PINK DISEASE (ACRODYNIA). A PHYSIOLOGICAL APPROACH (AN EVALUATION OF ADRENAL FUNCTION AND THE IMPORTANCE OF WATER AND ELECTROLYTE METABOLISM).

By Donald B. Cheek.

Appended Papers:

(1) PINK DISEASE: AN INVESTIGATION OF ITS CAUSE AND TREATMENT.

(2) PINK DISEASE: ITS NATURE PREVENTION AND CURE. (CASE REPORTS ONLY).

(3) THE IMPORTANCE OF THE SUPRARENAL GLAND IN DISEASES OF INFANCY AND CHILDHOOD WITH PARTICULAR REFERENCE TO PINK DISEASE.

(4) PINK DISEASE: THE MANIFESTATION IN OLDER CHILDREN AND THE ESTIMATION OF THE BLOOD ADRENALINE CONTENT.

(5) EVIDENCE OF ADRENAL CORTICAL FUNCTION IN PINK DISEASE.

(6) EXTRA RENAL BASIS OF ALKALOSIS IN POTASSIUM DEFICIENCY.

(7) A METHOD FOR THE ESTIMATION OF THE BROMIDE SPACE.

PINK DISEASE (INFANTILE ACRODYNIA) - A PHYSIOLOGICAL APPROACH. (AN EVALUATION OF ADRENAL FUNCTION AND THE IMPORTANCE OF WATER AND ELECTROLYTE METABOLISM)

A THESIS

BY

DONALD B. CHEEK.

Foreword.

This thesis represents the results of research carried out over a period of 3 years. Firstly is presented the results of biochemical, physiological, clinical and pathological investigations into the nature of pink disease. Secondly this thesis embodies the results of work done in the field of electrolyte and water metabolism performed in the Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut, the United States of America. This latter year's work was carried out under the guidance of Professor Daniel C. Darrow and with the aid of a medical research fellowship (Rotary International Foundation).

Research concerning pink disease was initially begun as a private endeavour, but subsequent support of this work was given by the National Health and Medical Research Council of Australia, and also by the University of Adelaide. In the earlier stages of this work the author was given facilities to work in the Department of Physiology of this university. Although assistance, facilities and nursing staff were not at hand for complete metabolic studies at the

Adelaide Childrens Hospital, the valuable cooperation and assistance of the Board of Honouries of this hospital, and of Mareeba Babies Hospital are gratefully acknowledged. Proper facilities for plasma mineral determinations were available only at the Waite Agricultural Institute in Adelaide, and these were generously carried out by the Division of Soils of the Commonwealth Scientific and Industrial Research Organization.

Interest in the problem of pink disease began while this author was a Resident Medical Officer at the Adelaide Childrens Hospital. Such interest was fostered by cases of this disease which were presented during this period of clinical training, and by the exasperating and pernicious nature of this obscure condition.

A child of five and a half years of age suffering from "florid" pink disease - a considered rarity - came under the care of this author. This led to a study of the age incidence of this disease, which in itself proved stimulating and provoking (vide reference (1 a) - this paper is not appended).

A physiological approach (which had hitherto been neglected) was the course chosen by this author. At the outset it seemed that there could be some disturbance of electrolyte or mineral metabolism, perhaps related to that important mediator of homeostasis, the adrenal gland. It was then, with these ideas in mind, that this author began work in the laboratory of the Adelaide Childrens Hospital, some little time before help, information, or financial aid were sought.

It should be pointed out that because pink disease is uncommon,

the undertaking to study this disease was not simple, and often necessitated long journeys into all parts of the State of South Australia.

The results of this work have been published in full, and are submitted as five appended papers. The interpretation of the results obtained has hitherto been to some extent difficult, and has caused some speculation and confusion.

It is fit and proper, therefore, that acknowledgment should be made to Professor Daniel C. Darrow and Robert E. Cooke of the Yale University School of Medicine; for these men, authorities in the field of electrolyte and water metabolism, have been of help in elucidating the interpretation of results obtained. They have therefore led this author to a correct approach to the available evidence, which is the context of this thesis. Recent studies have failed to confirm the consistently low sodium concentrations reported by this author. Since the method used was not as reliable as other methods, it will be considered that some of these values are erroneously low and conclusions will not be based on these findings. For obvious reasons, therefore, the case reports alone of the second appended paper are presented, and the theoretical interpretations and hypothesis of primary adrenal glandular involvement and insufficiency are excluded. In the light of subsequent work, such a hypothesis is untenable. Reference to any significance relating to constantly low serum sodium values is likewise deleted.

It is felt that this work which yields a new approach to pink disease also provides important facts which subsequently will fit into the correct aetiological pattern when the exact state of body water and electrolyte has been defined.

The sixth appended paper in which this author has been a collaborator, is based on some 2,500 separate chemical determinations.

The seventh appended paper is an independent project undertaken, and it is felt that this should prove of future value in both the clinical and research field. Although research into the field of electrolyte and water metabolism has provided this author with an insight into this particular field, it has been unfortunate that the extreme rarity of pink disease in the state of Connecticut has prevented the present extension of this clinical project.

Throughout the duration of the study of pink disease, this author wishes to acknowledge the help and inspiration of a friend and colleague, Basil S. Hetzel, and also the encouragement given by Professor Sir C. Stanton Hicks.

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A THESIS

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DONALD B. CHEEK.

Introduction.

There are few diseases in paediatrics which present such a complexity of symptoms, and few other conditions reveal such a picture of prolonged stress and misery. Doubtless there are few other circumstances where the paediatrician feels more embarrassed at his own therapeutic inactivity and more uncertain of his ability to retain the confidence and cooperation of the parents.

Clinicians from all countries of the world have been impressed by some particular aspect of the bizarre pattern of this symptomatology. They have respectively placed emphasis on different aspects (appended paper 1). They have endeavoured to support a virus aetiology - either neurotrophic or respiratory, to prove a vitamin deficiency, to incriminate a toxin or poison, or to fall back on allergies, to single out some particular region of the central nervous system or of the autonomic nervous system, or indeed, as recently, to suggest psychosomatic origins (1). But surely, if nothing else, this modern age has taught us to examine disease processes in terms of altered physiological conditions, and to fortify our knowledge of symptomatology and

our clinical approach by means of biochemical assay. By such methods we may not uncover or directly discover aetiological factors; but we can often glimpse for a moment the "before" and "potential after", which may lead us to draw a rough map of the uncharted sea of a particular disease. The study of the sea within us, "the milieu interieur", is often illuminating.

In regions where this disease is relatively much more common, as in Australia, the condition presents an aggravating problem. It is not the purpose of this manuscript to consider in any detail the possible aetiological causes, the literature concerning which is voluminous, and has been reviewed elsewhere (appended paper 1), but to direct attention to alterations in physiology and to the departure from homeostasis, which has previously been the particular interest of the author. It will be obvious that such knowledge in respect to this disease is not complete, but it is hoped that enquiry will be stimulated on the part of others to pursue further this work and this approach, which has hitherto been neglected.

HISTORICAL

It was unfortunate that the first clinical description of this disease given by Selter, of Germany, in 1903 aroused such little interest (2). He presented eight female children about two years of age suffering from a condition he termed "trophodermato-neurosis." Following Selter, silence reigned until the year 1914 when the Australian physician, Swift (3), gave an accurate description of the condition in its infantile manifestation. Prior to Swift's paper, the condition had not entirely escaped attention in Australia, as Charles Clubb of Sydney had already coined the name "Pink Disease", while William Snowball of Melbourne talked of the syndrome "raw beef hands and feet" as far back as 1883. As a result of this stimulus, Wood, in conjunction with Hobhill-Cole (4), presented a study of 91 cases at the first Australian Medical Congress following the first world war, in 1920.

This disease was independently described by Bilderback of the United States of America in 1920 at Seattle. Washington. His excellent description clearly defined the manifestation in children as well as in infants. In the same year, Weston of Columbia contributed the name "Acrodynia" (5), and soon reports and observations became numerous. In the following year an important paper was published by Brown, Courtney, and MacLachlan of Toronto, Canada (6), while in England Weber (7) drew attention to this disease, and it was described in France by Comby (8), and for the second time in Germany by Feer (9) in 1922.

AETIOLOGICAL POINTS

It is known that the sexes are equally affected. The question of seasonal incidence has through the years been a matter of some argument. But it has been shown in America (1), in Australia (11), and in England (12) that the seasons play no part. However, geographical location definitely does influence the incidence of this disease. Pink disease is not uncommon in Australia - any large hospital there will have at least one or two cases, either as outdoor or indoor patients, attending. In the state of South Australia, with a population approximating half a million inhabitants, there are at least 80-100 patients each year suffering from this condition; whereas in the state of Connecticut in the United States of America, with a much larger population, it is rare for one case to present in a year. It has been my impression from my own study of this disease (and from interrogation) that it is much more common in Australia than in America, England, Canada, or Europe. Perhaps more interesting still is the regional distribution of this disease within countries themselves, which has been so clearly shown.

From information gained through hospital records (1 a), and through a study of cases, it has been shown repeatedly that 80-90% of cases in Australia occur between six months and two years of age. From other data it is apparent that the same holds for Canada (13) and England (12). It is of some interest that the curve of the age incidence follows so closely that of infantile allergic phenomena (14) (1 a). Eosinophilia, if looked for in pink disease with appropriate methods, will sometimes be found (appended paper 4). In any large series of cases one finds occa-

sionally the occurrence of more than one case in the same family of siblings; and it has also been the experience of the author to find recurrence of this disease, which has already been pointed out by other clinicians (2 a).

The brightest and most hopeful aetiological fact in the history of the disease has been the discovery of an increased amount of mercury in the urine of these children with pink disease, findings which have been provided by the work of Warkany and Hubbard of Cincinnati (15) (16), and other workers (17) (18) (19) (20). In their most recent paper, Warkany and Hubbard show significant amounts of mercury in the urine of 38 out of 41 cases. These workers have gone far to establish an "idiosyncrasy" to mercury, as an important factor in our present understanding of the causation of pink disease. However, as to why such an idiosyncrasy should occur, or what factors are related to it, are unknown. The fact that some children are given mercury after the true onset of the disease in the belief that "teething troubles" are being treated causes some confusion and a difficulty in interpreting the significance of the urinary mercury estimation. Normal children of the same age group who have ingested non-toxic forms of mercury can excrete equal amounts without manifesting this disease. This emphasizes that the crux of the matter rests with elucidation of the "idiosyncrasy." This is also emphasized by the failure of dimercaprol (B.A.L.) to produce consistently effective results (15) (20).

The consideration of the role of mercury is discussed further later (see below).

SOME OBSERVATIONS RELATING TO SYMPTOMATOLOGY

The complexity of symptoms and signs and the bizarre nature of these impress all paediatricians and physicians who have met, and do meet cases of this fascinating disease. It is not intended that a detailed description be given of these well known phenomena, (vide appended paper 1) but rather to briefly point out some relevant aspects which have been obtained by experience with over one hundred cases.

I. The infantile form

The paediatrician or physician is consulted because of a sudden change of well being on the part of the infant. There may be a history of a cold, or of recent infection associated with rhinitis. Irritability is present and the little patient, usually aged five or six months or older, refuses to take his food. Teething is in progress, and the mother often ascribes the state of her infant to this. Indeed it is often not until weight loss becomes remarkable, which is early, that the family doctor becomes suspicious of a more pernicious condition. The paediatrician is sometimes confronted with the child which has been referred to him from the ophthalmologist, the mother having consulted an eye specialist in the belief that photophobia is the main cause of the condition.

Examination at this juncture reveals that sweating has become noticeable, and that the hands and feet of the infant show a slight pinkness, and perhaps an unusual cold and moist feeling. The infant markedly resents examination and tends to burrow his head into the pillow away from the light. The skin temperature is usually normal,

but may be depressed or elevated one degree. The mother complains that the child cries most of the night; that the stools have become constipated, or (and this is less common) that one or two relaxed bowel actions have been passed. If the opportunity presents itself, tachycardia and hypertension can be clearly defined in all cases.

Following this insidious onset the classical picture develops. Anorexia becomes marked, sweating profuse, and the hypertrophy of the sweat glands which is seen microscopically is emphasized by miliarial or sudaminal rashes which spread across the trunk of the body. As the disease progresses hypertension becomes easily detectable. The muscles are soft to the feel and the hands and feet previously pink some of the time, become pink, swollen, oedematous, cold, and moist most of the time. That they are causing the infant distress is made obvious by the fact that one may observe the little patient to rub them together. Any previous confusion of these symptoms with "measles" is now dispelled. The mother is generally very distressed; she is tired and exhausted from sleepless nights and from frequent fruitless efforts to force feed her infant. The resentment of the infant toward the mother is to her a matter for further concern. The prescribed sedatives of common usage in their usual dosages, fail to produce their expected effect. The household is in a state of some disruption, and although textbooks warn against the dangers of secondary infection, the paediatrician more often than not admits the child to a hospital or to a nursing home to relieve the harassed parents for at least some of the time. Respiratory infection is the chief danger (1 a). The weight chart reaches a steady low level with the passing of

the weeks. With the shedding of the skin from the hands, which starts between the fingers, the tender raw beef hands and feet become manifest. The layers of skin become more delicate with each successive shedding. During periods of atmospheric heat, which is badly tolerated by these children, crying gives way to listlessness, apathy and exhaustion. The hot, dry climate of Australia is singularly deleterious. There is always the potential danger of shock collapse and death. During periods of atmospheric cold the hands frequently become cyanosed. This picture of extreme misery and stress usually continues for several months, even up to 18 months, or longer. However, a minority of cases may be mild, with only 2 or 3 months illness. Diarrhoea presents in a smaller number of cases, and in the opinion of this author, is not usually of infective origin. The prognostic significance of this is grave.

As insidiously as they came, so the symptoms gradually disappear; the irritability subsides, sleep improves, feeding is no longer forced, and the first smile appears on the little face. Recovery begins. This is complete in the vast majority of cases. However, a state of "athrepsia" or "failure to thrive" may continue in some infants for several months - long after the extremities and cutaneous signs have disappeared (appended paper 4). In other cases the condition persists in a milder form throughout several years of childhood (appended paper 4). Bronchiectasis in pre-adolescence is sometimes a sequel of pink disease. Indeed, in Australia pink disease is the commonest cause of bronchiectasis.

