred on purina in the stomach and the excretion rate, so that both were completely normal. By six hours very little barium was left in the tract (figure 12 l). Slight abnormalities in the small intestine persisted as described above.

In the animals which were used for force-feeding experiments, improvement on diet A allowed ad libitum occurred and further improvement was produced by purina feeding. These results are comparable to those described above.

(e) Potassium concentration in body fluids.

In all the rats showing gastrointestinal changes from potassium depletion, the serum potassium concentration was lowered to values less than 3.5 meq per litre. However, the number of determinations performed was limited. The average value in the rats fed diet B ad libitum for 20 days was 3.0 meq, and when the deficiency was an average of 57 days, only a slight further decrease in the concentration occurred, to 2.73 meq per litre (Table VII). In a few animals on which serum potassium was determined at 39 and 69 days, the concentration was reduced by only 0.2 meq, which is not statistically analysable because of the small series. In force-fed rats serum potassium was 2.42 meq per litre about the time defecation ceased, or prior to this, and was not reduced further with the development of severe changes. Serum potassium during the stage of very severe distension tended to be elevated, probably because of the stress, as was discussed in Part I. DCA decreased the serum potassium, when diet A was fed, to concentrations epproximately as low as did the low potassium regime (diet B). The serum potassium concentration was decreased to an extremely low value of 1.91 meq/litre in one rat fed diet B ad libitum and given DCA (figure 11n rat 110).

As shown in figure 1 to 4 and Table VII, loss of potassium was greatest in force-fed rats during the first few days of potassium depletion than thereafter. Approximately one third of the body potassium was lost in 9 days in rats fed diet B.

It was inadvisable to risk loss of the animals by taking serum samples because of the small volume of blood in these animals, and because blood was obtained from the heart under anesthesia. The heart of potassium-deficient animals develops serious lesions and thus cardiac puncture was not advisable. In addition, the potassium-deficient rats proved to be particularly sensitive to anesthesia. Several died after doses one half or less than that necessary to anesthetize normal rats, and in others, anesthesia was prolonged for many hours. In the electrolyte balance studies blood sampling was not often performed because the anesthesia affects the excretion of electrolytes.

Summary of Experimental Observations.

The movements of the alimentary canal have been studied in rats by means of the X-ray technique. The gastrointestinal motility of normal animals fed purina was determined, and the effects of feeding a balanced synthetic diet (A), and of potassium deficiency have been investigated both in force-fed rats and in rats eating ad libitum.

The barium tests performed prior to the experiments, on rats maintained on purina, form the criteria of the normal tone and motility of the digestive canal, and, of the rate of transit of barium. By five hours barium was evacuated completely from the stomach, about one half was excreted and the rest was mainly in the large intestine. The barium was normally present in a continuous stream within loops of the small bowel.

When the rats were given the normal diet A ad libitum for two months, very slight changes occurred only in the small intestinal pattern. In rats force-fed this diet in fluid form intestinal changes were more evident and gastric tone was reduced. The changes which occurred in the small intestine consisted of some breaking up of the barium column so that barium was no longer in a continuous stream. Radiologists call this abnormal pattern "abnormal segmentation of barium". It was not marked in the case of the control animals. The radiologists believe the tone is irregular, that is, that areas of increased constriction of wall are interspersed between areas of either normal or reduced tone. There was

evidence of this on some X-rays obtained throughout the experiment.

Severe potassium deficiency resulted in a marked reduction of tone and motility of the gastrointestinal tract, and in a pronounced prolongation of the transit time of the barium. The changes occurred regardless of whether potassium depletion was produced by a low potassium intake or by DCA injections. The symptoms produced by potassium deficiency were extremely severe in force-fed animals. Defecation ceased in about 6 to 10 days and in all rats death occurred in less than two weeks from tremendous distension of the gastrointestinal tract. In rats fed ad libitum the symptoms produced by three months of potassium depletion were not as severe as those occurring in the force-fed animals in a week. In the force-fed animals, unabsorbed food, fluid and gas filled the distended tract. In several rats maintained on the low potassium diet ad libitum, clear fluid was present throughout the alimentary canal as determined by inspection. Gas accumulation was usually not marked in these rats. The abdominal blood vessels in both groups of animals were engorged, and this occurred in animals in which the intestine was flaccid, but not necessarily markedly distended.

The sequence in which gastrointestinal symptoms developed as a result of potassium deficiency was estimated in both force-fed and normally-fed rats. In force-fed animals

abnormalities in the small intestinal pattern (mainly "abnormal segmentation of barium") developed first, followed by gastric hypotonicity and pylorospasm, some reduction in motility in the various organs and slowing of the transit of barium. Soon after cessation of defecation, both tone and motility throughout the whole gut became greatly reduced. The typical changes which occurred at this stage and earlier are shown in figures 9 and 10 for rats which were not moribund. In rats fed ad libitum the symptoms observed and rate at which they developed, were much more variable. The sequence in which the deficiency condition in the gastrointestinal tract developed was estimated mainly from changes in the small bowel, and from the time required for transit of barium through the various digestive organs. In rats maintained on the low potassium diet, first motility was reduced in the small bowel, and the transit time of barium through the small bowel was correspondingly increased. The intestinal loops were often thin and ribbon-like, which may possibly be due to reduced diameter, that is, hypertonicity, or perhaps more likely to incomplete filling of the loops as was discussed. Later "abnormal segmentation of barium" occurred and only after this stage was tone reduced. By an average of 41 days of deficiency tone was reduced throughout the small bowel and barium filled continuous loops of hypotonic gut. In the small intestine and throughout the whole alimentary canal tone and motility became progressively reduced and transit of barium was

prolonged until the typical condition of severe potassium deficiency occurred in an average of 61 days, (figure 1li,j,k). Pylorospasm usually occurred in addition to gastric atony. In several animals tremendous distension of the cecum was the most prominent abnormality. Thus in general the sequence and type of change produced by the two methods of feeding were similar, but the changes produced were accentuated when the rats were force-fed.

In potassium deficient animals injections of potassium had a striking effect on the day of administration. In both force-fed animals and rats eating in the normal manner, rhythmic movements and peristalsis were initiated, or increased in vigour, within an hour or so after potassium administration, and the excretion of barium in twenty-four hours was restored almost to normal.

In chronic experiments, potassium added to the food in normal amounts restored the motility almost to the normal within five days in rats fed ad libitum. In the group force-fed the normal potassium diet A, improvement was remarkable, even in four days, in comparison with the extremely severe changes observed during potassium deficiency. However, in terms of the proper control (diet A force-fed prior to potassium depletion), restoration was not as complete as in the above group of animals eating normally. When these animals were fed ad libitum, restoration of motility was similar to that observed in rats fed ad libitum throughout the whole experiment.

Following the initial stimulation of motility almost to normal, as described above, a variable degree of regression in the improvement of the motility of the gastrointestinal tract occurred. The main abnormality was a variable effect on the X-ray pattern of the small intestine. Barium tended to be separated into isolated masses in the small bowel. In addition, gastric tone was somewhat reduced and gastric evacuation time was increased, since small amounts of barium were retained in this organ at five hours. The rate of excretion remained normal in some animals and was reduced in others. However, these are small changes in comparison with those observed prior to potassium therapy.

On purina the gastrointestinal tone, motility and evacuation of barium was improved over that observed during the synthetic dietary regime. In the few cases which were not improved chronic infection was detectible. However, the same type of abnormality in the barium pattern in the small intestine persisted, although to a smaller degree, even on the purina diet. This may have been due to the handling and to the various experimental techniques and have not been tested as yet.

The serum potassium was low in all animals showing moderately severe or severe changes in the gastrointestinal tract. The concentration was lower in force-fed animals depleted of potassium for a week than in the rats, normally fed, depleted of potassium for two months.

DISCUSSION

The experiments which have shown that prolonged depletion of body potassium produces very severe gastrointestinal disturbances, have been summarized in the last section. These changes result primarily from a marked hypotonicity and hypomotility of all parts of the alimentary canal. Since paralysis is produced by potassium deficiency in skeletal muscle, as well as functional and anatomical abnormalities in cardiac muscle, it is not surprising that paralysis occurs in the smooth muscle of the digestive tract. Whether the reduced contractility is muscular or nervous in origin will be considered below.