II. The older child

The manifestation in older children is not so frequently encountered, and the diagnosis is often more difficult to make unless the classical picture of the extremities is exhibited. This is rare and has been encountered in only one case of five and one half years of age in the experience of this author (1 a). The thicker layer of skin of older children does not so readily reveal pinkness of the hands, but coldness and moistness are in evidence, and peeling of the skin generally occurs.

In contrast to the infant, the symptoms of lassitude, fatigue, and inertia predominate. A fractious or even violent nature of a child previously of a normal happy disposition is characteristic. The sick child stops walking and may only want to "sit all day", becomes asocial, and talks infrequently. Long periods of nocturnal wakefulness are experienced. Sweating, though abnormally increased, is not so much in evidence objectively. Food and fluid demand is minimal, and constipation is nearly always present. Marked photophobia does not so often present in such an obvious way, but is rather shown by a resentment to bright light, or by the child's insistence to undress in the dark. The diagnosis is not generally made until this picture has been present for some months, and until a failure to gain weight is of several months duration. Hypertension and hypotonia are detectable.

The course of the illness is extended over many months, usually more than a year, and it is of interest that allergic phenomena seem to be closely associated. Six out of eleven patients of this category reported showed either "wheezes in the chest" or recurrent bronchitis or actual asthma, skin rashes, and a significant eosinophilia was demonstrated in

ten out of eleven cases (appended paper 4). On the other hand, Holzel and James (20), by investigating a large series of cases, found no evidence that allergic disorders in pink disease are more common that in the normal child population.

IIa. The nervous child and its relation to pink disease in older children

The manifestation of pink disease in older children was adequately defined by the French physician, Rocaz (21), as the "forme nerveuse", and by the German writers as the "form fruste" (22). It has received comparatively little attention by the English speaking world.

It is of interest that such children as these are disturbed emotionally; they are "nervous children" and indeed, as pointed out previously by this author, they conform to the "nervous child syndrome" as described by Cameron in 1919 (23). Whether or not stress and psychosomatic changes can produce such organic changes is yet to be determined.

The nervous child syndrome is a somewhat nebulous condition and can probably be produced by psychological disturbances, as well as by organic disease. Since the classical description of this condition by Cameron (23), clinicians have not been successful in understanding or classifying this malady to our own satisfaction. Many of these older children with pink disease have been diagnosed by outside

opinion as examples of "the nervous child syndrome" (appended paper 4). This aroused the interest of this author.

Cameron (23) 30 years ago in his monograph on "The Nervous Child Syndrome" in discussing the poor muscle tone of these children, their restlessness and irritability, their disturbed sleep and failure to gain weight, their periodic bouts of urticaria, of asthma, or bouts of vomiting or mucus diarrhoea and acidosis, their over anxious and nervous temperament, their prostration and pallor and dark rimmed eyes, and their fat intolerance, asked the question, "What is the underlying cause of the often occurring state of collapse, physical and mental, of the nervous child? Why does he so often suddenly fall from a state of comparative well being to a deplorable condition of weakness, accompanied by loss of weight and loss of colour - with circumoral pallor and dark rimmed eyes - loss of muscular tone - and loss of co-ordination - so that at the worst his walk deteriorates to a mere shuffle and the frequency of his falls becomes a common complaint, loss of energy so that he lags behind on his walks abroad, loss of control - so that clamour, opposition and tears may become unceasing?"

Cameron sought to explain these phenomena thus:- "Certain diseased states are characterised by a definite hypoglycaemia, a definitely low blood sugar, in Addison's disease for example, in which the suprarenal glands are destroyed by caseation, there is invariably hypoglycaemia accompanied by great vasomotor weakness and

pallor, a low blood pressure, a rapid pulse, extreme amyotonia and muscle weakness, prostration and loss of energy and attacks of nausea and vomiting. We know that the suprarenal glands are exhausted and that their function becomes impaired under the stress of great exertion, great emotional excitement and persistent infection. These are the same conditions which lead to the exhaustion of the carbohydrate reserves, and the fall of blood sugar to the lowest level, and they are the same conditions which in practice we are forced to regard as productive of the bouts or attacks of pallor prostration and vomiting of the nervous child. If speculation permitted where certainty is not yet, we may surmise that in Addison's disease we are observing the effects of the gross destruction of the suprarenal medullary tissue; in an attack of cyclical vomiting we are encountering the temporary exhaustion and functional impairment of the same gland."

The fat intolerance of some types of the "nervous child syndrome" is familiar to us. A full blown picture of failure of proper fat and carbohydrate absorption is what has been called the coeliac syndrome. More recent work on coeliac disease emphasizes a disturbance in carbohydrate metabolism (26).

There is no conclusive evidence that would lead one to believe that there is any real insufficiency of the adrenal gland in the "nervous child syndrome." It is reasonable to believe that emotional stress and nervous exhaustion make great demands on the adrenal gland. In many cases of the nervous child syndrome, there is a disturbance in carbohydrate metabolism. Administration of whole adrenal extract, which

contains "cortisone", would seem therefore to be of some benefit in the restoration of homeostasis and in the production of clinical improvement.

The literature concerning "athrepsia" and the use of adrenal hormone has been reviewed elsewhere (appended paper 3). More recently pure cortisone has become available. It is now an everyday observation that many malnourished children, or children suffering from anorexia associated with disease processes (for example nephrosis) may show striking improvement in respect to appetite and well being when given this hormone. The relationship between the adrenal gland, stress, and pink disease is discussed further later, and has been considered in detail in a previous paper (appended paper 3).

It is of interest that Kenneth Cameron and Leys (1) recently investigated the family setting and environment of six unselected cases of pink disease. They concluded that: "The evidence of stress in the family situation of these cases is striking and indeed more obvious than that in the families of many children attending the psychiatric clinic." There was a distinct lack of maternal warmth in all cases, and five out of six were obviously unwanted children. The stress of emotional deprivation is thus emphasized as being important in the aetiology of this disease.

Bernheim and Confavrecia (27) have reported on the beneficial effects of electric shock therapy with induced convulsions, as a means of control of the mental and emotional disturbances in these older children with pink disease.

THE ROLE OF MERCURY

Perhaps the most promising theory of causation of pink disease at the present time is the hypothesis of Warkany (16). A definite relationship between mercury and pink disease is becoming accepted. Investigations performed at Great Ormand Street, London, have led to the conclusion (18): "In view of the conflicting evidence, the theory that ingestion of mercury is the cause of pink disease should be given the Scottish verdict of "not proven"."

Warkany (15) has found this metal to be present in 92.7 per cent of his cases investigated, Fanconi and Botsztejn (17) in 19 out of 20 cases, and Holzel and James (20) in 61 out of 94. Warkany noted that occasionally in the same patient the excretion of mercury varied greatly from one sample of urine to another, and sometimes the urine specimen was free of mercury, or contained very small amounts. It has not been possible to find a correlation between the amounts excreted and the severity of the disease. However, there is little doubt that patients with pink disease excrete more mercury than normal control children.

Normal children can absorb and excrete small amounts of mercury without showing any signs or untoward symptoms. Hence, Warkany suggests that an individual susceptability ("idiosyncrasy") to mercury intoxication exists in the children who develop pink disease:- "Just as some persons react with asthma or hay fever to environmental conditions well tolerated by others."

In other words, it is suggested that we are dealing with a syndrome akin to drug sensitivity or to an allergic phenomenon. Since the

recurrence of this disease is the exception and not the rule, it must be further postulated that we are not dealing with a permanent idiosyncrasy.

The observation that the interval between the administration of mercury and the onset of the disease varies from one week to several months also requires elucidation. This was noted in the cases of Warkany and those reported by Fanconi and Botsztejn (17).

It is suggested by this author that as mercury essentially leads to denaturation of cellular protein and destruction of cells, adequate attention should be given to the possibility of sensitization to cellular protein liberated by mercury ingestion. That is, a state of autosensitization should be considered; just as recent research work on nephritis leads us to believe that the nephrotic syndrome is maintained by autosensitization to proteins arising from disintegration of the basement membrane of the glomerulus (Proceedings of the Society for the Study of Nephrosis, New York, December, 1951).

Warkany, possibly through a lack of clinical material, has been unable to demonstrate any association of allergic phenomena with pink disease. He gives an impressive comparison of the symptomatology with that of mercury poisoning. He fails to point out, however, that many phenomena which are characteristic of mercury poisoning, such as loss of teeth, gangrene of the mucous membranes, necrosis of jaw bones, and tremour, are rare in pink disease. On the other hand, several features of pink disease, such, for example, as hypertension, are not recognized symptoms of mercurial poisoning.

It is sometimes dangerous to compare diseases of known aetiology, such as mercury poisoning, with diseases of unknown origin. Although pink disease shows nearly all the features of mercury poisoning in its different phases, the same could be said of other agents. In particular, one might mention, for example, a disturbance of porphyrin metabolism. Indeed hypertension, tachycardia, sweating, abdominal pain, muscle weakness, photosensitivity, psychological change, oedema, cachexia, oliguria, peripheral neuritis with parathesia, albuminuria and constipation are all included as concomitant features (28). Metallic poisoning itself is one of several factors that can give rise to disturbances in porphyrin metabolism (29).

It is perhaps of interest that cursory attention to this fact showed a slightly raised coproporphyrin excretion in four children studied with pink disease as compared with 12 normal children (appended paper 4). From observing numerous samples of plasma taken from these cases, it became apparent to this author that a peculiar orange-brown tint was often revealed, which allowed one to distinguish it with the naked eye from samples taken from normal children. Spectrographic absorption tests revealed this to be due to an excess of oxyhaemoglobin in the plasma. Whether an intravascular haemolysis due to haemoconcentration or toxic factors occurs was not determined.

Fanconi (17) and his coworkers regard pink disease as a "neuro-allergic condition." Landolt and Egli (30), also of Switzerland, found allergic disorders in 32 per cent of former patients who had had pink disease. However, Hozel and James (20) found no evidence, in their cases,

of an allergic response to mercury.

These latter workers demonstrated a mild response to dimercaprol in 12 out of 15 infants, but a good response in only 3. They showed that in the district around Manchester, where pink disease is common, the administration of "teething powders" is less frequent than in the county of Warwick, where the disease is rare. Over 1500 interrogations were made in each district. In the 3 cases quoted that did respond to dimercaprol, the characteristic clinical picture of pink disease was not manifest. These cases also exhibited a rise in serum-cholesterol. This was regarded as evidence that chronic ingestion of mercury produces changes in the serum-cholesterol, probably due to renal damage, simultaneously with symptoms like those of pink disease. The finding of significantly low blood cholesterol levels has been the experience of this author (appended paper 4).

Interest in the role of mercury stimulated this author to investigate by animal experiment the effect of calomel on extracellular electrolyte equilibrium.

Five male rats were selected, weighing two hundred grams, and placed on an ordinary diet, but also given calomel. This was partially suspended with "bentonite" in their drinking water. The calomel produced an occasional loosely formed stool, but never diarrhoea. At the end of four weeks and six weeks respectively, the following serum determinations were made by lightly anaesthetizing the animals, and by drawing blood from the abdominal aorta.

Rat No.	Duration of Calomel Ingestion in Weeks	HCO_3 Milli-equiv. per litre	PH	Na Milli-equiv. per litre	K Milli-equiv. per litre	Cl Milli-equiv. per litre
1	4	20	7.37	143	4.0	101
2	4	21.5	7.44	140	4.2	103
3	4	21.8	7.49	141	3.9	101
4	6	14.7	7.21	139	3.8	112
5	6	15.2	7.26	140	4.0	110

It is apparent that the two animals so treated for six weeks developed an acidosis, doubtless due to the usual mercurial action of increasing the sodium excretion, relative to chloride.

If mercury is important in the aetiology of pink disease, and indeed the evidence is strong, we can in no way assume that this disease is due to clearly defined poisoning. The role of mercury is probably only one factor which disturbs the equilibrium of either enzymal or hormonal systems, and possibly penetrates the natural defense mechanisms against allergic reactions.

THE AUTONOMIC NERVOUS SYSTEM

Feer (31) was the first to emphasize that a possible overaction of the sympathetic nervous system existed in pink disease, and many others have supported this view that the hypertension, tachycardia, sweating and occasional hyperglycaemia provided evidence of such a disturbance. Stolz (32), and also Albertini (33) claimed to have found hyperplasia of the chromaffin system. Blackfan and McKhann (37) noted that the pulse rates of patients with acrodynia differed from those of normal subjects in that they did not vary with crying or during sleep. Day et alii (34) measured the electrical impulse of the heart beat with a special recording device. They noticed the effect on the heart rate produced by changes in emotion and following the injection of the drugs atropine, adrenaline, ergotamine tartrate, and mecholyl. These authors reached the conclusion that emotional disturbances failed to evoke certain normal responses from the vegetative nervous system in pink disease. They thought that this failure of the sympathetico-adrenal response to emotion could well arise from the fact that the sympathetic nervous system in a patient with acrodynia might already be overstimulated to such an extent that it could no longer react to further stimulation. It was suggested that a possible similarity existed between children with infantile acrodynia and subjects with pheochromocytoma, although an alternative view was also taken that the hypertension and tachycardia possibly resulted from widespread vasoconstriction due to some obscure toxin.

Glanzmann (22) also suggested that the adrenal medulla produced an increased secretion of adrenaline in pink disease. He investigated

this by noting the changes in the iris of an enucleated frog's eye which had been placed in Ringer's solution, to which had been added plasma samples from patients with pink disease. These plasma samples were found to have a strong mydriatic effect detectable even in a dilution of one in a million. No effect was found with plasma samples from normal children. Glanzmann suggested that a raised blood adrenaline level was responsible for these effects.

The sympathetic branch of the autonomic nervous system has received the most comment. It has never been shown, however, how hypertension, tachycardia, and hyperglycaemia which are features of sympathetic nervous stimulation can be reconciled with salivation, rhinorrhoea, muscle weakness and sweating, which are all indicative of stimulation of the parasympathetic system.

It is of interest that newer knowledge concerning the behavior of certain poisons (the organic phosphates) offers one possible explanation of the above phenomenon. The destruction of serum cholin-esterase can lead to both strong parasympathetic stimulation as a result of the accumulation of acetyl choline (muscle paralysis, areflexia and hypersecretion); and at the same time the adrenal medulla is stimulated with the liberation of adrenaline. The cholinergic, preganglionic fibers of the splanchnic nerves are stimulated by acetyl choline. These fibers supply the adrenal medulla. Hence hypertension, tachycardia, and hyperglycaemia can be concomitant features. Such a combination of effects is seen in poisoning with parathion (p. nitrophenyl diethyl thiono-phosphate) or "nerve gas", which is extremely lethal in small amounts (35). Thus attention is again

directed to a toxic agent.

It has been shown by this author that there is no gross or remarkable rise in the blood adrenaline in pink disease. Eighteen cases were investigated along with 24 normal children (appended paper 4).

CARBOHYDRATE METABOLISM

It has not been the experience of this author to find high glucose tolerance curves as described by other workers (36) (22) (37). Nor have the blood sugar levels, fasting or non-fasting, been above normal limits. Published and unpublished glucose absorption curves have shown, in some cases, a flattened contour. Such alterations in the behavior of glucose absorption could find explanation in the work of Clark and McKay (38). These workers demonstrated alterations in carbohydrate metabolism in the presence of electrolyte disturbances and reductions of blood volume. (Vide glucose absorption curves appended paper 2.)

THE PERIPHERAL VASCULAR SYSTEM

The extremities are painful with subjective parathesia, and show coldness, oedema, erythema and sweating with cyanosis during cold atmospheric temperatures. The circulation through the extremities is retarded. Shedding of the skin occurs and sometimes, but infrequently, ulceration leads on to gangrene. Skin color is dependent on capillary filling, and examination of the nail bed by direct microscopy has revealed to this author a capillary bed which is open and apparently engorged with red blood corpuscles.

That the permeability of the capillary walls is altered is evidenced by the oedema present in the hands and feet. Indeed this phenomenon was clearly shown in the face and scalp of one infant observed (appended paper 2).

The systolic and diastolic pressure is raised. There is tachycardia and a slow circulation through the extremities, so that it is reasonable to presume that there is a widespread arteriolar spasm in this condition.

The extremities manifest the "susceptible state of Lewis", as pointed out previously (appended paper 1). This state is known to be related to the effects of histamine and anoxia. It is of interest that resistance to histamine is lowered by an upset of the electrolyte balance, analogous to those induced by excision of the adrenal glands (39) (40). Such resistance is restored by the administration of desoxycorticosterone acetate (41).

THE IMPORTANCE OF WATER AND ELECTROLYTE METABOLISM IN PINK DISEASE

Braithwaite (42) of Leicester, in an endeavour to evaluate the relationship of this disease to light and vitamin D was led to determine blood calcium, phosphorus and haemoglobin levels. He found that some of his cases revealed slightly elevated calcium levels, and that the haemoglobin was consistently raised. The latter observation he interpreted in terms of water loss due to sweating.

Because preliminary investigations by this author (appended paper 1) into the blood picture of 10 cases of pink disease (aged 6 months to 2 years) revealed a mean erythrocyte count of 5,400,000 cells, and a mean white cell count of 13,500 cells, careful haematocrit, haemoglobin and plasma protein estimations were undertaken in a further series of 23 cases (appended papers 2 and 4). That haemoconcentration is a consistent, significant, and an appreciable finding is evidenced by the values obtained.

23 Cases

mean haematocrit	45%
mean haemoglobin	14.6 grams %
mean plasma protein	7.3 grams %

These values exceed those for normal children of the same age. Some individual cases may show haematocrit readings as high as 55%, a haemoglobin of 17 grams % and a plasma protein above 8 grams % (venous blood).

It is little wonder, then, that earlier clinicians such as Van Bogaert (43), Bruckner (44) and Rocaz (21) mentioned polycythaemia of 6 to 9 million cells; but perhaps it is a little surprising that a

persistently raised white cell count was interpreted in terms of leucocytosis, and causative infection. The absence of a febrile state, of neurological sequellae, the non-contagious nature, and the sharply demarkated age grouping, might have aroused suspicion. Attention to the differential white count reveals no significant shift in Arneth count (appended paper 1), and modern methods have been significantly unsuccessful in the detection of infective agents (45). The evidence, in the absence of starvation, for consistent, definite, and convincing neuropathological changes at autopsy, is lacking (46)(47). Cerebral congestion and oedema is present.(48). Examination of pathological material has also led this author to the same conclusions. Thorough microscopical investigation of the spinal cord and nerves revealed no significant changes in 2 cases (appended paper 1).

Anaemia and hypoproteinaemia is expected along with the associated anorexia. A rise of the reticulocyte count may alone reveal this latent anaemia. The estimated haemoconcentration takes no account of anaemia, so, if anything, such simple methods underestimate, to some extent, the loss of water from the blood volume. More detailed methods, as yet not undertaken, may show an appreciable deficit of extracellular space; and it may not be so surprising that shock collapse and death sometimes supervene (vidi appended paper 7).

Although these particular infants and children are dehydrated, we do not find thirst as a prominent symptom; nor is thirst by any means commensurate with the amount of fluid lost through the kidney in the earlier stages, or through the skin in the established condition.

This observation is also emphasized by Craig (49). Anorexia, muscle pains, loss of weight, weakness and oliguria, are prominent symptoms of the usual clinical picture. In older children, the region around the eyes is often sunken with darkened shadows. Collapse and shock are a potentiality. These are all features of hypotonic dehydration.

In the early stages of this condition, diuresis is characteristic (49a), while in the established case, oliguria is the usual finding. Rocaz (21) made the remark, "the urine is usually scanty, dark in colour, of low specific gravity and charged with urea and salts." It has been the experience of this author (1 a), and that of others (37) (50), to find albumen in the urine on several occasions. Indeed, in one case observed by this author, haematuria, albuminuria, and casts were found in the absence of infection, and apparent from the outset of the disease.

In the series of older children reported (appended paper 4), suffering from pink disease over a period of several months, 48 hour urine collections were carried out. Taking 6 cases of about the same age (2 1/4 to 3 years), or cases 2 to 6 inclusive, and case 8 of this series, it is seen that the mean urinary 24 hour volume is 270 cc. or 21 cc. per kilogram per day (mean weight equals 13 kilograms). It was known by this author that the fluid intake was close to 2 pints (or approximately 1000 cc. per day). This figure is close to the requirement necessary for life itself under normal circumstances, and was barely supplemented by the food intake, which was very diminished. The chloride loss through the urine alone represents 2.4 milli-equivalents per kilogram per day, or 31 milli-

equivalents per child per day. It is known that under normal circumstances the loss of chloride from the body as a whole is covered by an intake of only 1.5 to 2 milli-equivalents per kilogram per day* ( ). Doubtless additional loss of chloride through the sweat over a prolonged period leads as well, in these children under discussion, to a deficit of total body chloride.

Although direct investigation of the sweat was not undertaken, it is clear from the above that some 70% or more of fluid intake of these children was being lost as "insensible water loss." (Actually, it should under normal conditions account for no more than about 40%.)* We know that alteration of acid base balance does not appear to modify the composition of the sweat, which is in contrast to the urinary pattern. Evidence is suggestive that alteration of cell tonicity appears to effect the rate of secretion of sweat, but not its composition (51) (52) (53) (54). In the presence of hypotonicity of the extracellular fluid the rate of secretion of sweat is increased (53) (54). After the administration of salt there is a diminution of sweating, and the loss of sodium, potassium, and chloride is reduced (54). The secretion of sweat is important in the process of heat exchange, but apparently it does not play a major role in maintaining homeostasis. It seems that in adrenal insufficiency the loss of sodium and of chloride through the sweat are slightly increased (55). These electrolytes are decreased in the sweat content in salt depletion (56) (57). This latter phenomenon, however, depends on the prior development of a negative salt balance, rather than

*Darrow, D. C. and Cooke, R. E. (Personal communication)

on adaptation to prevent its onset (57) (58).

There is little or no information in the paediatric literature pertaining to the physiological process of sweating in children, except for the important work done by Cooke, Pratt and Darrow (59). For losses of insensible water of the order which has been referred to, these workers showed a loss of 11.3 and 8.8 milli-equivalents of chloride per litre of sweat in 14 and 16 month old infants. The concentration of electrolytes in the sweat increases as age progresses. It is much higher in adults (1 to 2 milli-equivalents of chloride is lost per kilogram of body weight per day). At temperatures lower than 84° F., one-fourth of the calorie production is dissipated by evaporation of water. When the temperature rises above 84° F., sweating occurs. Above 93° F., all heat loss is by sweating*. The occurrence of noticeable sweating during the Australian summer is therefore not surprising. In winter sweating must lead to a drop in body temperature or to a rise in metabolism.

Enough has been said to indicate that the fluid loss through the sweat in pink disease is considerable, and the electrolyte losses require study. In Australia the importance of a hot and dry climate cannot be neglected, during the course of pink disease, for sweating is more profuse and prolonged than in any other paediatric condition.

Investigation of the plasma electrolytes in the series of 23 cases previously referred to (appended papers 2 and 4), exhibited low plasma sodium and chloride levels. The mean value for the plasma sodium level (124 milli-equivalents per litre) shows a deficit of 9 milli-equivalents per litre below the lower limit of normal (133 milli-equivalents per litre). It was unfortu-

* Darrow, D. C. Personal Communication

nate that the method used had not previously been adapted to plasma sodium estimations. (This method involved ashing of the plasma and determination by emission spectrophotometry. The measurement of the intensity of the sodium band on a photographic plate was carried out with the aid of a photo-electric galvanometer.) Later work by Williams (60) and his co-workers in Melbourne, while demonstrating a significant deficit of serum chloride in 22 cases, and also haemoconcentration, revealed low serum sodium levels in only 2 cases. Their sodium method employed was by gravimetric analysis, using 0.2 cc. of serum. It has been the experience of other workers to find a small percentage of lowered serum sodium values in this disease (61); but the majority of values are normal (20) (60).

The rise in haematocrit, haemoglobin and plasma protein with normal plasma sodium concentration, or with low concentration, indicates a low plasma volume, and a loss of plasma chloride and sodium.

As a whole the loss of sodium and chloride in the extracellular fluid in many examples is the same order of magnitude as the loss of plasma volume. However, adequate data are not available to establish the magnitude of the deficit of sodium and chloride in the body as a whole in pink disease. But there is evidence that chloride deficiency may be considerable in some cases. This has also been suggested by therapeutic studies of the use of sodium chloride with and without desoxycorticosterone acetate (appended papers 2 and 4).

Southby of Melbourne (62), states that "in babies very sick with pink disease there has been a complete absence of hydrochloric acid from the gastric juice." He interprets this as evidence to support the concept that achlorhydria offers easy passage to a hypothetical virus, which

possibly causes pink disease. It has been my own observation that cases of pink disease subjected to gastric lavage are liable to collapse and die a few hours later. In the presence of the alarming complication of diarrhoea, hypotonic solutions of chloride and sodium demonstrate a rapidly deleterious effect on the infant with pink disease. Proper attention to electrolyte therapy may be of great value (see appended paper 2, cases 15 and 16).

THE ADRENAL GLAND

On the basis of this loss of plasma sodium and chloride, it was at first thought by this author that the condition of pink disease might be associated with a relative adrenal insufficiency of the so-called "salt and water corticosteroids." It was thought that such an insufficiency could possibly be occasioned by specific and non-specific "stress producing agents" (from emotional changes to mercury poisoning). The malady was thought to be prolonged by the actual stress of the disease itself, which is so apparent clinically.

Such a concept was to borrow directly from the work of Selye (63), and indeed almost all the biochemical changes and observable features of the alarm reaction are manifest by the condition of pink disease (appended paper 3).

The age incidence of this disease falls predominantly in that period of development when the adrenal gland undergoes remarkable changes, the reasons for which are as yet still obscure, and have again recently been convincingly demonstrated by the work of Takka (64). Conditions which are referable to a disturbance of the "salt and water hormone" have of late received much attention in the paediatric literature (vide appended paper 3). The concept of this author was strengthened by the fact that the first 16 cases treated by the oral administration of 6 to 8 grams of salt, with or without 2 to 4 milligrams of desoxycorticosterone acetate, gave very encouraging results clinically. Some of these cases in a matter of days showed striking and even dramatic improvement. Anorexia disappeared, sleep became undisturbed, and irritability gave place to a happy

disposition. Older children lost their lassitude and became noticeably energetic. It seemed that an illness of many months had been reduced to a matter of weeks. Such striking improvement with this therapy has since been reported by some other clinicians (65) (66).

As pointed out by Homer Smith (67), most of the physiological disturbances seen in adrenal insufficiency appear to be secondary to oligaemia, since they can be partly or wholely alleviated by the administration of sodium chloride. It is natural that oligaemia or chloride and sodium depletion, from any cause, produces a picture resembling adrenal insufficiency (68).

Later therapeutic investigations undertaken, using salt and steroid in a series of 70 cases, revealed that some showed little or no improvement: "the photophobia, pinkness of the hands, loss of weight and muscle weakness of the infant sometimes seem to be little, if at all mitigated." This evidence supported by hormonal investigation led to the conclusion that changes in electrolyte and water equilibrium found their origin outside of the adrenal gland, and it was suggested that attention be given to the kidney tubule (appended papers 4 and 5).

In view of the close resemblance between most aspects of the symptomatology of this disease and "adrenal stress" it was necessary to undertake hormonal determinations to further elucidate this aspect.

Investigation of 12 cases using the technique of Venning (69) revealed normal glucocorticoid excretion in the urine. The 17 ketosteroid determination was found to be slightly but significantly raised, and the reaction of the circulating eosinophile cells to adrenaline was

found to be normal in response. The estimation of the blood adrenaline content using the technique of Raab (70) as modified by Shaw (71) has already been referred to.