Changes in the secretion of juices and the absorption of food and fluids probably contributed to the disturbances which developed in these depleted rats, although these factors have not been examined. Decreased absorption was particularly evident in force-fed rats, for in these animals the flaccid tract was distended with incompletely digested food, with gas and fluid constituents. It is probable that absorption of water and associated electrolytes and of digested food substances was inhibited because of the hypomotility of the muscular walls. The gas accumulated in the gut may have resulted from a similar cause, since in intestinal distension of various types in man, the gas is derived mainly from swallowed air. (234). It is not likely that accumulation of fluid in the lumen was due to an increase in digestive secretions. Both secretion and absorption are dependent upon the blood flow in the mucosa. The flow is normally aided by the rhythmical contractions of the muscular walls which act as a type of auxiliary pump to fill and empty vessels, and aid venous return of blood. In the force-fed rats, the distension of the flaccid walls produced by the administered fluid and food probably also inhibited the flow of blood through the walls, and acted to reduce motility further. Thus, from analysis of the several facts observed and deduction from the physiological properties of smooth

muscle and of the digestive tract, it seems probable that four factors, poor tone, poor motility, reduced blood flow and therefore of intestinal absorption, and the distension produced both by the administered food and the unabsorbed intestinal food, fluid and gas, all enter into a vicious cycle in the force-fed animals which quickly leads to death. In the rats fed ad libitum the factor of distension is not prominent, since the stomach and intestines are not suddenly distended with food. The animal appears to eat in accordance with the reduction in functions of the digestive organs, for food does not accumulate in the gut. The absorption of fluid is probably slow since it was observed that clear fluid filled the alimentary canal in several animals even when no food was present. The motility was greatly reduced but the walls were not distended appreciably. Without this factor of distension to close the vicious cycle, death did not usually occur.

The changes in gastrointestinal motility produced in animals fed a low potassium diet ad libitum are considered to be more specifically due to absence of this ion, than are those produced in force-fed animals. Experiments reported have shown that force-feeding is a stress which cannot be tolerated by the gastrointestinal tract in unadapted potassium-depleted rats, but which is well tolerated by the unadapted normal animal. Thus this technique probably could be used as a method of testing the capacity of the stomach and intestine to respond to stress of distension.

The possible influence of barium on the secretions and absorption of fluid and on the movements must not be neglected. This substance is present in an insoluble suspension. It has a high specific gravity and tends to clump. It seems to be mildly stimulating to the movements of the digestive tract (138). There was a tendency for barium to adhere to the stomach walls in animals which were otherwise not appreciably affected, and to remain there for prolonged periods. This may have been due to the properties of barium

or possibly indicates abnormality in mucus secretion in the upper part of the stomach on which the barium adhered.

Force-feeding of the normal control diet A, itself, produced abnormalities in the stomach and small intestine, but these were slight in comparison with those produced by potassium deficiency. They were mainly due to changes in the tone of these organs. The changes observed were dependent upon the introduction of the food, rather than upon the factors of introducing a tube into the stomach bidaily or of distending the stomach suddenly with water. However, since water runs out of the stomach quite rapidly, perhaps the addition of some colloidal agent would provide a better control of the factor of distension than water alone. Since these rats were eating normally of the purina diet, the factor of psy chnic disturbances produced by the abnormal feeding of food by tube was not controlled. However, in view of the rapid effect which force-feeding has upon unadapted potassium-deficient rats and of the gastric distension produced by sudden filling of the stomach with food, the changes are regarded as primarily due to distension.

Another balanced diet (E) of entirely different composition was force-fed to several rats. This consisted primarily of whole milk powder and sucrose. Barium tests performed on these animals showed that gastric distenston was marked and prolonged, pylorospasm occurred, and the motility of the small intestine was reduced. The difference between the effects of force-feeding diets E and A were considered as possibly due to a higher content of fat, which was mainly butter fat rather than vegetable fat. The abnormalities produced by this technique of feeding suggest the inadvisability of performing experiments, especially metabolic balance studies, unless the effect of the diet force-fed is tested by the radiological method.

The effect of subcutaneously injected potassium was detected in less than an hour, even in twenty minutes in two rats examinated at this time. No experiments were performed to analyse the effect of potassium deficiency and of therapy with potassium in these experiments and no microscopic sections were made, but the possible means of action of these procedures will be considered. Potassium treatment would be expected to exert two effects, one directly on the cell membranes from a sudden increase in the extracellular fluid concentration, and the other, from a diffusion into the cells to restore intracellular concentration to normal. This was discussed in the review of in vitro experiments. Subcutaneously injected potassium first diffuses through extracellular fluid and then fairly rapidly into tissues. Diffusion is complete by four hours in muscle and by about two hours in the gastrointestinal tract. This has been shown with the use of radioactive potassium (235, 236). In view of the rapidity of the effect, the initial stimulation of intestinal motility produced during the first half hour or so by potassium is probably primarily a surface phenomenon due to increased extracellular potassium concentration. The serum potassium was elevated and remained slightly above normal for a day at least. In the frog (220) the optimal concentration for maximal irritability of skeletal muscle is above normal and possibly this is the case for the smooth muscle in the digestive organs of the rat. The rapid effect of potassium is analogous to its effect in relieving muscle paralysis in DCA-treated animals and in periodic familial paralysis. In the course of the next several hours tonus gradually recovered, rhythmic contractions remained vigorous and strong waves of peristalsis resulted in the excretion of most of the barium within twenty-four hours. Untreated rats retained most or all of the barium in this time. This sustained effect may be considered to be due to increased capacity of the cells to contract because of improvement in the intracellular

composition, since it seems unlikely that abnormal cells would continue to show such vigorous activity and since potassium is known to diffuse readily into the cells during this time.

The therapeutic effect of potassium may be due to either an effect on the muscle cells or on the nervous elements supplying these muscle , but the end result is increased contractility of the muscle. If some nerve cell degeneration were responsible for the severe distension produced by potassium depletion, potassium would not be expected to have so rapid an effect in increasing motility of the muscle as it does. On the other hand, it is known that skeletal muscle function in potassium-deficiency can be rapidly restored by potassium. Since it is probable that permanent changes were not produced by potassium deficiency in these experiments, any changes which may have occurred in muscle or nerve cells were reversible.

It may be that potassium deficiency and therapy influence the release of acetyl choline either peripherally or centrally in the nervous system, and thereby affect transmission of impulses to the muscles. It is well known that acetyl choline is liberated at vagus nerve endings and at synapses, and plays a role in transmitting impulses. It is also known that the release of acetyl choline is stimulated by small amounts of potassium but inhibited by large amounts (224).

The maximum effect of potassium therapy in rats fed ad libitum was obtained within a week when potassium was added to the diet. In one animal, motility was increased above normal in two days, but it regressed rapidly four days later despite continued treatment with potassium. In this animal potassium was added to the food and also given by stomach tube, but the total amount was comparable to that obtained from a diet of purina. The

initial stimulatory effect may be comparable to that discussed for the first day, since the serum potassium was still elevated 5.57 meq per litre after the barium test. The usual effect of potassium added to the food was almost complete restoration to normal of the tone and motility of the gastrointestinal tract in the first week of treatment. No potassium determinations were performed in these animals, but after the first couple of days they may be considered analogous to the other rats fed on the normal control diet A in which the serum potassium was normal at 4.8 meq per litre. Thereafter, the stimulatory effect of potassium wore off somewhat, so that some abnormalities in the small intestine became evident. What the cause of this regression was, was not determined. It appears to be related chiefly the same deficiency in the diet, since when the animals were placed on purina diet, the tone and motility was restored almost to the normal pre-experimental controls. Certain slight abnormalities persisted in the small intestinal pattern as shown from the barium distribution, but these may have been due to the experimental procedures of handling, X-raying, etc. Why the regression in improvement occurred after the first week on diet A, after almost complete recovery was obtained, is unknown unless the restoration of the serum potassium to normal from the very low values observed during the deficiency was a stimulus to which the rats later became acclimatized. In rats force-fed diet A the recovery of the gastrointestinal tract was not as complete as in rats fed ad libitum. When these animals were then fed diet A or purina, they behaved similarly to the rats fed ad libitum throughout the experiments. This shows that permanent changes were not produced by force-feeding, and indicates that with continuance of the factor of distension from the force-fed diet did not permit the muscle walls to recover maximally.

It must be emphasized that the criteria of normal behaviour in the

barium test have been derived from the pre-experimental X-ray examinations. At this time the animals had not been handled often or subjected to the experimental proeedures of being taped to a board, fasted, treated with barium and X-rayed. In addition, they had not been in the laboratory for more than a month. The housing conditions were not ideal and some infections did occur, A number of animals examined at the beginning and not used during the experiment also developed slight abnormalities in the gastro-intestinal motility, particularly in the small intestine. Therefore, it is probably not valid to assume that the muscular and nervous functions of the tract remain unaltered by the experimental procedures. The slight changes observed in the small intestinal pattern in rats which had recovered on purina diet may be due to the experimental conditions and not to incomplete recovery from potassium deficiency. This view is favoured since, as mentioned above, abnormalities were sometimes observed in rats housed in the laboratory but not used for experiments, and since the fasting and the taping of animals to boards for X-rays several times in the course of the experiment are emotional stresses.