The possible relationship between pink disease and Selye's alarm reaction has been considered. The investigations of this author led Selye himself to discuss pink disease in his treatise on stress (72), and to include it as a disease of adaptation. Bearing all the available evidence in mind, it is difficult to believe that an alteration of the vegetative adrenal system is of more than secondary importance in this disease. That this mechanism is involved to some extent seems likely. The rise in 17 ketosteroid excretion is significant. The failure to demonstrate an elevation in urinary glucocorticoid levels is not disturbing. The work of Nichols and Miller (73) has shown that in some conditions of sweating the loss of glucocorticoid through the sweat may be significantly high, even though urinary excretion is normal. The conclusions of Bornstein and Trewella seem therefore somewhat premature (74). Indeed, as pointed out by Sayers (75), until we know more concerning tissue utilization of adrenal hormone from blood assay, the value of glucocorticoid excretion will remain of limited value. The presence of an electrolyte disturbance itself throws a definite load on the adrenal gland, and herein probably lies one mode of involvement of adrenocortical function.

THE POSSIBLE INTERPRETATIONS OF IONIC CHANGES

With salt and steroid treatment any small series of cases may show little alteration in the course of this disease (appended paper 4), but the spectacular improvement of some cases in the series of this author made it impossible to disregard these results as being purely fortuitous. In retrospect it would seem that any beneficial effect of sodium chloride and/or of desoxycortico-sterone to some cases, may rest in the fact that some of the symptoms of pink disease are referable to a chloride deficiency and to hypotonic dehydration. With expansion of blood volume and return of chloride to the body, clinical improvement is often manifest. However, this is not always so (vide below). Because controls were not carried out, the value of this therapy is still open to question.

Alteration of the electrolyte pattern and the above observed phenomena are further clarified by the data of Williams and his co-workers (60). Their data shows strikingly high serum bicarbonate values. Nineteen out of 22 cases were investigated using capillary blood. So high are many of their values (40 milli-equivalents or 90 volumes per cent) that individual values are even more significant than the presence of a mean value of 30 milli-equivalents per litre. Actually, these workers apparently calculated on the basis of 22 cases instead of 19, and they obtained a normal mean value. They surprisingly overlooked the significance of their own data. This was pointed out recently by this author (7a).

Neither vomiting nor diarrhoea are prominent symptoms to account

for this alkalosis. Nor is it likely that sweating per se could produce it. There are many who have championed the thesis that pink disease is due to a disturbance of the central nervous system and/or of the hypothalamus (21). We are aware of the condition of "cerebral salt wasting." More recently it has been shown that tuberculous meningitis can give rise to alterations of the plasma electrolytes, not unlike that which is present in the disease under discussion (76). As emphasized earlier, pathology has produced little to support this approach.

It is relevant, however, before passing on to discuss the significance of the elevated bicarbonate level, to consider the recent important information concerning "salt losing conditions."

Marritt and Fremont-Smith (77) showed that the usual low chloride values found in the spinal fluid in cases of meningitis, including those of tuberculous origin, can be ascribed to a decrease in plasma chloride and to a general hypo-osmolarity and hypoelectrolytaemia of the body fluids.

Such findings are familiar to us in adrenal disorders (appended paper 3), primary renal disturbance, lobar pneumonia, certain febrile conditions, and in congestive cardiac failure (78) (79).

Sunderman (80) demonstrated that in the earlier stages of pneumonia on a low intake of chloride the body is unable to conserve this ion, and conversely, if the patient is given excess chloride, there is a diminished capacity to excrete it.

Similarly, Winkler (81) showed that in pulmonary tuberculosis a comparable relationship held for chloride.

Rapaport et al (82) observed a reduction in serum chloride, a lesser fall in sodium and an increased bicarbonate level in children with peritonitis, and these authors noted that contrary to what they might have expected, a large intake of salt did not restore ionic equilibrium.

Rapaport and his co-workers (76) carried their studies further by investigating three patients with tuberculous meningitis. Again they clearly demonstrated that specific renal defects involving loss of chloride and sodium can result in the face of subnormal plasma chloride levels.

In their cases there was impairment of the ability of the kidney either to clear or to conserve chloride in a normal manner. The normal response of a disappearance of chloride from the urine during hypochloraemia was absent. In these cases an elevation of plasma bicarbonate was significant, as well as the reduced levels of serum chloride and sodium. In one of the three cases a very low serum potassium level was also apparent, and these authors being aware of the work of Darrow (83) (84), realized that "a decrease of the intracellular potassium concentration is an unavoidable correlate of the reduction of the concentration of the extracellular ions."

The presence of significant amounts of chloride in the urine, in the presence of hypochloraemia, has been noted by this author in cases of pink disease (appended papers2 and 4). To quote but one case, we can consider that of G. C., aet. 2 2/3 years (appended paper 2). The weight of this child equalled 10 kilograms. The blood chloride was 425 milli-

grams per cent (normal equals 450-500 milligrams per cent). Haematocrit equalled 48 per cent and plasma protein 8.1 grams per cent. The 24 hour urine volume was 325 cc., and contained 2 grams of chloride. She therefore excreted 5.6 milli-equivalents of chloride per kilogram per day. This value is remarkably high (as mentioned in other cases cited previously) and was at a time when fluid intake was minimal.

The electrolyte disturbance in pink disease is most likely related to an alteration of the renal regulation of acid base equilibrium augmented by electrolyte losses in the sweat.

Firstly one should consider that anorexia being marked the intake of potassium must be very low. In animals low intake of potassium combined with high intake of sodium chloride can produce marked alkalosis, and this deficit of potassium leads to a loss of chloride (83) (84). Secondly, and this would seem even more relevant, deficit of chloride can lead to a loss of cellular potassium (83) (84). Cellular potassium deficiency is not necessarily characterized by a fall in serum potassium. Some of the serum values in pink disease are low (3 milli-equivalents per litre) (60).

Darrow was able to demonstrate by animal experiment that in alkalosis produced by depletion of chloride, the serum potassium concentration is low or normal. Muscle potassium is low, and intracellular sodium is high. The loss of intracellular potassium occurred despite abundant potassium in the diet. It was suggested therefore that during clinical alkalosis there was an intracellular loss of potassium and an increase in cellular sodium in the presence of normal renal function (84).

Later work involving induced deficits of chloride, sodium, and potassium ions, respectively, showed a high degree of correlation between the concentration of bicarbonate in the serum, muscle potassium and intracellular sodium. Serum bicarbonate varied directly with intracellular sodium and inversely with muscle potassium. Muscle potassium and intracellular sodium showed inverse relationships. It was considered that the existing ionic relations could be regarded as a biological equilibrium which is attained when renal adjustment is made in the presence of a deficit of sodium, of chloride, or of potassium.

More recent work in which this author has been concerned (8a) (see appended paper 6) more adequately defines these relationships and reveals important scientific facts. This particular study of tissue and renal adjustment to single ion deficiencies has produced considerable data and information previously not anticipated. A large series of rats were rendered potassium deficient, and alkalotic, through the use of a low potassium diet, and balanced salt instead of drinking water. Injections of desoxycorticosterone acetate were also given to increase potassium loss. These animals were then divided into two groups. The first group (K), was given potassium chloride, 3 millimols per kilogram, twice a day, by injection for one week. Group two (Na) received 3 millimols of chloride (as sodium chloride) in the same way and dosage. Urine, blood and tissue analyses were undertaken before and during this therapy. The group (Na) animals demonstrated some renal compensation for the alkalosis by excreting urine of a high pH and low titratable acidity. The group (K) animals excreted urine of low pH, high titratable acidity and a high ammonia content.

* Solution containing 150 milli-equivalents of sodium, 120 milli-equivalents of chloride and 50 milli-equivalents of acetate plus bicarbonate.

The urinary bicarbonate values closely followed the pH in both groups. Despite the absence of a renal compensatory mechanism in the group (K) animals, at the end of one week the extracellular alkalosis was corrected, and muscle composition was normal. On the other hand, although in the group (Na) animals there was some renal compensation for alkalosis, at the end of one week it was found, by serum analysis, to be aggravated, and muscle composition was shown to be grossly abnormal with respect to intracellular electrolytes.

Obviously, then, in alkalosis and potassium deficiency the administration of potassium leads to correction of the ionic changes by exchange of ions between the extra- and intracellular phase. This is an extra-renal occurrence. During potassium deficiency and alkalosis sodium replaces potassium inside the cells, but there is not complete reciprocity between these cations, only about 2/3 of the potassium being replaced by sodium. If, then, administration of potassium can bring about ionic equilibrium, it is clear that unless there is some radical change of ionic structure within the cell itself, there is approximately 1/3 of the lost potassium cations to be accounted for inside the cell during this alkalosis. Thus, the evidence is extremely suggestive that hydrogen ions as well as sodium ions enter the cell to replace potassium; and during correction of the induced alkalosis, hydrogen ion moves out of the cell along with sodium. Thus, this study has shown again the importance of giving potassium in clinical alkalosis. It also reveals the way in which potassium can restore ionic equilibrium.

It has been known clinically that sodium chloride is of benefit in many cases of alkalosis. It is also known that this is not so under all

circumstances (i.e. when cellular potassium deficits are present). It is not suggested here that potassium chloride alone may always be effective in reducing alkalosis clinically. The truth of the matter seems to be that a combination of ions is the rational procedure clinically.

With respect to pink disease, the above research is important, for the data of Williams and his co-workers (60) draws attention to metabolic alkalosis of a significant duration. Their data offers a definite challenge for further elucidation of the behavior of the electrolytes, sodium, potassium and chloride. Provided their determinations are accurate, they confirm beyond doubt the presence of an electrolyte disturbance and reveal an acid-base imbalance (Darrow, personal communication).

It should be pointed out that if cellular potassium depletion exists in pink disease, this could well explain the variability of therapeutic results observed after giving sodium chloride.

It is of interest to refer back 30 years ago to the early paper of Brown, Courtney and MacLachlan (6), which contains the only complete and single metabolic studies in the literature). Metabolic investigations on a 3 year old girl, receiving a reasonable caloric intake of 75 calories per kilogram, showed a negative balance for potassium, (1.104 milli-equivalents per day) for sodium, (0.135 milli-equivalents per day) and for nitrogen (0.158 grams per day). Ninety-eight per cent of the sodium and nitrogen loss occurred through the urine, and 94 per cent of the potassium loss. Sweating was neglected. When one considers this, and also the duration of the disease, these values may well be significantly large

over a long period.

Attention has recently been directed to alkalosis resulting from mercurial diuretics (85). Here chloride is said to be excreted in excess of sodium. In view of the work of Warkany (15), this aspect also obviously requires elucidation.

Clearly, our knowledge of the altered electrolyte pattern in pink disease is as yet incomplete, but obvious clues are now before us. The magnitude of chloride, sodium and potassium loss must be determined, the role of mercury clarified, and the nature of the disturbance of renal function and acid base imbalance defined in terms of modern concepts.

Restoration of homeostasis by appropriate electrolyte therapy in states of prolonged ionic disturbance, such as the interesting and contrasting condition of "renal acidosis" (86), can go far toward neutralizing the undesirable effects of disease.

SUMMARY

In conclusion, this work has revealed the following:

1. In pink disease there is a depletion of chloride and sodium ions in the vascular space; neither the chloride nor the sodium ions rise concommitantly with the existing haemo-concentration.

2. There is some contraction of the blood volume and some loss of water from the blood stream. We are apparently dealing with extra-cellular hypotonic dehydration.

3. There is a significant and surprising amount of water lost as "insensible water loss." There is probably appreciable loss of electrolyte through the sweat glands.

4. There is evidence that the kidneys are excreting significantly high amounts of chloride - more than can be ascribed to intake.

5. The existence of alkalosis has been pointed out by this author, and the possibility of cell potassium deficit as a sequal of chloride depletion is presented. The use of sodium chloride therapeutically may be of value in some cases. The question is unsettled.

6. The role of the adrenal gland (cortex and medulla) has been evaluated from all aspects; and also a comprehensive analysis of the literature relating to known disturbances of the adrenal gland in childhood has been presented. Attention to the effect of stress has been originally described and discussed.

7. Evidence is suggestive of an alteration of blood pigment metabolism in this disease. This is suggested by the presence of free oxy-haemoglobin in the plasma, and a slightly raised coprophoryrin excretion. The state of the capillaries has been defined by direct microscopy, and reference to the vascular system has been made in terms of the available evidence.

8. Certain clinical observations have been made with respect to cases of older children, which have not aforeto been realized.

9. The above electrolyte changes throw some light on the understanding of this disease, and there is reason to believe that further attention to electrolyte disturbance and deficits of chloride, sodium, and potassium, will lead to further and more clearly defined therapeutic aids.

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PINK DISEASE: AN INVESTIGATION OF ITS CAUSE AND TREATMENT.

(Preliminary Report.)

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PINK DISEASE OR INFANTILE ACRODYNIA: ITS NATURE, PREVENTION AND CURE.

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SYDNEY
AUSTRALASIAN MEDICAL PUBLISHING COMPANY LIMITED
Seamer and Arundel Streets, Glebe, N.S.W.
1950

Cheek, D. B. with technical assistance from Stace, H. C. (1950). Pink disease: an investigation of its cause and treatment. (Preliminary report). *The Medical Journal of Australia*, *1*(4), 101-107.

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THE IMPORTANCE OF THE SUPRARENAL GLAND IN DISEASES OF INFANCY AND CHILDHOOD WITH PARTICULAR REFERENCE TO PINK DISEASE.*

By DONALD B. CHEEK

THE ADRENALS OF THE FOETUS.

The development of the suprarenal gland is interesting as a study in evolution and emphasizes the change in anatomy that follows in response to changing environmental demands. In fishes the cortex and medulla are separate. At the amphibian stage the two structures are approximate; in birds they become intermingled, and in mammals they are one. As the constant salt and water environment of the fish has been by the process of evolution exchanged in man for a constant "milieu interieur," it is reasonable to suppose that this important organ of vegetative regulation grows and develops in a manner to meet the new requirements.

Whether or not the foetus is furnished with an active endocrine system was a controversial subject for a considerable time. Recent evidence has definitely established the presence of an actively-functioning system—certainly in the case of the adrenal glands. The exact stage at which secretory activity begins is not known. It is known that the adrenal medulla is very late in development both anatomically and functionally. There is little medullary tissue during the early stages of foetal life; the pheochrome cells which are grouped around the veins are poorly differentiated and adrenalin secretion is not likely to be important. The cells of congenital tumours of this structure are of the most primitive type (sympathoblastoma).

On the other hand the adrenal cortex displays a different state of affairs, for during the last third of foetal life there is an excessive growth of this structure which strongly suggests important functional demands. On examination the cortex of the foetus shows a narrow peripheral zone which represents the permanent cortex, and a large thick central layer which rapidly undergoes involution immediately after birth. It is noteworthy that this involution occurs in all infants excepting those with congenital heart defects which lead to inadequate oxygenation of the blood. It would seem that the foetal cortex is functionally related to the oxygen transport and exchange in circumstances of low oxygen pressure and special foetal haemoglobin (C. Stanton Hicks, personal communication).

Further evidence that the foetal cortex must be physiologically active is furnished by the fact that patients with Addison's disease and rheumatoid arthritis both improve during pregnancy. Admittedly in the latter condition it may well be that the maternal adrenal gland is functioning more normally. In both cases following parturition, an exacerbation occurs, and in the case of Addison's disease there is always the potential danger of a fatal crisis.

Very little is known about the relationship of the foetal cortex to salt and water balance. We know that the salt and water content of the foetus is high and that following involution at birth there is a progressive loss of water and salt from the infant. Whether there is a relationship between these two phenomena we can but guess. There is also the significant fact that in anencephaly we find a prenatal involution of the foetal cortex. In man alone during the second half of foetal life a characteristic convolution of the cortex of the gland occurs which greatly increases the surface area. This fact in itself emphasizes the great importance of this tissue to the body. There is a striking parallelism between cerebral and suprarenal development, seen not only in the convolution which occurs in both tissues, but in the fact that they are both so rich in lipoids. The fact that in anencephaly there is a marked concomitant hypoplasia of the foetal cortex may indicate that this structure is of great importance in the development of cells, particularly those of the brain (Bourne, 1934).

It is noteworthy that infants born of mothers with an eclampsia should have an enlargement of the adrenal cortex (Parkes, 1941).

The endocrine system is an integrated one and the body functions as a total organism. We know, for instance, that the thyroid is antagonistic in its action to the adrenal gland,

* A portion of a thesis which was submitted with appended papers to the University of Adelaide for the degree of Doctor of Medicine, August, 1950. Under the circumstances, the references and bibliography are printed as given by Dr. Cheek, without corresponding reference numbers in the text.

hence we find that in conditions of aplasia of the thyroid in the newborn there is a hyperplasia of the adrenal cortex.

THE ADRENAL CORTEX OF THE INFANT.

At and after birth the greatly enlarged suprarenal cortex undergoes dramatic and sudden degenerative changes. This hyperaemic involution of the innermost layer of the cortex extends during the second month outwards into the cortical cells in which degenerative changes continue to take place (Elliot and Armour (1911), Kern (1911), Thomas (1911)).

From the second month to the end of the first year of life fatty and colloid degeneration progresses throughout all layers of the cortex except in the outermost thin layer. With the process of reabsorption fibrous tissue is laid down and comes to surround the primitive medulla to form a capsule. During the second year of life regeneration of both cortex and medulla begins and the fibrous tissue capsule surrounding the medulla begins to be invaded by the now rapidly-developing phæochrome tissue until it comes into contact with the outer adrenal cortical tissue. Full development of the cortex is reached at the eighth year of life (Husnot, 1908, cited by Goldzieher) and the medulla reaches full growth at adolescence (Lewis and Pappenheimer (1916)).

The facts that regeneration definitely begins at the second year and that full cortical development is reached at the eighth year, are significant anatomical facts which correlate with the clinical observations under review (*vide* Cheek and Hicks, 1950).

ADDISON'S DISEASE.

Addison's disease is uncommon in childhood. Jaudon (1946) has given an impressive review of 100 cases of this disease reported since the year 1856.

He described the case of a 3-year-old girl who suddenly became ill at 4½ months of age with a high fever, cough, and a generalized maculopapular rash. These symptoms subsided, but two weeks later she developed anorexia, became listless and intermittently drowsy, began to vomit once or twice a day, and developed diarrhoea with marked loss of weight. Her fair skin became dark and at 6½ months, when she was admitted to hospital, it was found that the serum carbon dioxide-combining power was 42 volumes per cent and the non-protein nitrogen was 80 milligrammes per cent. Parenteral therapy gave dramatic relief, only to be followed by a relapse on cessation of treatment, with the reappearance of dehydration, anorexia, and listlessness. The Wassermann reaction was negative, but the intradermal tuberculin test was strongly positive. It was found that a servant living in the same house as the child had pulmonary tuberculosis. The diagnosis of Addison's disease was considered in the child and 2 milligrammes of desoxycorticosterone acetate was given daily. After 20 days of treatment the child was normal in all respects. The non-protein nitrogen had fallen from 64 to 40 milligrammes per cent, the serum chloride had risen from 520 to 630 milligrammes per cent, the sodium from 300 to 350 milligrammes per cent, and the potassium had fallen from 28 to 21 milligrammes per cent. This child remained well with injections and the case was studied for two years. The skin pigmentation increased to almost negro black.

Jaudon, as stated previously, reviewed 100 cases of Addison's disease in childhood from the year 1856. He classified 62 as proved, 33 as probable, and 15 as doubtful. He found that the incidence was twice as common in males than females (which is similar to the distribution in adults), that 85 per cent manifested gastro-intestinal symptoms, and that 87 per cent were of tuberculous origin.

SOMATO-SEXUAL DISTURBANCES IN INFANTS AND CHILDREN.

The clinical manifestations of an oversecretion of the androgen factor of the adrenal cortex due to tumour or hyperplasia are more than twice as frequent in the female as in the male child.

Most of the somato-sexual abnormalities result from a disturbance or dysfunction of the adrenal cortex. The clinical picture induced by sexual cortical hyperfunction depends on the age and sex of the patient, and on whether the excess is androgenic or oestrogenic, since the adrenal cortex develops from the gonadoplasmic mesoderm which is capable of elaborating both androgens and oestrogens. Androgenic hyperfunction in childhood tends to accelerate the onset of puberty, maleness being superimposed on genetic females and accentuated in genetic males. Oestrogen hyperfunction is very rare and presents an opposite clinical picture.

Cortical hyperfunction may be self-limited or indefinitely prolonged and may or may not be associated with hyperplasia, or neoplasia, benign or malignant.

In cases of somato-sexual abnormalities a considerable number show no macroscopic enlargement of the adrenal gland, even though clinical

and laboratory tests indicate hyperactivity. In other cases benign hyperplasia or neoplasia may be present.

Melicow and Cahill (1950) concluded from their recent work on somato-sexual abnormalities that the true hermaphrodite is probably a mutant, and may be the result of a genetic error and possess gonads of both sexes. The so-called "pseudohermaphrodite," on the other hand, is generally one sex but somatically resembles the opposite sex. Experimental evidence suggests that this may be the result of an ante-natal endocrine disturbance (Green and Burill, 1940).

Melicow and Cahill have concluded that somato-sexual aberrations caused by excess androgen are usually of adrenal origin.

Butler, Ross, and Talbot (1939) presented an interesting case of a 20-months-old baby boy who, from the age of 2 weeks, had developed macrogenitosomia with brownish pigmentation of the skin, and a tendency to sudden collapse on restriction of fluids or on withholding additional salt from the diet. During these periods of collapse the patient would become dehydrated, manifesting a lowering of the sodium and an elevation of the potassium in the serum. Excessive amounts of oestrogen and androgen were excreted in the urine. The child was maintained in a fairly good nutritional balance by the addition to the diet of 2 or 3 grammes of salt and 1 to 2 grammes of sodium bicarbonate daily. This was the first and is the youngest reported case of macrogenitosomia associated with hyperplasia of the androgenic tissue and with typical signs and symptoms of adrenal insufficiency.

Wilkins, Fleischmann, and Howard (1940) presented a similar case. Owing to poor appetite this child was difficult to feed, but he did have a marked craving for salt, refusing to eat any but highly savoury foods. The penis and scrotum were enlarged from birth, and at 15 months of age pubic hair began to grow. At 3½ years the boy was defective mentally. The skin was brown in colour, the genitalia were enlarged, and there was a fasting non-protein nitrogen blood level of 99 milligrammes per cent. The haemoglobin was raised to 16.3 grammes per cent, and renal function was satisfactory. The blood pressure was 95/45, and the serum sodium was 255 milligrammes per cent. Shortly after this latter estimation the child had a convulsion and died. At post-mortem the adrenal cortex was found to be composed largely of androgenic or prenatal zone cells, which apparently replaced other elements of the cortex. The authors concluded that this was the first case to be presented in which there was substantial proof that death from adrenal insufficiency resulted from the destruction of the electrolyte-controlling elements of the adrenals by hyperplasia of the androgenic zone. They also concluded that this case lent support to the theory that the androgenic zone had a different function from that portion of the cortex which controls the electrolyte balance.

Gallais (1912) reported a girl with the adrenogenital syndrome who showed muscle weakness and pigmentation; and Priesel (1931) mentions "strong pigmentation of the skin" in the case of a 5-year-old girl with hermaphroditism.

Hoag (1923), in a review of 22 cases of malignant hypernephroma in children with virilizing effects, reported that "a few showed distinct pigmentation of the skin"; and Grollmann (1936) stated that "certain other clinical symptoms described as associated with adrenal virilism are nausea, vomiting, weakness, and rarely pigmentations of the forehead and border of the axillae. These are symptoms of Addison's disease and are due to the encroachment of the malignant androgenic zone on the true cortical tissue."

Thelander and Chofflin (1941) presented a further case which at the age of 8 days developed diarrhoea, vomiting and loss of weight. The child was given salt and water solution by mouth, a blood transfusion, and parenteral fluids. At 1 month of age a sudden collapse occurred and the blood sugar fell to 17 milligrammes per cent. The patient was restored by desoxycorticosterone; these injections were continued and the child remained well. The penis was large at birth, but at 6 months it became extremely enlarged, and pubic hair developed, together with a low deep voice.

Dijkhuisen and Behr (1940) reported a 2-weeks-old boy with pseudohermaphroditism, who became fatally ill with vomiting, diarrhoea, and dehydration. The condition was thought to be intestinal obstruction, and no post mortem was obtained. Some time later the brother of the child was admitted to hospital, with the same clinical history. The same diagnosis was again made and again death followed the same sequence of events. At post-mortem, both adrenals were found to be very enlarged and convoluted, their weight being 22 grammes. Later these authors presented a third case of a few-days-old infant with fatal diarrhoea and vomiting. The adrenal glands at autopsy together weighed 24 grammes and the thymus was enlarged. A fourth case reported by

Dijkhuisen and Behr was that of a 5-weeks-old girl suffering from diarrhoea and vomiting, who became excessively emaciated and finally died at 3½ months of age. The only distinctive features at autopsy were very large hyperplastic adrenal glands weighing together 34 grammes. These four cases were reported as deaths secondary to hyperfunction of the adrenal cortex, but it is considered by other authors that these cases represent instances of severe cortical insufficiency due to hyperplasia of the androgenic zone. Unfortunately, no blood chemical estimations were made.

Congenital insufficiency of the adrenal cortex associated with virilism has been recently discussed by Darrow (1944). He reviewed the work of others which in his opinion pointed to the fact that the clinical picture of increased androgenic activity with or without adrenocortical insufficiency was associated with persistence of the foetal (androgenic) cells of the adrenal cortex. He presented a case which he believed fell into this category. The patient was a male child who weighed 3,100 grammes at birth, and from the start displayed no appetite. By the eleventh day he began to vomit, became dehydrated, and collapsed. In spite of repeated clyces, rehydration could not be established, and the child remained emaciated and very sick for several weeks. The tissues were limp and the skin hung in loose folds. The loss of weight was particularly obvious in the subcutaneous fat over the buttocks and thighs. The red blood count was 6,500,000/c.mm. and the haemoglobin 19 grammes per cent. The 17-ketosteroid excretion was 2.5 milligrammes. If clyces were stopped the child vomited, became weak, and anorexia developed, with rapid respirations and cyanosis.

Because of the low serum sodium and chloride values and because of the high potassium level, adreno-cortical insufficiency was diagnosed. Adreno-cortical extract (Eschatin), 4 cc. four-hourly, were given, but it was found that clyces were still necessary to maintain hydration. No significant change occurred in the plasma electrolytes.

Clinical improvement was obtained when 3 grammes of salt were given daily with the food; later desoxycorticosterone acetate was given, and finally an implant made. It was noticed that the penis of the child had become large and growth of hair on the sides of the face, although fine, was more plentiful than usual at six months of age. The face of the child was always a deeper red than that of other babies. He sweated a great deal and was a poor sleeper, often being restless.

By deliberately withholding treatment several Addisonian crises were precipitated. Of particular interest in this case were the electrocardiographic tracings which showed full and sharp T_2 waves, a finding that is also seen in adrenalectomized dogs with a potassium level equal to 10 milliequivalents per litre (Winkler *et al.*, 1939).

This patient, a boy, showed no signs of sexual disturbance at birth, and this is usual—signs of sexual precocity in the male infant do not as a rule develop until later. This is in contrast to the disease in females, in whom pseudo-hermaphroditism generally gives an early clue to some disturbance of adrenal function and, if combined with signs of adrenal insufficiency also, makes the diagnosis of affected girls obvious. The signs of hypertrichosis, and so on, in the female hermaphrodite will then develop later. Darrow drew attention to the puzzling fact that in his case there was little hypoglycaemia, a fact which emphasized his claim that this type of adrenal insufficiency was of quite a different nature from that due to the absence of cortical cells. He was "tempted to surmise that owing perhaps to overproduction of androgens normal cortical compounds are not handled in the usual fashion and perhaps never reach the kidneys in effective amounts."