Since the movements of the digestive tract were restored more completely or permanently by purina than by diet A, there was some deficiency in the diet if the differences in physical consistency of the two diets are neglected. This was so in spite of the addition of yeast extract equivalent to 4 grams of yeast per 100 grams of dry diet A, and all the vitamins required for normal motility in dogs (237) except inositol. Inositol presumably was present in the extract but may not have been present in adequate amounts. When normal rats were maintained on diet A ad libitum for two months, slight changes occurred in the distribution of barium throughout the small bowel, but the stomach and excretion rate were normal. These changes were much less pronounced than those observed in rats recovering for several weeks

on diet A, in which the same reduction of tone in the stomach also occurred. It is possible that the animals recovering from potassium deficiency are more sensitive to deficiencies in the diet. In addition, in the latter animals the total duration of feeding on synthetic diets (B and A) was three to three-and one-half months, whereas the controls were fed diet A for two months.

Potassium may play a role in clinical cases of gastrointestinal distension, particularly in obstruction, and in clinical cases of chronic diarrhea or vomiting. In cases when distension is severe, especially from obstruction in the tract, fluids and gases are absorbed poorly (234). The ions which are present in the fluid are lost from the body by secretion. Of particular interest are the gastric juices in which the potassium concentration is more than twice that of the serum (238). When the fluid and gases accumulated in the distended tract are syphoned by suction, which is the usual clinical treatment, or when they are vomited, potassium is lost from the body in excess of sodium and water. In addition, sodium chloride is usually infused intravenously. Falconer et al (41) has observed that the saline infusions tend to decrease the serum potassium concentration even in normal individuals, and suggests the use of potassium in the solution infused. He found that the serum potassium tended to be low in gases of intestinal obstruction, and obtained some evidence that the infusion of Ringer's solution instead of saline was of value in a few cases which responded poorly, from a clinical point of view, to saline infusions. In cases of diarrhea from non-tropical sprue, Harrison et al (239,240) have observed very low values of serum potassium concentrations, as low as 1.1 meq in one case. Since experiments in rats restored the motility of the gastrointestinal tract so effectively, it is possible that there may be some clinical application of these observations, at least in cases when potassium is lost from the body. There is also a possibility that small doses may stimulate tone and

movements in certain types of distension, such as post-operatively, even when the potassium from the tissues is not detected.

The gastrointestinal changes observed in potassium deficiency are similar in some respects to those produced by other deficiency conditions, Many investigators have observed that vitamin B deficiency in man and animals produces changes in the stomach and intestine. The type of change produced depends upon the duration and severity of the deficiency. In the early stages, hypermotility and hypertonicity are usually observed in the intestines, while as the deficiency state advances, hypomotility and later hypotonicity develop.(241). Abnormal patterns in the small intestine usually occur, which include abnormal "segmentation" a term which the radiologists use to indicate irregular areas of constriction or even spasm of the walls of the intestines. In most cases the gastric tone is reduced while peristalis is less affected at first, but is eventually reduced. Plyoro-spasm is a frequent occurrence as the deficiency state advances. Martin et al (237) observed that inositol deficiency in dogs produced marked irregularities in tone and barium distribution in the small intestine and a decrease in tone in the stomach.

Similar deficiency states occur in hypoproteinemia, icterus, anemia etc. (241).

Thus there is considerable similarity between the changes produced by potassium deficiency in rats and those produced by vitamin B deficiency and other deficiency states in man and animals. No stage of hypermotility was observed but the animals were not examined prior to 20 days. It is possible that there is some common cause responsible for the abnormalities. In man degenerative changes in the intrinsic nerve plexuses of the gastrointestinal tract are associated with the clinical deficiency states, particularly with vitamin B deficiency. In the rats, since recovery from potassium deficiency

was so prompt and may be considered almost complete, it is obvious that permanent changes in nervous elements cannot be responsible for the symptoms observed. The symptoms were more severe in the rats than those which have been observed from other types of deficiencies. It is possible that both types of deficiencies may affect the intermediary metabolism of cells. Potassium is an essential ion for certain enzymatic systems responsible for muscular contraction and transfer of energy in cells (242, 243). In many of the enzymatic reactions of the cell vitamins of the B complex are also essential, and serve as co-enzymes. Deficiency of either may thus interfere with either muscular or nervous activities.

In moderately severe or severe potassium deficiency in the experiments reported, the blood vessels of the digestive tract and the abdomen were congested, and were pulsating strongly. The venous blood appeared well-oxygenated, except in animals in poor condition. This tendency for the blood to pool in the lower part of the body may have been due to two factors. It is quite possible that the tone of smooth muscle was reduced by potassium deficiency, just as the tone of the smooth muscle in the gastrointestinal tract is reduced. This would cause vascular dilatation. In addition, cardiac function is reduced by prolonged potassium deficiency and pathological lesions are severe (35,45). These were observed grossly in the experiments reported. Liebow et al (7/) has observed heart failure in a few potassium deficient rats. Thus, it is possible that weakened cardiac function decreased the venous return of blood to the heart and contributed with poor tone in the blood vascular musculature, to the dilatation and pooling of blood in the lower part of the body.

CONCLUSIONS

The effect of prolonged potassium deficiency on the motility of the gastrointestinal tract and of subsequent therapy with potassium has been investigated in intact rats by means of the X-ray technique. Experiments were conducted both in rats force-fed the synthetic diets and in animals eating in the normal manner, ad libitum. Potassium depletion was produced by a low potassium diet or by DCA. The results have been summarized in the Experimental Observations (page 165).

Rats fed the control diet A develop very slight changes in the small intestinal pattern. Those force-fed this diet showed more appreciable changes, but these were slight in comparison with those produced by potassium deficiency.

Severe potassium deficiency produced marked hypotonicity and hypomotility both in force-fed rats and rats fed ad libitum. In the former these changes led to death in less than two weeks from extreme distension of the whole digestive tract. Less marked changes were produced in rats fed ad libitum for two or three months, and these did not cause death. The motility was reduced prior to the reduction of tone. Evidence indicated that the effect of DCA was similar to that of a low potassium regime.

The effect of potassium therapy on the condition of the gastrointestinal tract was striking. The abnormalities were promptly relieved by potassium therapy and restored almost to normal in a week. Subsequent regression in improvement was discussed as possibly due to a deficiency in diet A, since almost complete normal motility was observed on purina diet. The slight abnormalities which persisted on purina feeding in the small intestine were discussed as probably caused by experimental procedures and housing for long periods.

Therefore, it seems probable that the severe changes produced by

potassium deficiency can be completely reversed. It is concluded that no detectible permanent changes were produced by force-feeding *per se*.

The possible mechanism responsible for the changes produced by potassium deficiency and subsequent therapy with potassium have been discussed.

BIBLIOGRAPHY

1a. Winter, H.A. - "The influence of method of administration upon the actions of progesterone and desoxycortisterone acetate". Montreal Physiol. Soc., November 1941.

1b. Winter, H.A. - "The effect of hypophysectomy and thyroidectomy on the hypochloremic response of rats to DCA". Presented in this form at the Federation Soc. Exp. Biol. and Med. in Boston, 1942.

1c. Winter, H.A. - "Factors influencing the diuretic effect of progesterone and desoxycorticosterone acetate". Fed. Proc. 1, no. 11, 1942.

2a. Steiger, M. and Reichstein, T. "Desoxycorticosterone (21-oxy-progesteron) aus 5-3-oxy-aetiocholensauere". Helv. Chim. Acta, 20, 1164, 1937.

2b. Reichstein, T. von Euw, J. "Ueber Bestandteile der Nebennierenrinde: Isolierung der Substanzen Q (desoxy-corticosteron) und R sowie Weiterer Stoffe". Helv. Chim. Acta, 21, 1197, 1938.

3. Thorn, G.W., Howard, R.P., Emerson, K., and Firor, M. - "Treatment of Addison's disease with pellets of crystalline adrenal cortical hormone (synthetic desoxycorticosterone acetate) implanted subcutaneoulsy". Bull. Johns Hopkins Hospital, 64, 339, 1939.

4. Cleghorn, R.A., Fowler, J.L. and Wenzel, J.S. - "The treatment of Addison's disease by synthetic adrenal cortical hormone (desoxycorticosterone acetate". Can. Med. Assoc. J., 41, 226, 1939.

5. Ferrebee, J.W., Ragan, C., Atchley, D.W. and Loeb, R.F. - "Desoxycorticosterone esters. Certain effects in the treatment of Addison's disease". J.A.M.A., 113, 1725, 1939.

6. Loeb, R.F., Atchley, D.W., Ferrebee, J.W. and Ragan, C. - "Observations on the effect of desoxycorticosterone esters and progesterone in patients with Addison's disease". Tr. Assoc. Am. Physicians, 54, 285, 1939.

7. Ferrebee, J.W., Ragan, C., Atchley, D.W., and Loeb, R.F. - "A comparison of certain effects of desoxycorticosterone acetate, corticosterone and cortical extract on patients with Addison's disease". Endocrinology, 27, 360, 1940.

8. Thorn, G.W., Howard, R.P. and Emerson, K. - "Treatment of Addison's disease with DCA, a synthetic adrenal cortical hormone (preliminary report)". J. Clin. Inves. 18, 449, 1939.

9. Thorn, G.W., Dorrance, S.S. and Day, S. - "Addison's disease. Evaluation of synthetic DCA therapy in 158 patients". Ann. Int. Med., 16, 1053, 1942.

10. Thorn, G.W., Engel, L.L., and Eisenberg, H. - "The effect of corticosterone and related compounds on the renal excretion of electrolytes". J. Exp. Med., 68, 161, 1938.

11. Steiger, M. and Reichstein, T. - "Partial synthesis of a crystalline compound with the biological activity of the adrenal cortical hormone". Nature, 139, 925, 1937.

12. Harrison, H.E. and Harrison, H.C. - "Effect of desoxycorticosterone acetate upon blood sugar and electrolyte of adrenalectomised rats". Proc. Soc. Exp. Biol. & Med., 42, 506, 1939.

13. Thorn, G.W. and Eisenberg, H. - "Studies on desoxycorticosterone". Endocrinology, 25, 39, 1939.

14. Mason, H.L. - "Chemistry of the adrenal cortical hormone". Endocrinology, 25, 405, 1939.

15. Kuizenga, M.H., Nelson, J.W. and Cartland, G.F. - "Comparative parenteral and oral assays of adrenal cortical hormone substances". Am. J. Physiol., 130, 298, 1940.

16. Wells, B.B. and Kendall, E.C. - "A qualitative difference in the effect of compounds separated from the adrenal cortex on distribution of electrolytes and on atrophy of the adrenal and thymus glands of rats". Mayo Clin. Staff Meet. Proc., 15, 133, 1940.

17. Kendall, E.C. - "The adrenal cortex". Arch. Path., 32, 474, 1941.

17a. Pfiffner, J.J. - "The adrenal cortical hormones". Advances in Enzymology, 2, 325, 1942.

18. Kuhlman, D., Ragan, C, Atchley, D., and Loeb, R. - "Toxic effect of desoxycorticosterone esters in dogs". Science 90, 496, 1939.

19. Ferrebee, J.W., Parker, D., Carnes, W.H., Gerity, M., and Atchley, D.W. - "Certain effects of desoxycorticosterone: The development of "diabetes insipidus" and the replacement of muscle potassium by sodium in normal dogs". Am. J. Physiol., 135, 230, 1941.

20. Tooke, T.B., Power, M.H. and Kepler, E.J. - "The tolerance of patients suffering from Addison's disease to potassium while such patients are being treated with DCA". Mayo Clin. Staff Meet. Proc., 15, 365, 1940.

21. McCullagh, E.P. and Ryan, E.J. - "The use of desoxycorticosterone acetate in Addison's disease". J.A.M.A., 114, 2531, 1940.

22. Goodof, I.I. and MacBryde, C.M. - "Heart failure in Addison's disease with myocardial changes of potassium deficiency". J. Clin. Endoc., 4, 30, 1944.

23. Long, C.N.H., Katzin, B. and Fry, E. - "The adrenal cortex and carbohydrate metabolism". Endocrinology, 26, 309, 1940.

24. Ingle, D.J. - "The work performance of adrenalectomized rats treated with corticosterone and chemically related compounds". Endocrinology 25, 405, 1939.

25. Thorn, G.W., Koepf, G., Lewis, R.A. and Olsen, E.F. - "Carbohydrate metabolism in Addison's disease". J. Clin. Inves. 19, 813, 1940.

26. Lewis, R.A., Kuhlman, D., Delbue, C., Koepf, G. and Thorn, G. - "The effect of the adrenal cortex on carbohydrate metabolism". Endocrinology 27, 971, 1940.

27. Lucas, G.H.W. - "Blood and urine findings in desuprarenalised dogs". Am. J. Physiol., 77, 114, 1926.

28. Loeb, R.F. - "Chemical changes in the blood in Addison's disease". Science, 76, 420, 1932.

and Leland, J.

29. Loeb, R.F., Atchley, D.W., Benedict, E.M./- "Electrolyte balance studies in adrenalectomised dogs with particular reference to the excretion of sodium". J. Exper. Med., 57, 775, 1933.

30. Harrop, G.W., Soffer, L.J., Ellsworth, R., and Trescher, J.H. - "Studies on the suprarenal cortex. III Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog". J. Exper. Med., 58, 17, 1933.

31. Gamble, J.L. and Ross, S.G. - "The factors in the dehydration following pyloric obstruction. J. Clin. Invest. 1, 403, 1925.

32. Gamble, J.L. - "Extracellular fluid and its vicissitudes". Bull. Johns Hopkins Hosp., 61, 151, 1937.

33. Gamble, J.L. - "Renal defense of extracellular fluid: Control of acid-base excretion and factors of water expenditure." Bull. Johns Hopkins Hosp. 61, 174, 1937.

34. McQuarrie, I., Johnson, R.M. and Ziegler, M.R. - "Plasma electrolyte disturbance in patient with hypercorticoadrenal syndrome, contrasted with that found in Addison's disease". Endocrinology, 21, 762, 1937.

35. Darrow, D.C. and Miller, H.C. - "The production of cardiac lesions by repeated injections of desoxycorticosterone acetate". J. Clin. Invest. 21, 601, 1942.

36. Hartman, F.A., Smith, D.E. and Lewis, L.A. - "Adrenal functions in the oppossum". Endocrinology, 32, 340, 1943.

37. Heppel, L.A. - "The electrolytes of muscle and liver in potassium depleted rats". Am. J. Physiol., 127, 385, 1939.

38. Miller, H.C. and Darrow, D.C. - "Relation of muscle electrolyte to alterations in serum potassium and to the toxic effects of injected potassium chloride". Am. J. Physiol., 130, 747, 1940.

39. Durlacher, S.H. and Darrow, D.C. - "The effect of depletion of body potassium in the time of survival after nephrectomy and ureteral ligation". Am. J. Physiol., 136, 577, 1942.

40. Hegnauer, A.H. - "The effect of a low potassium diet and of desoxycorticosterone acetate on the cation content of rat erythrocytes and muscles". Am. J. Physiol., 150, 353, 1943.

41. Falconer, M.A., Osterberg, A.E. and Bargen, J.A. - "Intestinal obstruction in man. Alterations in the serum bases and their significance". Arch. Surg., 38, 869, 1939.

42. Orent-Keiles, E. and McCollum, E.V. - "Potassium in animal nutrition". J. Biol. Chem., 140, 337, 1941.

43. Rakoff, A.E., Paschkis, K.E. and Cantarow, A. - "A typical response of the rabbit to desoxycorticosterone acetate". Science, 96, 322, 1942.

44. Darrow, D.C. - "Changes in muscle composition in alkalosis". J. Clin. Invest., 25, 324, 1946.

45. Miller, H.C. and Darrow, D.C. - "Relation of serum and muscle electrolytes, especially potassium, to voluntary exercise". Am. J. Physiol., 132, 801, 1941.

46. Buell, M.V. and Turner, E. - "Cation distribution in the muscles of adrenalectomized rats". Am. J. Physiol., 134, 225, 1941.

47. Selye, H. and Dosne, C. - "Changes produced by desoxycorticosterone overdosage in rats". Proc. Soc. Exp. Biol. & Med., 44, 165, 1940.

48. Selye, H. and Dosne, C. - "Action of DCA and progesterone on blood and tissue chloride of normal and adrenalectomised animals". Am. J. Physiol., 132, 522, 1941.

49. Ragan, C., Ferrebee, J.W., Phyfe, P., Atchley, D.W. and Loeb, R.F. - "A syndrome of polydipsia and polyuria induced in normal animals by DCA". Am. J. Physiol., 131, 73, 1941.