At a conference of the New York Hospital-Cornell Medical Centre in January, 1949, another case of adreno-cortical insufficiency associated with virilism was presented by Tepper (1949). Similar clinical and bio-chemical findings were reported. This male infant had a malnourished and dehydrated appearance, with rapid respiration, irritability, vomiting, cyanosis, and a disappearance of the subcutaneous layers of fat which had set in shortly after birth. Intravenous fluids were necessary to maintain hydration. The penis of the child was slightly enlarged, and the red blood count was 6,400,000/c.mm. and the haemoglobin value 22 grammes per cent. The serum sodium was low (130 milliequivalents per litre) and the serum potassium was raised. With added dietary salt (5 grammes) and sodium lactate (0.5 grammes), together with hormone therapy (4 milligrammes of desoxycorticosterone acetate daily), the child showed remarkable improvement which was only maintained by continued treatment. With full suprarenal extract (22 cc. per day) improvement was not quite so definite or dramatic. Repeated electrocardiograms showed changes

which were interpreted as being due to changes in serum potassium.

The 17-ketosteroid excretion was 5.7 milligrammes per 24 hours, and the injection of 8.5 milligrammes of adreno-corticotrophic hormone caused no decrease in circulating eosinophils. Barnett, in discussing this case, and the few others reported in the literature, made some interesting comments. The raised 17-ketosteroid excretion in his opinion was definite evidence of increased androgen activity. He pointed out that congenital adreno-cortical hyperplasia occurred in siblings, manifesting itself by similar findings in brothers or as pseudohermaphroditism in sisters, in a large proportion of cases. Barnett also pointed out that relatively enormous doses of desoxycorticosterone acetate (4 milligrammes) had been given in the case under discussion and that this alone had produced a significant therapeutic result. It was also pointed out at this conference that there seemed to be at least three distinct functions of the hormones of the adrenal cortex and that desoxycorticosterone acetate was at least one which controlled the electrolyte and water balance of the body, adequate evidence for this having been obtained from other cases and from the patient under discussion. The 17-ketosteroids were definitely associated with the secondary sexual characteristics. It was thought reasonable to deduce that hyperplasia of the androgenic cells was actually responsible for a failure of function of the other cells. It was also concluded that apparently the 11-oxysteroids, which are concerned with sugar-fat-nitrogen metabolism, are not greatly concerned.

The most recent cases have been reported by Zuelzer and Blum (1949) and by Mazursky (1950). The latter author investigated his case for uric acid excretion and found that it was abnormally high. This evidence is suggestive of a rise in gluco-corticoid liberation and excretion.

My own work on pink disease has led me to believe that the desoxycorticosterone factor is the significant hormone which is mainly disturbed. Here again relatively large doses of this hormone are necessary for therapeutic results, and although these infants and children exhibit symptoms attributable to an underaction of the vegetative adrenal mechanism, particularly in respect to the corticosteroid that controls sodium and water balance, together with (in some cases) eosinophilia, they do not exhibit any obvious disturbance in carbohydrate metabolism as judged by blood sugar levels. There is possibly some association between children with pink disease and these infants reported with macrogenitosomia praecox. There are to my knowledge no reported cases of virilism occurring with pink disease, although Edmonds,* of Perth, claims to have seen such a case. I myself have observed hyperplasia of the mammary glands in one female infant.

A case coming under the notice of the present writer is that of an infant boy who, following a normal delivery, refused food, became dehydrated, developed circulatory collapse, and died 16 days after birth. At post-mortem the adrenal glands were grossly enlarged and greyish in colour but did not show the usual internal haemorrhages. The mother of this child had previously lost two children in exactly similar circumstances, but unfortunately post-mortem examinations were not made. Two of her other children evinced evidence of suprarenal cortical dysfunction: one died of pink disease at the age of 9 months, and the other, the only surviving child, now aged 2 years, has shown a persistent failure to thrive.

A photograph and microphotograph of one of the adrenal glands from this case are shown (Figs. 1 and 2). An attempt was made to extract 17-ketosteroid from the other adrenal. Each gland weighed 13.5 grammes and Miss Joan Cleland, M.Sc., extracted 6 milligrammes of androgen from one of the glands.

*Personal communication.

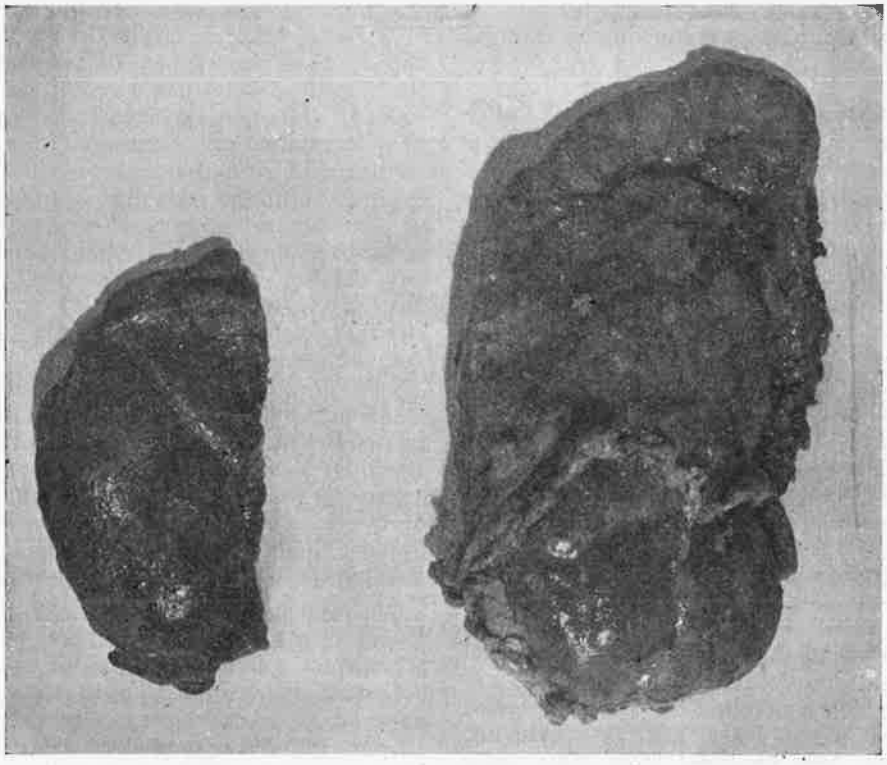

Fig. 1.—One of the large suprarenal glands of the infant referred to, as compared with the usual size found at post-mortem in newborn infants.

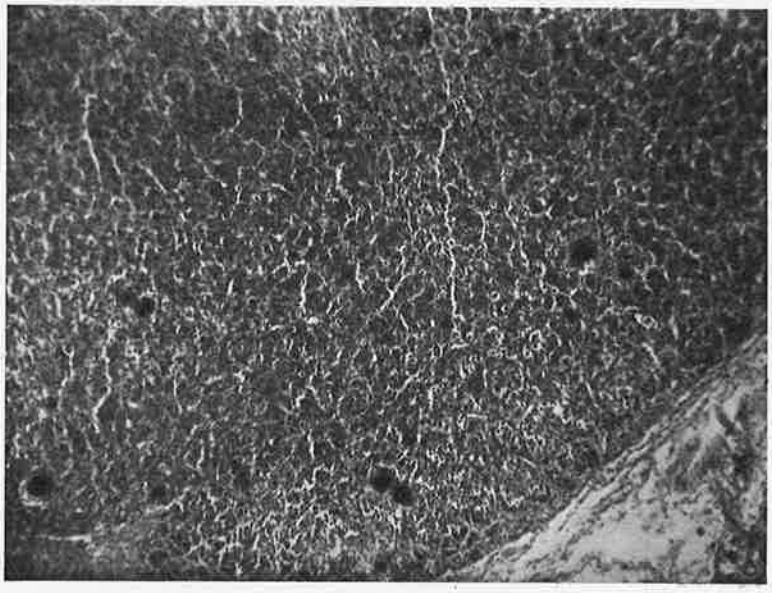

Fig. 2.—Microphotograph (low power) showing marked hyperplasia of the adrenal cortex. It is believed that this hyperplasia is confined to the deeper layers of the cortex ("X zone or androgenic layer") and is responsible for the macrogenitosomia. The clinical manifestations, nevertheless, are mainly those of adrenal insufficiency because the cortical cells concerned with electrolyte balance are actually embarrassed by the preponderant androgenic zone. Dean *et al* (1948) considers that the outer layer (*zona gomerulosa*) is concerned with this electrolytic control: changes in the blood Na/K ratio produce changes in these cells detectable under polarized light.

THE INFANT HERCULES TYPE OF THE ADRENO-GENITAL SYNDROME.

These male children of the "pocket Hercules type," a name suggested by Anthony White in 1809, are conspicuous in their unduly rapid growth. The long bones increase in length, the muscles hypertrophy, and adipose tissue is laid down. The deposition of adipose tissue is mainly on the trunk, the extremities remaining free; this gives a plethoric effect which, when combined with distended cutaneous vessels on the face, may present a striking picture (Fraser (1940); Gross (1940)).

These children have an adult appearance with advanced development of their genitalia (which may be functionally active), with hypertrichosis, and other secondary sexual characteristics. Interestingly enough, their muscular development is so advanced that they may be able to perform extraordinary feats of strength and consequently, even though the syndrome is rare, it has attracted attention and stimulated clinical curiosity down the years.

The essential abnormality as pointed out by Dods and Jeremy (1938) is a gross disturbance in the time factor of growth and development: changes which would normally occur at adolescence and later are projected into the early years of life.

CUSHING'S SYNDROME.

Although this is a comparatively rare condition, cases are sometimes seen in children.

These patients differ from the children with excessive adreno-genital activity in that they present a predominating adreno-metabolic disorder. Only some patients with Cushing's syndrome show sexual changes due to concomitant androgenic activity, so a careful distinction must be drawn between these two classes. Indeed, as has been pointed out, with hyperplasia of the androgenic zone, one expects to find adrenal cortical insufficiency, whereas in this instance we have an occasional increase in androgenic activity together with an overactivity of the true suprarenal cortex (11-oxysteroids). A child suffering from Cushing's syndrome presents the characteristic moon face, short thick neck, obese trunk, muscle weakness, lassitude, emotional instability, low basal metabolic rate and lymphocyte count. The blood pressure and red blood cell count are both raised.

ADRENAL CYSTS WITH ADRENAL INSUFFICIENCY.

A unique case of an infant with adrenal insufficiency and an underlying cystic condition of the adrenal glands was recently reported by Moore and Cermak (1950).

The child, a male infant weighing 3,320 grammes, had a normal birth. Seventeen days after birth he passed watery and green stools associated with vomiting and dehydration. Rehydration could not be established by parenteral therapy and his condition steadily deteriorated. The serum sodium was 132 milliequivalents per litre (normal 135).

The child was given only 0.5 cc. of Eschatin every four hours as an empirical measure together with intravenous saline therapy. Only slight improvement resulted and oral feeds were reinstituted. At the end of the fifth week of life the child was gravely ill. His complexion was grey, and the diarrhoea and vomiting had increased. Parenteral and oral fluids up to a total of 750 cc. per day were continued. At this stage the authors realized as a result of reading Jaudon's work that they had a case of adreno-cortical insufficiency, and the infant was given 3 milligrammes of desoxycorticosterone acetate together with 2 grammes of salt by mouth. The result according to the authors was dramatic, and within three weeks the child gained 700 grammes in weight. He became active, began to smile, and cried more strongly. Investigations disclosed a serum sodium of 126 milliequivalents per litre, with a carbon dioxide combining power of 28, and an eosinophilia of 17 per cent. With treatment the sodium rose to normal. The child continued to do well and was discharged at the age of 4 months weighing 4,000 grammes. Later, however, the steroid injections were reduced. The child developed a secondary infection and became ill, with vomiting, diarrhoea, collapse, a raised temperature, and finally death in a state of vascular shock.

At post-mortem the adrenals were enlarged, weighing 10 grammes each, and a cyst was found to occupy three-quarters of the diameter of the right gland, whilst two cysts occupied the same area on the left. Microscopically, three layers of the adrenal cortex were differentiated and histologically there was sufficient cortical tissue to maintain life. The cystic spaces were lined by compressed cortical cells with a thin fibrous membrane. The authors concluded that the child had had adrenal insufficiency since birth, that the dose of adreno-cortical extract given initially was far too small, and that the cysts had probably arisen from unresolved adrenal haemorrhage following birth. They suggested that if hormone therapy had not been cut down the child might have survived longer; the added

stress of infection, however, had quickly led to a fatal result. These authors in a search of the literature found only one case similar to their own. This was quoted by Tyson in 1888 as being a case of Dr. Belaieff's: it was a male infant with greyish discolouration of the skin, who, in spite of a good fluid intake, died at 2 months of age. At post-mortem numerous cysts were present in the adrenal glands.

ACUTE ADRENAL INSUFFICIENCY.

Acute adrenal insufficiency produces the most striking effect if the hormonal requirements of the body are raised whilst the capacity for adaptation to the inadequate hormone supply is minimal. This set of circumstances follows in the newborn upon destruction of the adrenals by haemorrhage, and may produce the fulminating symptoms of what Goldzieher (1945) called "pseudopneumonia of the newborn." In this condition, manifestations suggestive of pneumonia develop within a short time of birth, with rapid respiration and fever, in the absence of any physical signs of pulmonary involvement. Anorexia, apathy, cyanosis, and a generalized petechial rash also are usual features. The abdomen may become distended and a unilateral or bilateral mass in the renal region may become palpable. Convulsions, vomiting, jaundice, and diarrhoea may supervene. In some cases shock, collapse, and death all occur within a few hours. The tendency to develop severe manifestations of acute insufficiency is but slightly diminished in the older infant: such an adrenal insufficiency as a result of an overwhelming septicaemia produces the clinical picture of the so-called "Waterhouse-Friderichsen" syndrome. In severe childhood infections such as diphtheria the manifestations of adrenal insufficiency are often overlooked and are too often attributed to the septic or toxic process.