50. Harkness, D.H., Muntwyler, E., Mautz, F.R. and Mellors, R.C. - "Electrolyte and water exchange between skeletal muscle," available (thiocyanate) fluid, "and plasma in the dog following the administration of DCA". J. Lab. Clin. Med., 28, 307, 1942.

51. Muntwyler, E., Mautz, F.R. and Griffin, G.E. - "The distribution of water and electrolytes in muscle of dogs treated with DCA following injections of glucose and glucose containing potassium chloride." J. Biol. Chem., 156, 469, 1944.

52. Mulinos, M.G., Spingarn, C.L., and Lojkin, M.E. - "A diabetes insipidus-like condition produced by small doses of desoxycorticosterone acetate in dogs". Am. J. Physiol., 135, 102, 1941.

53. Clinton, M. Jr., Thorn, G.A., Eisenberg, H. and Stein, K.E. - "Effect of synthetic desoxycorticosterone acetate therapy on plasma volume and electrolyte balance in normal dogs". Endocrinology, 31, 378, 1942.

54. Thorn, G.W. and Emerson, K. - "The role of gonadal and adrenal cortical hormones in the production of edema". Ann. Int. Med., 14, 757, 1940.

55. Harrop, G.A., Nicholson, W.M. and Strauss, M.J. - "Studies on the suprarenal cortex. V. Influence of the cortical hormone upon excretion of water and electrolyte in suprarenalectomized dogs". J. Exp. Med., 64, 233, 1936.

56. Ziegler, M., Anderson, G.A. and McQuarrie, I. - "Effects of DCA on water and electrolyte content of brain and other tissues". Proc. Soc. Exp. Biol. & Med., 56, 242, 1944.

57. Dean, R.B. - "The determination of chloride in single isolated muscle fibres". J. Biol. Chem., 137, 113, 1940.

57a. Amberson, W.R., Nash, T.P., Mulder, A.G., and Binns, O. - "The relationship between tissue chloride and plasma chloride". Am. J. Physiol., 122, 224, 1938.

58. Hastings, A.B. and Eichelberger, L. - "Exchange of salt and water between muscle and blood. I. The effect of an increase in total body water produced by the intravenous injection of isotonic salt solution". J. Biol. Chem., 117, 73, 1937.

59, Fenn, W.O. - "Electrolytes in muscle". Physiol. Rev., 16; 450, 1936.

60: Manery, J.F., Danielson, I.S., and Hastings, A.B. - "Connective tissue electrolytes". J. Biol. Chem., 124, 359, 1938.

61. Yannet, H. and Darrow, D.C. - "The effect of depletion of extracellular electrolytes on the chemical composition of skeletal muscle, liver and cardiac muscle". J. Biol. Chem., 134, 721, 1940.

62. Darrow, D.C. - "Tissue water and electrolyte". Ann. Rev. Physiol., 6, 95, 1944.

63. Boyle, P.J. and Conway, E.J. - "Potassium accumulation in muscle and associated changes". J. Physiol., 100, 1, 1941.

64. Boyle, P.J., Conway, E.J., Kane, F. and O'Reilly, H.L. - "Volume of interfibre spaces in frog muscle and the calculation of concentrations in the fibre water". J. Physiol., 99, 401, 1941.

65. Muntwyler, E., Mellors, R.C. and Mautz, F.R. - "Electrolyte and water equilibrium in the dog. I. Equilibria in the blood in adrenal insufficiency". J. Biol. Chem., 134, 345, 1940.

66. Conway, E.J. and Hingerty, D. - "The influence of adrenalectomy on muscle constituents". Biochem. J., 40, 561. 1946.

67. Darrow, D.C. - "Effect of low potassium diet and desoxycorticosterone on the rat heart". Proc. Soc. Exper. Biol. & Med., 55, 13, 1944.

68. Sykes, J.F. and Alfredson, B.V. - "Studies on the bovine electrocardiogram. I. Electrocardiographic changes in calves on low potassium rations". Proc. Soc. Exper. Biol. & Med., 43, 575, 1940.

69. Darrow, D.C., Harrison, H.E., and Taffel, M. - "Tissue electrolytes in adrenal insufficiency". J. Biol. Chem., 130, 487, 1939.

70. Liebow, A.A., McFarland, W.J. and Tennant, R. - "The effects of potassium deficiency on tumor-bearing mice". Yale J. Biol. & Med., 13, 523, 1941.

71. Follis, R.H. Jr., Orent-Keiles, E., and McCollum, E.V. - "The production of cardiac and renal lesions in rats by a diet extremely deficient in potassium". Am. J. Path., 18, 29, 1942.

72. Thorn, G.W. - "Desoxycorticosterone." J. Mount Sinai Hospital, 8, 1177, 1942.

73. Ingle, D.J. and Thorn, G.W. - "A comparison of the effects of 11-dehydrocorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone in partially depancreatized rats". Am. J. Physiol., 132, 670, 1941.

74. Thorn, G.W. and Engle, L.L. - "The effect of sex hormone and allied substances on the renal excretion of electrolytes". XVI Internat. Physiolkong, Zurick, 1938.

75. Thorn, G.W., Garbutt, H., Hitchcock, F. , and Hartman, F.A. - "The effect of cortin upon the renal excretion of sodium, potassium, chloride, inorganic phosphorus and total nitrogen in normal subjects and in patients with Addison's disease". Endocrinology, 21, 203, 1937.

76. Thorn, G.W., Engel, L.L. and Lewis, R.A. - "Effect of 17-hydroxy-corticosterone and related adrenal cortical hormone on sodium and chloride excretion". Science, 94, 348, 1941.

77. Thorn, G.W. and Engel, L.L. - "The effect of sex hormones on the renal excretion of electrolytes". J. Exper. Med., 68, 299, 1938.

78. Sykes, J.F. and Moore, L.A. - "Lesions of the Purkinje network of the bovine heart as a result of potassium deficiency". Arch. Path., 33, 467, 1942.

79. Follis, R.H. Jr. - "Histological effects in rats resulting from adding rubidium or cesium to a diet deficient in potassium". Am. J. Physiol., 138, 246, 1943.

80. Follis, R.H. Jr. - "Effect of exercise in rats fed a diet deficient in potassium". Proc. Soc. Exper. Biol. & Med., 51, 71, 1942.

81. Thomas, R.M., Mylon, E. and Winternitz, M. - "Myocardial lesions resulting from dietary deficiency". Yale J. Biol. & Med., 12, 345, 1940.

82. Durlacher, S.H. and Darrow, D.C., and Winternitz, M. - "The effect of low potassium diet and of DCA upon renal size". Am. J. Physiol., 136, 346, 1942.

83. Heppel, L.A. - "The diffusion of radioactive sodium into the muscles of potassium-deprived rats". Am. J. Physiol., 128, 449, 1939.

84. Moehlig, R.C. and Jaffe, L. - "Syndrome simulating diabetes insipidus in dogs induced by desoxycorticosterone acetate. J. Lab. & Clin. Med., 27, 1009, 1942.

85. Carnes, W.H., Ragan, C., Ferrebee, J.W., and O'Neill, J. - "Effects of desoxycorticosterone in albino rats". Endocrinology, 29, 144, 1941.

86. Ruegamer, W.R., Elvehjem, C.A. and Hart, E.B. - "Potassium deficiency in the dog". Proc. Soc. Exper. Biol. & Med., 61, 234, 1946.

87. Smith, S.G. - "Potassium vs. biotin in the treatment of progressive paralysis in dogs". Proc. Soc. Exper. Biol. & Med., 63, 339, 1946.

88. Aitken, R.S., Allott, E.N., Castleden, L.I., and Walker, M. - "Observations on a case of familial periodic paralysis." Clinical Sc., 3, 47, 1938.

89. Pudenz, R.H., McIntosh, J.F., and McEachern, D. - "The role of potassium in familial periodic paralysis". J.A.M.A., 111, 2253, 1938.

90. Thorn, G.W. - "Effect of adrenal cortical hormone on renal excretion of electrolytes in normal subjects". Proc. Soc. Exper. Biol. & Med., 36, 361, 1937.

91. Clinton, M. Jr., and Thorn, G.W. - "Effect of DCA administration on plasma volume and electrolyte balance of normal human subjects". Bull. Johns Hopkins Hosp., 72, 255, 1943.

92. Loeb, R., Atchley, D., Gutman, E. and Jillson, R. - "On the mechanism of sodium depletion in Addison's disease". Proc. Soc. Exper. Biol. & Med., 31, 130, 1933.