Goldzieher and Gordon (1932) classified cases of adrenal insufficiency in infancy into two groups which in their opinion should be carefully distinguished, viz., those with symptoms due to adrenal haemorrhage, and those with symptoms referable to adrenal insufficiency alone.

1. Symptoms of acute adrenal insufficiency—
   (*a*) Endocrine symptoms: Rapid respiration, high temperature, metabolic changes, convulsions, cyanosis, and purpura.
   (*b*) Associated symptoms: Gastrointestinal disturbances (vomiting, diarrhoea, abdominal pain); nervous manifestations (coma, convulsions, twitchings).
2. Symptoms due to haemorrhage—
   (*a*) General symptoms: Shock, collapse, weak and irregular pulse, pallor, cold extremities.
   (*b*) Local symptoms: Distension of abdomen, palpable tumour in one or both kidney regions, peritoneal irritation, haemoperitoneum.

It is interesting that convulsions, coma, and nervous symptoms impressed earlier observers so much that they felt called upon to distinguish a separate group of nervous symptoms in acute adrenal insufficiency. It is agreed by all writers that the actual mechanism of these manifestations is not clearly understood, but one might mention in passing that the same symptoms are noted in severe pink disease, which is characterized by a severe electrolyte and water disturbance, and possibly by turgor of the brain cells.

Whereas acute suprarenal insufficiency in the newborn is due to haemorrhagic involution of the foetal cortex, in older infants it is invariably precipitated by septic infection and it is relevant to this thesis to consider this phenomenon a little more closely.

Little-Bamatta or Waterhouse-Friderichsen Syndrome.

Although the term "Waterhouse-Friderichsen syndrome" is applied to all cases of adrenal haemorrhage in both newborn and older children, this is unfortunate for both the reasons stated above and because their description of the syndrome applies only to the meningococcal septicaemic type of adrenal haemorrhage.

Little (1901) was the first to describe the syndrome. He published four cases and gathered eight others from the literature. Waterhouse published a single case and added nothing more to our understanding of the syndrome. Similarly, although Friderichsen published some cases, Bamatta emphasized the significance of the meningococcal infection, and altogether it seems reasonable to call the entity the "Little-Bamatta syndrome."

All infections regardless of their aetiology produce changes in the adrenal glands. Bacterial emboli readily settle in the capillaries of the adrenals and may lead to thrombosis, infarction, or toxic necrosis. Meningococci, streptococci, staphylococci, and the influenza bacillus seem to have a special affinity for the adrenal glands. With few exceptions, however, the meningococcal organism is the one generally involved. Symptoms of restlessness and headache appear together with some gastro-intestinal

upset such as vomiting. Cyanosis, which is generally marked, rapidly develops, and petechial haemorrhages become widespread. The temperature swing, which may be below normal at the start, becomes of the "septic," spiking type, and may reach 108°F. McLean and Caffrey (1931) obtained positive blood cultures in 83 per cent of their cases. The pulse rate is raised, but the circulation begins to fail and later a feeble pulse and a failing blood pressure occur. Air hunger with rapid respiration may occur and assume a Cheyne-Stokes character, frequently with a fatal outcome.

ACUTE ADRENAL INSUFFICIENCY IN CHILDREN.

A good example of this syndrome is to be found in diphtheria. There is no adequate explanation in the text books of paediatrics as to why some few cases of diphtheria become toxic or malignant in character—a severity that is generally manifested so early in the illness that serum therapy usually comes too late to save life (Behr, 1941).

Peripheral circulatory collapse is in these cases the outstanding manifestation of acute adrenal failure and is demonstrated by a fall in blood pressure, by cold clammy extremities, and by a failing pulse. Anatomical examination of the heart does not offer satisfactory evidence of serious myocardial impairment (Goldzieher, 1945).

Electrolyte behaviour in diphtheria has never been investigated, but increase in non-protein nitrogen in the blood has been found and the higher the level the worse the prognosis (Bennholdt *et al.*, 1938).

ADRENAL INSUFFICIENCY IN EARLY DAYS OF LIFE.

Bruch and McCune (1936) who studied the blood of 72 newly born and normal infants were unable to demonstrate any changes in the plasma sodium, plasma protein, total base or cell volume indicative of the physiological state of adrenal insufficiency as compared with the changes found in Addison's disease or in adrenalectomized dogs. They did, however, report a haemoconcentration and a definitely raised non-protein nitrogen in the first few days of life and were unable to explain these findings.

Miller (1941) extended this work and considered that a state of adrenal cortical insufficiency did exist. He made a study of 60 infants during the first 10 days of life and interestingly enough showed that the usual weight loss of the infant which occurs during the first few days of life could be reduced by the the use of desoxycorticosterone acetate. Miller again reported the presence of haemoconcentration, and a rise in protein and non-protein nitrogen during the first four days of life.

In a later study of shock in the early days of life, Miller found that the plasma sodium in some cases was at the lowest level of normality (310-320 milligrammes per cent) and interpreted his findings as evidence of adreno-cortical insufficiency. He observed that severe shock was six times more frequent in infants with a serum sodium below 327 milligrammes per cent than in those with a serum sodium either equal to or greater than this amount. He also observed that the maintenance of a normal blood sugar level was important. If hypoglycaemia was present the prognosis was bad. He considered that even in a normal delivery the infant's blood findings were suggestive of shock, but the clinical picture of this condition would only become apparent if trauma to the infant became excessive during labour.

Akerren (1945) investigated the cause of death in premature and newborn infants and discussed the conflicting opinions regarding this subject. The hypothesis was advanced that the low condition which often precedes death was nearly always due to shock and adrenal insufficiency. In autopsies he found the most typical and constant finding was a definite macroscopic and microscopic hyperaemia in the liver and extremely wide intra-acinous capillaries; these findings were also present in other organs in some cases. He found without exception that a good result was obtained by the injection of adrenal extract in premature or newly-born children in poor condition. The change of colour from a greyish hue to a rose colouration of the skin was in his opinion very noticeable.

Berlind (1941) gave desoxycorticosterone acetate together with infusions of saline to 12 newly-born infants suffering from foetal shock. These infants he described as being limp and traumatized, with diminished muscular tone, a pale greyish colour, and irregular respiration. There was either a rapid pulse rate or an abnormally slow or irregular one. He reported excellent results with this therapy. No biochemical investigations were carried out.

Despite all the evidence hitherto reported for hypoadrenalism in the early days of life, it was left to Jaudon (1946-48), by carefully correlating clinical, biochemical, and therapeutic results, firmly to establish its reality in some children. Jaudon has brought forward substantial evidence to show that a hypofunction of the

adrenal cortex produces disturbances in the metabolism of young infants which is more of a physiological than pathological nature.

The group of apparently physically normal premature and full-term infants frequently referred to as "constitutionally inferior" because of an inability to thrive despite adequate medical and nursing care, attracted his attention. He noted that this type of infant was weak, would refuse food, and manifested some of the symptoms of Addison's disease. It was known that in Addison's disease there is a diminished retention of sodium by the kidneys and a decreased concentration of sodium in the blood whilst there is an increase in the plasma protein and in the excretion of chloride. There is also a decrease in plasma volume and an increase in the non-protein nitrogen. Clinically, characteristic findings are a steady loss of weight, dehydration, refusal of food, vomiting, muscle weakness, and oliguria. Jaudon accordingly sought for similar evidence in this group of weakly newborn infants. His first series of nine cases were characterized by debility, weakness, impending shock, dehydration, collapse, occasional hypoglycaemia with lowered glucose tolerance, diarrhoea, vomiting, irritability, and acidosis.

He was able to show that the administration of cortical extract and/or desoxycorticosterone acetate produced therapeutic results and he was able to detect changes in the blood which supported the view of an adrenal insufficiency. He concluded that each of the cases reported presented some phase of hypofunction of the adrenal cortex, and considered this hypofunction to be physiologically due to the changes occurring in the adrenal gland during the neonatal period.

Two years later, Jaudon (1948) published a second paper and his work led him to realize that infants who, during early life, presented symptoms of protracted gastro-intestinal disturbance, who failed to gain weight, who had an unexplained tendency to dehydration, and who responded unsatisfactorily to accepted therapeutic measures, were suffering from adreno-cortical insufficiency. This disturbance of adrenal function was in Jaudon's opinion a fractional and transitory disturbance which was related to the "salt and water" hormone. He presented five cases of young infants who suffered from vomiting, failure to gain weight, and periods of unexplained dehydration, accompanied by biochemical changes indicative of one or other phase of hypofunction of the adrenal cortex. These cases all responded to salt and desoxycorticosterone acetate, and in some instances treatment was maintained over a period of several months. He found an elevation of the non-protein nitrogen, a moderately low serum sodium and chloride during periods of haemoconcentration, a low carbon dioxide-combining power, together with a high chloride excretion. Jaudon therefore claimed to have demonstrated a physiological hypofunction of the adrenal cortex of a transitory and fractional nature, since he believed that the "salt and water hormone" was predominantly involved.

A similar conclusion has been reached concerning my own work on pink disease.* Here for the first time a clinical study relating to a hypofunction of the adrenal gland has been conducted throughout the months and years of childhood, and provides a natural corollary to the work of previous investigators. Provenzano (1950) recently presented in Boston the case of a newborn infant who manifested in life a hypofunction of the adrenal cortical tissue which was seen to have an anatomical basis on post-mortem examination. The size, weight, and microscopic findings indicated a marked hypoplasia of the adrenal glands.

MARASMUS.

Hislop (1938) treated marasmus by the injection of adrenal cortical extract. He considered that it was rational to draw a parallel between marasmus and adrenal insufficiency. It had been shown that adrenalectomized animals did not gain weight in comparison with normal animals, whilst both were receiving the same amount of food (Simpson, 1934). This inability to make use of food was in his opinion a concomitant feature of marasmus.

Marriot (1920) investigated marasmus and found that "from the metabolic standpoint the most outstanding features of this condition are an inability to utilize food and a negative nitrogen and mineral salt balance." A negative nitrogen and mineral salt balance are cardinal features of adrenal insufficiency and of experimental salt depletion (McCance, 1936). Hyperaemia or haemorrhage is not uncommonly found at autopsy in the adrenals of infants who have died from severe diarrhoea and vomiting. This has sometimes been alleged to be due to intestinal intoxication from gastroenteritis. That there is a link between actual vitamin deficiency and adrenal function is shown by the work of

* Paper read before The Australian Pediatric Association in Brisbane, 29th May, 1950.

Lockwood and Hartmann (1933), and Simpson (1934). These workers showed that in vitamin deficiency degenerative changes occur in the adrenal gland. In relation to infection and intoxication, Jaffe (1926) stated that "in acute infections and intoxication pathological changes regularly occur in the suprarenals, varying in intensity from congestion and oedema to haemorrhage and necrosis."

Hislop treated 19 cases of infantile nutritional disorders with suprarenal extract and reported favourable results.

Szaz (1939) similarly treated 20 patients and included salt and glucose with the suprarenal extract. He also reported favourable results, and, in addition, described the occurrence of cyanosis in the acute stage of his cases.

Prolonged starvation from any cause may result in a severe atrophy which is spoken of as "the marantic state" and in experimental animals leads to changes in the structure of the adrenal gland. Severe wasting is also noticed in coeliac disease, even though the intake of food may not be poor in quantity or in composition, and this marantic state may develop more rapidly than can be explained by the actual nutritional deficiency. Loss of intracellular water accounts for this, and one of the first steps in therapy is the restoration of hydration of the tissues. Our attention therefore is directed again to salt and water metabolism, and to the adrenal gland. More concerning this important subject, especially in relation to coeliac disease, has been said in unpublished papers submitted with this thesis (Cheek, 1950a).

PHYSIOLOGICAL INVOLUTION OF THE ADRENAL CORTEX.

The physiological involution of the foetal cortex is an established fact to which hitherto little biological significance has been given. A probable explanation of this involution by different authors is as follows: *in utero* the foetus depends on the maternal blood supply for oxygen, and greater amounts of cortical hormone are required for the foetal tissues to utilize this oxygen at the low gas tension which is present in the foetal circulation. After birth when normal respiration is established there is no longer need for increased cortical hormone and consequently involution takes place. It has already been pointed out that this does not occur in infants with congenital malformation of the heart; similarly it has been shown that animals exposed to low atmospheric pressure develop cortical hyperplasia (Armstrong and Heim, 1938).

It is also becoming realized that in severe anaemia in the newborn, especially in blood destruction due to severe incompatibility (erythroblastosis foetalis), hypofunction of the vegetative adrenal mechanism is possibly present.

It is reasonable to deduce from the foregoing facts that, if physiological involution proceeds too rapidly or too far, in some instances a state of deficiency may be manifested in the early days of life. In any case there must be a state of marginal sufficiency which although adequate under normal circumstances will be unable to cope with sudden increased demands due to stress of some type.

No doubt nature had provided other temporary adjustments which help to safeguard survival and ease the burden on the adrenal cortex in the maintenance of tissue metabolism, and no doubt the previously unexplained large salt reserve of the infant is an example of this. Until the seventh or eighth year of life a limited cortical reserve power seems to prevail and the child's existence is characterized by instability with respect to its environment and by marked susceptibility to infections. It can thus be seen why at the sixth month of life when the foetal salt reserve of the infant is exhausted that so-called pink disease may appear, and it is not until the seventh or eighth year of life that this manifestation can no longer be found. We are all familiar with the common statement of the practitioner on the child who fails to thrive: "If the child reaches 8 years of age he will grow out of it."

No better illustration of the importance of the adrenal gland can be found than in the hydrolability of the infant and the ever-threatening danger of dehydration. The newborn infant contains twice as much water in proportion to body weight as the adult and the body distribution of this fluid is different: the extracellular water exceeds the intracellular water, in contradistinction to the adult in whom the extracellular water is only half the amount of the intracellular (Sterns, 1939). It can thus be seen that a disturbance or shift of the normal excess extracellular fluid will produce a pronounced physiological disturbance. Such a state of affairs occurs in pink disease.