93. Crisømon, J.M., Crisømon, C.S., Calabresi, M. and Darrow, D.C. - "Electrolyte redistribution in cat heart, and skeletal muscle in potassium poisoning". Am. J. Physiol., 139, 667, 1943.

94. Darrow, D.C. - "Congenital alkalosis with diarrhea". J. Pediat., 26, 519, 1945.

95. Swingle, W.W., Parkins, W.M. and Remington, J.W. - "The effect of DCA and of blood serum transfusion upon the circulation of the adrenalectomized dog". Am. J. Physiol., 134, 503, 1941.

96. Winter, C.A. and Ingram, W.R. - "Observations on the polyuria produced by desoxycorticosterone acetate". Am. J. Physiol., 139, 710, 1943.

97. Selye, H., and Bassett, L. - "The effect of desoxycorticosterone and testosterone on water and chloride metabolism." Proc. Soc. Exper. Biol. & Med., 45, 272, 1940.

98. Harned, A.S. and Nelson, W.O. - "The relation between desoxycorticosterone acetate and diabetes insipidus". Fed. Proc., 2, 19, 1943.

99. Rice, K.K. and Richter, C.P. - "Increased sodium chloride and water intake of normal rats treated with desoxycorticosterone acetate." Endocrinology, 33, 100, 1944.

100. Anderson, E., Haymaker, W., and Joseph, M. - "Hormone and electrolyte studies of patients with hyperadrenocortical syndrome (Sushin's syndrome)". Endocrinology, 23, 398, 1938.

101. Walters, W., Wilder, R.M., and Kepler, E.J. - "The suprarenal cortical syndrome; report of 2 cases with successful surgical treatment". Proc. Staff. Meet. Mayo Clinic, 9, 661 & 400, 1934.

102. Kepler, E.J. - "Report of 4 cases: Symposium: Polyglandular dyscrasies involving abnormalities of sexual characteristics". Proc. Staff Meet. Mayo Clinic, 8, 102, 1933.

103. Kepler, E.J., Kennedy, R.L.J., Davis, A.C., Walters, W., and Wilder, R.M. - "Suprareno-cortical syndrome and pituitary basophilism; presentation of 3 new cases". Proc. Staff Meet. Mayo Clinic, 9, 169, 1934.

104. Willson, D.M., Power, M.H. and Kepler, E.J. - "Alkalosis and low plasma potassium in a case of Cushing's syndrome: a metabolic study". J. Clin. Invest., 19, 701, 1940.

105. Cluxton, H.E., Bennett, W.A., Power, M.H., and Kepler, E.J. - "Cushing's syndrome without adenomatous or hyperplastic changes etc.". J. Clin. Endoc. 5, 61, 1945.

106. Siebeck, R. - "Physiologie des Wasserhaushaltes". Handb. d. norm. u. path. Physiol., Part 2, 17, 161, 1926.

107. Skelton, H. - "The storage of water by various tissues of the body". Arch. Int. Med., 20, 140, 1927.

108. Darrow, D.C. - "Body-fluid physiology: The relation of tissue composition to problems of water and electrolyte balance". New Eng. J. Med., 233, 91, 1945.

109. Harrison, H.E., Darrow, D.C., and Yannet, H. - "The total electrolyte content of animals and its probable relation to the distribution of body water". J. Biol. Chem., 113, 515, 1936.

#60

110. Manery, J.F. and Danielson, I.S. - "Connective tissue electrolytes". J. Biol. Chem., 124, 359, 1938.

111. Manery, J.F. and Hastings, A.B. - "The distribution of electrolytes in mammalian tissues". J. Biol. Chem., 127, 657, 1939.

112. Fenn, W.O. - "Potassium in physiological processes". Physiol. Rev., 20, 377, 1940.

113. Miller, H.G. - "Potassium in animal nutrition. II. Potassium in its relation to the growth of young rats". J. Biol. Chem., 55, 61, 1923.

114. Greenberg, D.M. and Cuthbertson. - "Dietary chloride deficiency and alkalosis in the rat". J. Biol. Chem., 145, 179, 1942.

115. Smith, P.K. and Smith, A.H. - "Electrolytes in the serum of the rat". J. Biol. Chem., 107, 673, 1934.

116. Van Slyke, D.D. and Hiller, A. - "Application of Sendroy's iodometric chloride titration to protein-containing fluids". J. Biol. Chem., 167, 107, 1947.

117. Sendroy, J. Jr. - "Microdetermination of chloride in biological fluids, with solid silver iodate. II. Titrimetric analysis". J. Biol. Chem., 120, 405, 1937.

118. Peters, J.P. and Van Slyke, D.D. - "Quantitative Clinical Chemistry". vol. 2. Baltimore, The Williams & Wilkins Co., 1932.

119. Patterson, J. - "Determination of chlorides in body fluids". Biochem. J., 22, 758, 1928.

120. Snedecor, G.W. - "Statistical methods, applied to experiments in agriculture and biology". Ames, Iowa State College Press, 1940.

121. Winkler, A.W., Elkington, J.R. and Eisenman, A.J. - "Comparison of sulfocyanate with radioactive chloride and sodium in the measurement of extracellular fluid". Am. J. Physiol., 139, 239, 1943.

122. Crandall, L.A. Jr., and Anderson, M.X. - "Estimation of the state of hydration of the body by the amount of water available for the solution of sodium thiocyanate". Am. J. Dig. Dis. & Nutrition, 1, 126, 1934.

123. Lavietes, P.H., Bourdillon, J. and Klinghoffer, K.A. - "The volume of the extracellular fluids of the body". J. Clin. Invest. 15, 261, 1936.

124. Rosenbaum, J.D. and Lavietes, P.H. - "Lipoid-thiocyanate in serum". J. Biol. Chem., 131, 663, 1939.

125. Ashworth, C.T., Muirhead, E.E., Thomas, O.F., and Hill, J.M. - "An analysis of the thiocyanate method for determining the distribution of the body fluids". Am. J. Physiol., 139, 255, 1943.

126. Wilde, W.S. - "Entrance of chloride with potassium into live rat muscle fibers - Cl-space error". Science, 98, 202, 1943.

127. Zwemer, R.L. and Pike, F.H. - Ann. N.Y. Aca. Sci., 37, 257, 1938. quoted by Fenn (112).

128. Baetjer, A.M. - "The diffusion of potassium from resting skeletal muscles following a reduction in blood supply". Am. J. Physiol., 112, 139, 1935.

129. Bisgaard, J.D., McIntyre, A.R. and Osheroff, W. - "Studies of sodium, potassium and chlorides of blood serum in experimental traumatic shock, shock of induced hyperpyrenia, high intestinal obstruction, and duodenal fistulas". Surgery, 4:, 528, 1938.

130. Houssay, D.A., Marenzi, A.D., and Gerschman, R. - "Potassium sanguin et mecanisme sympathetique - adrenalino-hepatique". Compt. rend. Soc. de Biol., 124, 382, 383, 384, 1937.

131. Maximow, A.A. and Bloom, W., "A Textbook of Histology", Philadelphia, W.B. Saunders Company, 1938.

132. Cole, E.C. - "Textbook of Comparative Histology". Philadelphia, Blakiston Company.

133. McSwiney, B.A., and Robson, J.M. - "The response of smooth muscle to stimulation of the vagus nerve". J. Physiol., 68, 124, 1929.

134. Hill, C.J. - Phil. Trans. Roy. Soc., B, 215, 355, 1927.

135. McSwiney, B.A. - "Innervation of the stomach". Physiol. Rev., 11, 478, 1931.

136. Alvarez, W.C. - "An Introduction to Gastroenterology". 3rd Edition. New York, Hoeber, Inc., 1939.

137. Irwin, D.A. - "The anatomy of Auerbach's plexus". Am. J. Anat., 49, 141, 1931.

138. Sauer, M.E. and Rumble, C.T. - "The number of nerve cells in the myenteric and submucous plexuses of the small intestine of the cat". Anat. Rec., 96, 373, 1946.

139. Bayliss, W.M. and Starling, E.H. - "The movement and innervation of the large intestine". J. Physiol., 26, 107, 1900.

140. Bayliss, W.M. and Starling, E.H. - "The movements and innervation of the small intestine". J. Physiol., 24, 99, 1899 and 26, 125, 1900.

141. Alvarez, W.C., Hosoi, K. Overgard, A. and Ascanio, H. - "The effects of degenerative sections of the vagi and the splanchnics on the digestive tract". Am. J. Physiol., 90, 631, 1929.

142. Cannon, W.B. - "The motor activities of the stomach and small intestine after splanchnic and vagus section". Am. J. Physiol., 17, 429, 1906.

143. Cannon, W.B. - "The movements of the stomach studied by means of the roentgen rays". Am. J. Physiol., 1, 359, 1898.

144. Cannon, W.B. - "The movements of the stomach studied by means of the roentgen rays". Am. J. Physiol., 1, xiii, 1898.

145. Cannon, W.B. - "The movement of the intestines studied by means of the roentgen rays". Am. J. Physiol., 6, 251, 1902.

146. Cannon, W.B. - "Further observations on the movements of the stomach and intestines". Am. J. Physiol., 8, xxi, 1902.

147. Cannon, W.B. - "The passage of different foodstuffs from the stomach and through the small intestines". Am. J. Physiol., 12, 387, 1904.

148. Cannon, W.B. - "The influence of emotional states on the functions of the alimentary canal". Am. J. Med. Sci., 137, 480, 1909.

149. Cannon, W.B. - "The Mechanical Factors of Digestion". New York, E. Arnold, 1911.

150. Cannon, W.B. - "The importance of tonus for the movements of the alimentary canal." Arch. Int. Med., 8, 417, 1911.

151. Cannon, W.B. - "The relation of tonus to antiperistalsis in the colon". Am. J. Physiol., 29, 238, 1911.

152. Cannon, W.B. - "The nature of gastric peristalsis". Am. J. Physiol., 29, 250, 1911.

153. Cannon, W.B. - "Peristalsis, segmentation and the myenteric reflex". Am. J. Physiol. 30, 114, 1912.

154. Cannon, W.B., and Lyman, H. - "The depressor effect of adrenalin on arterial pressure". Am. J. Physiol., 31, 376, 1913.

155. Cannon, W.B. and Moser, A. - "The movements of the food in the oesophagus". Am. J. Physiol., 1, 435, 1898.

156. Evans, C.L. - "The physiology of plain muscle". Physiol. Rev., 6, 358, 1926.

157. Fischer, E. - "Vertebrate smooth muscle". Physiol. Rev., 24, 467, 1944.

158. Evans, C.L. - "Studies on the physiology of plain muscle. II. The oxygen usage of plain muscle and its relation to tonus". J. Physiol., 58, 22, 1923.

159. Bozler, E. - "An analysis of the properties of smooth muscle". Sympos. Cold Spring Harb., 4, 260, 1936.

160. Bozler, E. - "The mechanism of the inhibitory action of vasocilator nerves". Am. J. Physiol., 117, 457, 1936.

161. Magnus, R. - "Cholineand gastro-intestinal system". Muencher. Med. Wochschr., 72, 249, 1925.

162. Zimmerman, B., Frank, R. and Necheles, H. - "Effect of acetyl choline and of physostigmine on gastrointestinal motility. Observations of normal animals and of animals with experimental peritonitis". Arch. Surg., 33, 188, 1936.

163. Hukuhara, T. - "Die normale Duenndarmbewegung. (Mit Hilfe der Bauchfenstermethode und Kinematographie)". Arch. f.d. ges. Physiol., 226, 518, 1930-31.

164. Gunn, J.A. and Underhill, S.W. - "Experiments on the surviving mammallian intestine". Quart. J. Physiol, 8, 275, 1914.

165. Alvarez, W.C. and Mahoney, L.J. - "The myogenic nature of the rhythmic contractions of the intestine". Am. J. Physiol., 59, 421, 1922.

166. Evans, C.L., and Underhill, S.W. - "Studies on the physiology of plain muscle". J. Physiol., 58, 1, 1923.

167. Gasser, H.S. - "Plexus-free preparations of the small intestine; a study of their rhythmicity and of their response to drugs". J. Pharmacol. & Exper. Therap., 27, 395, 1926.

168. Ogata, S. - "The activity of the isolated uterus". J. Pharmacol. & Exper. Therap., 18, 185, 1921.

169. Kaminester, S. and Reynolds, S.R. - "Motility in the transplanted, denervated uterus". Am. J. Obst. & Gynec., 30, 395, 1935.

170. Hryntschak, T. - "Beitraege zur Physiologie des Ureters: I. Zur Harnleiterautomatie". Arch. f.d.ges. Physiol., 209, 542, 1925.

171. Alvarez, W.C. and Mahoney, L.J. - "Peristaltic rush in the rabbit". Am. J. Physiol., 69, 226, 1924.

172. Alvarez, W.C. - "Differences in rhythmicity and tone in different parts of the wall of the stomach". Am. J. Physiol., 40, 585, 1916.

173. Brown, G.L. and McSwiney, B.A. - "Reaction to drugs of strips of the rabbit's gastric musculature". J. Physiol., 61, 261, 1926.

174. Elliott, T.R. and Barclay-Smith, F. - "Antiperistalsis and other muscular activities of the colon". J. Physiol., 31, 272, 1904.

175. Garry, R.C. - "The movements of the large intestine". Physiol. Rev., 14, 103, 1934.

176. Kornberg, A. and Endicott, K.M. - "Potassium deficiency in the rat. V". Am. J. Physiol., 145, 241, 1946.

177. Schrader, G.A., Prickett, C.O., and Salmon, W.D. - "Symptomatology and pathology of potassium and magnesium deficiencies in the rat". J. Nutrition, 14, 85, 1937.

178. Heppel, L.A. and Schmidt, C.L.A. - "Studies on potassium metabolism of rat during pregnancy, lactation and growth." Univ. California Pub., Physiol., 8, 189, 1938.

179. Skinner, J.T. and McHargue, J.S. - "Response of rats to boron supplements when fed rations low in potassium". Am. J. Physiol., 143, 385, 1945.

180. Robertson, E.C. and Doyle, M.E. - "Intestinal stasis in low mineral diets". J. Nutrition, 9, 553, 1935.

181. Miller, H.G. - "Potassium in animal nutrition. IV. Potassium requirements for normal growth and maintenance". J. Biol. Chem., 70, 587, 1926.

182. Kupaloff, P.S. - "Die Chemischen Erreger der Magenkontraktionen. III. Über die Werkung von Kalium-chlorid und Calciumchlorid auf die Bewegungen eines Froschmagens". Arch. f. Physiol., 204, 483, 1924.

183. Vogt, M. - "Chronic suprarenal deficiency and its effects on the responses of the isolated intestine in the rabbit". J. Physiol., 102, 239, 1943.

184. Fowler, J.L.A. and Cleghorn, R.A. - "The response of splanchnic blood vessels and of the small intestine to vasoconstrictor influences in adrenal insufficiency in the cat". Am. J. Physiol., 137, 371, 1942.

185. Clarke, A.P.W. and Cleghorn, R.A. - "Chemical study of tissue changes in adrenal insufficiency and traumatic shock". Endocrinology, 31, 597, 1942.

186. Rose, W.B., Stucky, C.J., and Cowgill, G.R. - "Studies in the physiology of vitamins. X. Further contribution to the study of gastric motility in vitamin B. deficiency". Am. J. Physiol., 91, 531, 1930.

187. Rose, W.B., Stucky, C.J. and Cowgill, G.R. - "Studies in the physiology of vitamins. XII. The relation of gastric motility to anhydremia in vitamin B- deficient dogs". Am. J. Physiol., 92, 83, 1930.

188. Stiles, P.G. - "On the influence of calcium and potassium upon the tone of plain muscle". Am. J. Physiol., 8, 269, 1903.

189. Magee, H.E. and Reid, E. - "Studies on the movements of the alimentary canal. I. The effects of electrolytes on the rhythmical contractions of the isolated mammalian intestine". J. Physiol., 63, 97, 1927.

190. Jendrassik, J. "Beitraege zu einer Pharmakologie der Konzentrationsaenderungen ueber Wirkungen von Kalium, Calcium and Magnesium am Darm". Biochem. Z., 148, 116, 1924.

191. Amabache, N. - "Interaction of drugs and the effect of cooling on the isolated mammallian intestine". J. Physiol., 104, 266, 1946.

192. Whitehead, R.W. - "Responses of excised intestine to alterations of electrolyte concentration". Am. J. Physiol., 89, 253, 1929.

192a. Mathison, G.C. - "The effects of potassium salts upon the circulation and their action on plain muscle". J. Physiol., 42, 471, 1911.

193. Clark, A.J. - "The mode of action of potassium upon isolated organs". J. Pharmacol. and Exper. Therap., 18, 423, 1921.

194. Vogt, M. - "The site of action of some drugs causing stimulation of the circular coat of the rabbit intestine". J. Physiol., 102, 170, 1943.

195. Zondek, S.G. - "Über das Wesen der Vagus - und Sympathicusfunktion. Die Identität von Nerv- und Ionenwirkung". Biochem. Z. 132, 362, 1922.

196. Singh, I. - "A comparative study of the effect of the interaction of ions, drugs and electrical stimulation as indicated by contraction of unstriated muscle". J. Physiol., 96, 367, 1939.

198. Tate, G., and Clark, A.J. - "The action of Potassium and Calcium upon the isolated uterus". Arch. int. Pharm. Therap. 26, 103, 1932.

199. Jendrassik, L., and Annau, E. - "Beiträge zu einer Pharmakologie der Konzentrationsänderungen. III. Weitere Versuche über Kationenwirkungen". Biochem. Z., 162, 207, 1925.

200. Hogben, L.T. - "Studies on the comparative physiology of contractile tissue. I. The action of electrolytes on invertebrate muscle." Quart. J. Exper. Physiol., 15, 263, 1925.

201. Jannink, E.H. - "The influence of potassium on the intestinal movements". Onderzoek. Physiol. Lab. Utrecht, 5, 13, 1925. C.A. 20, 1462.

202. Burridge, W. - "An inquiry into some chemical factors of fatigue". J. Physiol., 41, 285, 1910.

203. Fenn, W.O., Wilde, W.S., Boak, R.A. and Koenemann, R.H. - "The effect of blood flow on potassium liberation from muscle". Am. J. Physiol., 128, 139, 1939.

204. Hegnauer, A.H., Fenn, W.O. and Cobb, D.M. - "Cause of rise in oxygen consumption of frog muscle in excess of potassium". J. Cell. and Comp. Physiol., 4, 505, 1934.

205. Carleton, B.H., Blair, H.A. and Latchford, W.B. - "Effects of chlorides of potassium, calcium and sodium on the excitability of muscle". J. Cell. and Comp. Physiol., 12, 223, 1938.

206. Lapicque, M. and Nattan-Larrier, M. - "Actions antagonistes du calcium et du potassium sur l'imbibition et la chronaxie musculaire". Comp. rend. Soc. de Biol., 94, 808, 1926.

207. Chao, I. - "Action of electrolytes on electrical stimulation of skeletal muscle". J. Cell. and Comp. Physiol., 6, 1, 1935.

208. Greisheimer, E. - "A quantitative study of the effects produced by salts of sodium, potassium, rubidium and calcium on motor nerve of frog". Am. J. Physiol., 49, 497, 1919.

209. Hoeber, R. and Strohe, H. - "Uber den Einfluss von Salzen auf die elektrotonischen ströme die Erregbarkeit und das Ruhepotential des Nerven". Arch. f. d. ges. Physiol., 222, 71, 1929.

210. Rosenberg, H. and Kitayama, K. - "Untersuchungen uber Nervenaktionsströme, das Verhalten der Negativitatswelle bei Uberschuss von Kalium- und Calciumionen". Arch. f. d. ges. Physiol., 223, 602, 1929.

211. Hooker, D.R. - "Saline perfusion of the respiratory center in frogs: the effect of calcium chloride and potassium chloride". J. Pharmacol. & Exper. Therap., 4, 443, 1913.

212. Heinekamp, W.J.R. - "The action of potassium salts on the medulla as shown by perfusion of the medulla of the terrapin (pseudomys troosti) with potassium salts". J. Pharmacol. & Exper. Therap., 19, 239, 1921.

213. Mullin, F.J., Hastings, A.B., and Lees, W.M. - "Neuromuscular responses to variations in calcium and potassium concentrations in the cerebrospinal fluid". Am. J. Physiol., 121, 719, 1938.

214. Walker, S.M. - "Enhancement and depression of neuromuscular transmission by intraarterial injection of potassium chloride in the dog". Proc. Soc. Exper. Biol. & Med., 64, 425, 1947.

215. Feldberg, W. and Vartiainen, A. - "Further observations on the physiology and pharmacology of a sympathetic ganglion". J. Physiol., 83, 103, 1934.

216. Brown, G.L. and Feldberg, W. - "The action of potassium on the superior cervical ganglion of the cat". J. Physiol., 86, 290, 1936.

217. Braun, L. - "Ueber die Wirkung des Kalisalze auf das Herz und die Gefaesse von Saeugethieren". Arch. f. d. ges. Physiol., 103, 476, 1904.

218. Camp, W.J. - "Potentiation of digitalis action by epinephrine and potassium". Arch. int. de Pharmacodyn. et de Therap., 61, 60, 1939.

219. Boehm, R. - "Ueber das Verhalten des isolierten Froschherzens bei reiner Salzdialt". Arch. f. exper. Path. u. Pharmakol., 75, 230, 1914.

220. Cicardo, V.H. and Marenzi, A.D. - "Variaciones de excitabilidad y permeabilidad de los corazones de los sapos a los iones potasio, sodio y calcio". Rev. Soc. Argen. Biol., 14, 59, 1938.

221. Hegnauer, A.H. - "Injury potential, oxygen consumption, and irritability in frog muscle as affected by potassium". Am. J. Physiol., 105, 47, 1933.

222. Beznak, A.B.L. - "On the mechanism of the autacoid function of parasympathetic nerves". J. Physiol., 82, 129, 1934.

223. Dawes, G.S. - "The vaso-dilator action of potassium". J. Physiol., 99, 224, 1941.

224. Mann, P.J., Tannenbaum, M. and Quastel, J.H. - "Acetylcholine metabolism in the central nervous system. The effects of potassium and other cations on acetylcholine liberation". Biochem. J., 33, 822, 1939.

225. Ferguson, J. - "A study of the nerve-free smooth muscle of the amnion of the chick". Am. J. Physiol., 131, 524, 1940.

226. Vogt, M. - "The site of action of some drugs causing stimulation of the circular coat of the rabbits intestine". J. Physiol., 102, 170, 1943.

227. Eichholtz, F., and Starling, E.H. - "The action of inorganic salts on the secretion of isolated kidney". Proc. Roy. Soc. B, 98, 93, 1925.

228. Cicardo, V.H. - "Liberacion de potassio durante la contraccion del estomago". Rev. Soc. Argent. Biol., 17, 81, 1941.

229. Cicardo, V.H. - "Desprendimiento de potasio por las mucosas excitadas". Rev. Soc. Argent. Biol., 19, 271, 1943.

230. Fenn, W.O. - "Factors affecting the loss of potassium from stimulated muscles". Am. J. Physiol., 124, 213, 1939.

231. Heppel, L.A. - "Effect of age and diet on electrolyte changes in rat muscle during stimulation". Am. J. Physiol., 128, 440, 1940.

232. Alvarez, W.C. and Hosoi, K. - "The latent period of intestinal muscle". Am. J. Physiol., 89, 201, 1929.

233. Alvarez, W.C., and Freedlander, B.L. - "The rate of progress of food residues through the bowel". J.A.M.A., 83, 576, 1924.

234. Wangensteen, O.H. - "Intestinal obstructions. A Physiological and Clinical Consideration with Emphasis on Therapy; including Description of Operative Procedures". Springfield, Illinois, C.C. Thomas, 1945.

235. Noonan, T.R., Fenn, W.O., and Haege, L. - "The distribution of radioactive potassium in rats". Am. J. Physiol. 132, 474, 1941.

236. Fenn, W.O., Noonan, T.R. and Haege, L. - "The exchange of radioactive potassium with body potassium". Am. J. Physiol. 135, 149, 1942.

237. Martin, G.J., Thompson, M.R. and Carvajal-Forero, J.de - "The influence of single and multiple B, complex deficiencies upon the motility of the gastro-intestinal tract". Am. J. Dig. Dis. 9, 268, 1940.

238. de Beer, E.J., Johnston, C.G. and Wilson, D.W. - "The composition of intestinal secretions". J. Biol. Chem. 108, 113, 1935.

239. Harrison, H.E., Tompsett, R.R. and Barr, D.P. - "The serum potassium in two cases of sprue". Proc. Soc. Exp. Biol. & Med. 54, 314, 1943.

240. Harrison, H.E., Harrison, H,C., Tompsett, R.R. and Barr, D.P. - "Potassium deficiency in a case of lympho-sarcoma with the sprue syndrome". Am. J. Med. 2, 131, 1947.

241. Golden, R. - "Abnormalities of the small intestine in nutritional disturbances: some observations on their physiological basis". Radiol. 36, 262, 1941.

242. Boyer, P.D., Lardy, H.A. and Phillips, P.H. - "The role of potassium in muscle phosphorylations". J. Biol. Chem. 146, 673, 1942.

243. Boyer, P.D., Lardy, H.A. and Phillips, P.H. - "Further studies on the role of potassium and other ions in the phosphorylation of the adenylic system". J. Biol. Chem. 149, 1943.