Reference to the work of others on the biochemical changes in the blood in the early days of life has been made. It is to be noted that with respect to the work of Bruch and McCune

the value of their findings is diminished by the lack of urinary chloride or sodium estimations, as the sodium reserve of the infant can be mobilized and drawn upon to make up for any temporary deficit. The rise of non-protein nitrogen which these workers demonstrated is much more significant.

Hartmann and Jaudon (1937) demonstrated the presence of a physiological hypoglycaemia in 286 newborn infants. In addition to this, the metabolism of the infant is in a precarious equilibrium which is readily upset by any incidental loss of salt or water. Chronic disease of the adrenals in adults may remain latent for a long time until some stress produces an acute breakdown. The existence of a barely marginal adrenal sufficiency in the newborn is the only logical and satisfactory explanation for their hydrolability, and susceptibility to infections and gastro-intestinal disturbances. This is in keeping with the fact that gastroenteritis of the infantile type is much more frequent in the summer and, as shown by Campbell and Cunningham, does not occur after the age of 2 years. The incidence of pink disease is also seen to subside at 2 years of age. This has been clearly shown by Southby (1948) and by the author (1949), and one would remind the reader that it is at this age that appreciable regeneration of the adrenal cortex occurs. One may therefore conclude that the salt and water balance of the growing infant becomes more stable at about this age and that intra- and extra-cellular water relationships begin to approach more those of the adult.

It has been shown conclusively by Conn and Louis (1950) that acclimatization to heat in the human individual consists of increased adrenal cortical function mediated by increased pituitary production of adrenocorticotropic hormone. It is, of course, known that the heat regulation of the body is dependent on the vegetative adrenal mechanism (Cramer, 1928). Conn and Louis also showed that the electrolyte composition of thermal sweat affords a constant index of adrenal production of desoxycorticosterone-like corticosteroids.

Hot atmospheric conditions obviously cause loss of water and salt through perspiration and therefore could easily upset a precariously balanced hormone equilibrium—which in Addison's disease is often manifested by a crisis—and may well explain the increased incidence of the florid picture of pink disease in this country. In view of the work of Conn and Louis it can be seen that in the presence of an underaction of the vegetative adrenal mechanism there is an increased loss of sodium and chloride in the sweat, and this would apply to infants with pink disease; but it is the conclusion of the author that sweating alone cannot account for the depletion of sodium in pink disease, although it might exacerbate it. There is primarily the lack of response of the vegetative adrenal mechanism, and this is borne out by the study of older children (Cheek, 1950a, in preparation) in which it was seen that the administration of salt was necessary, and (at the time of writing) is still necessary, to ensure their proper growth and development, even though their plasma sodium has not risen to completely normal levels. The most that we can say is that the sweating in pink disease is a manifestation of the disturbed vegetative adrenal mechanism, that it is the only available heat-regulating mechanism left to the child, and that it aggravates the already established salt loss.

The tendency for the normal infant to develop hyperpyrexia is yet another phenomenon connected with hydrolability. After a rapid loss of water and subsequent haemoconcentration the viscosity of the blood increases; its circulation is slowed down in the capillaries, and the essential mechanism of peripheral heat loss is interfered with. The increased temperature of these infants is a manifestation of the inability to lose heat rather than an increase in heat production. These are accepted facts which apply to the normal infant, but how well they apply to the case of pink disease with anhydraemia and haemoconcentration, cold clammy hands and feet, intolerance of heat, and a tendency to run a low-grade temperature which so many workers sought to explain in terms of increased heat production due to infection.

When loss of salt occurs in the infant as a result of sweating, diarrhoea, or vomiting, increased fluid intake alone does not compensate for the loss sustained, but aggravates it both in the normal, sweating infant and in children suffering from pink disease. In the words of Goldzieher (1945), "the analogy between the acute crisis of Addison's disease in the adult and the summer diarrhoea of the infant goes far beyond the similarity of salt and water metabolism. The inability to absorb carbohydrate and fat from the intestine is conspicuous in both conditions. The morbidity and mortality of small infants due to upper respiratory infections also resembles the decreased resistance of the Addisonian patient." One might remind the reader in passing of the same association of respiratory infection and diarrhoea with pink disease. From what has been

said it is obvious that the newborn child comes from the constant fluid environment of the uterus, during which time one phase of adrenal development has taken place—a phase that is no doubt related to the growth and development of rapidly-proliferating cellular tissues, and to the foetal oxygen partial pressure. At birth a new and vastly different environmental requirement calls for dramatic and sudden change in adrenal structure, and the second phase of adrenal development begins (Cheek and Hicks, 1950).

The successful adjustment to this new environment, and the child's survival, therefore depend to an appreciable extent on the development and adequacy of the vegetative adrenal mechanism; we could describe this as "physiological adaptation." As long as adaptation is maintained, the child will grow, develop, and thrive, and the integrity of the respiratory, cardio-vascular, and gastro-intestinal systems will be maintained together with hydration. The Spartans, who had other motives, placed their naked infants out on the hillside overnight and if they survived they expected them to grow into strong fighters. Perhaps this was an extreme example of adaptation! (Stanton Hicks. Paper presented to International Paediatric Congress, Zurich, 1950.)

Selye (1946) has given us a clearer conception of adaptation and the effects of environmental change and stress on the organism. Admittedly he and his fellow workers have been interested mainly in the overactive or imbalanced response of the adrenal mechanism as a result of stress, and have sought the clinical manifestations of a failure of proper adaptation. In this thesis, however, the importance is emphasized of considering a marginal sufficiency of adrenal function during infancy (and, to a lesser extent, in childhood) which may prove inadequate in conditions of stress.

THE ADAPTATION SYNDROME.

The rudimentary medulla and thin cortical structure of the adrenal glands in the young infant provide anatomical evidence for a physiological inability adequately to meet environmental demands and to establish "adaptation." Let us look then more closely into the "adaptation syndrome."

During the last 20 years sufficient evidence has been presented to establish the conception that many of the common diseases of human pathology whose aetiology has hitherto been unexplained may be the result, directly or indirectly, of non-specific deleterious factors present in the internal or external environment of the individual. These factors produce their results through the endocrine system of the body, with the pituitary, the adrenal glands, and the vegetative adrenal system as the main mediators. It is, therefore, necessary that one should have an understanding of the principles of the general adaptation syndrome. By means of this concept, Selye (1946) focused particular attention on the adrenal gland. By detailed physiological, biochemical, and pathological studies of the result of stress upon animals he was able to divide this general adaptation syndrome into three important stages. He defined this as follows: "The general adaptation syndrome is the sum of all non-specific, systemic reactions of the body which follow long-continued exposure to stress."

There are three stages of this:—

(1) The alarm reaction, subdivided into the shock and counter-shock phases.

(2) The stage of resistance.

(3) The stage of exhaustion.

The alarm reaction can be induced by any non-specific agent requiring a general adaptive adjustment, and is an illustration of the primary demand made on the vegetative adrenal mechanism by stress-producing agents. These agents include such widely-separated and variable factors as cold, solar and roentgen radiations, muscle exercise, infectious diseases, nervous shock, drugs, bacterial toxins, toxic substances, and so on. The alarm reaction is not necessarily a pathological phenomenon, and if the reaction is mild there is no shock in the ordinary sense of the word. A slight hyperglycaemia, tachycardia, and leucocytosis may be the only detectable findings. The symptoms of an alarm reaction in an organism represent "a call to arms" of the body's defence forces. Two more or less distinct phases, shock and counter-shock, arise, but the transition between them is not always sharp and some of their manifestations overlap. Stress, which elicits an increased blood adrenalin, ushers in the shock phase, and the counter-shock phase follows satisfactorily in the presence of adequate adrenal cortical activity. The following features of the shock phase were noted: tachycardia, decrease in muscle tone and body temperature, formation of gastric and intestinal ulcers, haemoconcentration, anuria, oedema, increased capillary permeability, decrease of blood chlorides and plasma sodium, acidosis, a decrease in blood sugar, leucocytosis, a rise in the blood potassium and phosphate, a decrease in blood volume, a

negative nitrogen balance, an increase in the ketone bodies in the blood, and a fall in the blood lipids and cholesterol. Allergic manifestations were prominent.

During the alarm reaction the adrenal cortex enlarges, the cells hypertrophy and discharge lipid granules, and an excess output of corticoid hormone occurs in the urine (Venning and Kazmin, 1946). The stage of alarm merges into the stage of resistance which is characterized by an increased resistance to the particular agent to which the organism is exposed. As a result of prolonged stress the stage of resistance gives place to the stage of exhaustion. "The stage of exhaustion represents the sum of all the non-specific systemic reactions which ultimately develop as a result of prolonged exposure to stimuli to which adaptation has been developed, but could no longer be maintained." In the stage of exhaustion the same features as set out above for the alarm reaction are again present, with a decrease in body weight.

Selye discovered that rats which were in the "stage of resistance" during an experimental "stress" developed nephrosclerosis and hypertension. This discovery led to the view that some imbalance in gluco-corticoid and mineral corticoid elaboration during the stage of resistance might well be responsible for some of the common human diseases of uncertain origin, (*e.g.*, hypertension, rheumatoid arthritis, nephritis, rheumatic fever, periarteritis nodosa, gout, etc.), and much work has been done and is being done to support this theory (Selye, 1949, 1950).

It is relevant to this present thesis to consider a little more closely the alarm reaction and the stage of exhaustion.

Selye noted that after adrenalectomy or hypophysectomy certain alterations occurred in the pattern of events in the course of the general adaptation syndrome. The shock phase was severe and the counter shock was negligible or absent, hypoglycaemia was more pronounced, and there was no lymphatic involution. Thus he showed directly the importance of sufficient adrenal tissue to maintain adaptation.

We have seen that the symptoms and signs of pink disease include tachycardia, decrease in muscle tone, scanty urine, haemoconcentration (and therefore decrease in blood volume), cellular oedema, decrease in the plasma sodium, a possible retardation of glucose absorption, leucocytosis, and lymphoid hyperplasia. The presence of ketone in the urine was noted by Rocaz (1934). A negative nitrogen balance was noted by Brown, Courtney and McGlauchlin (1921). We have seen, too, that gastric bleeding and inflammatory changes have been noted *post mortem*, and the association of allergy with this disease has been fully discussed (Cheek 1950a, in preparation).

Williams, of the Mayo Clinic (1945), after making a study of allergy concluded: "I have suggested that physical allergy is a perversion of a normal physiological reaction, the alarm reaction of Selye. Since I have shown that the hormones of the adrenal cortex can relieve the symptoms of physical allergy and these hormones control the permeability of cell membranes and electrolyte and water metabolism, and since intact adrenal function is necessary for the alarm reaction to take place, I feel that good indirect evidence that physical allergy is an alteration of this normal mechanism is at hand." My own work has shown me that children with the symptoms that I have come to regard as the manifestations of "pink disease" at a later age (the "nervous child," failure to thrive, etc.) have exhibited allergic phenomena. These children have lost their asthmatic attacks after the addition of 8 grammes of salt daily to their diet. In other words, the allergy has become quiescent. Continued administration of desoxycorticosterone would probably have been just as, or more, effective. In any case, these children confirm in a striking manner the statements of Williams and provide strong evidence to substantiate his vision.

The work of Selye is based on a purely academic study of animal experiments, and it cannot be assumed that the detailed pattern and sequence of events that have been followed in them will apply in any exact manner to the study of the human organism; but in the infant and child, on anatomical grounds, we could expect a lesser and more limited response, particularly in the first two years of life. This fact probably accounts for many of the phenomena seen at this age, especially the hydrolability and the often severe and protracted course of gastro-enteritis, whether due to true infection of the bowel or to a so-called "parenteral" infection (*vide* Cheek, 1950b). The parallelism between the findings in pink disease and "the alarm reaction" is striking, perhaps even more striking than in those conditions that have hitherto been described as possibly related to the "alarm reaction." As can be imagined, this is of the greatest interest to Selye himself (personal communication).

The progress of discovery, as a result of concentration upon narrow lines of research, is

slow, but it is interesting to recall the fact that Claude Bernard, the father of hormone physiology, emphasized the importance of the "milieu interieur"; in the work of Selye we find a fresh exposition based upon the accumulated knowledge of the intervening generations.

ADDENDUM.

Further work on the excretion of the neutral 17-ketosteroids in pink disease reveals that there is not the diminution of this factor in the urine that one might have expected in a condition of poor nutrition (Landau *et al.*, 1948). Indeed, evidence so far suggests the reverse—a slight increase in excretion. Biological assay of the gluco-corticoid excretion (performed through the help of a colleague, B. S. Hetzel, and his assistant, D. Hine) also suggests a slight rise in excretion of this fraction as well. It is hoped that further work will throw more light on this aspect.

In conditions of stress the production of the adreno-cortico-trophic hormone from the pituitary is raised; this induces an increased production of glycogenic hormone and an increased excretion of 17-ketosteroid. A normal fall in eosinophil count has been noted after the injection of small amounts of adrenalin (Cheek, 1950a). The association of glycosuria and hyperglycaemia in occasional cases of pink disease has been noted by previous workers (Rocaz, 1933) (Glanzmann, 1937). One explanation for such findings could be an increased production of the glycogenic hormone, but in the presence of an electrolyte disturbance one would also expect to find retarded glucose absorption from the gastro-intestinal tract (Clark and MacKay, 1942). Some evidence for such a retardation has been presented and similar curves have been obtained from other unpublished cases. The raised blood sugar levels are not satisfactorily explained by the theory of a persistent overaction of the sympathetic nervous system and a raised blood adrenalin, as suggested by several authors (*vide* Glanzmann, 1937). Biochemical estimation of the blood adrenalin, using the method of Shaw (1938) as modified by Raab (1941), reveals no gross difference in the level of blood adrenalin in 15 cases of pink disease and 15 normal children.

Since the completion of this manuscript the most interesting case report of Geppert *et al.* (1950) has appeared in the literature. These authors present the records of a child with hypoplasia of the adrenal glands whom they carefully followed from birth until death at 20 months of age. Conclusive clinical, biochemical and post-mortem evidence has been produced to show that this case had insufficiency mainly of the glycogenic or "S" hormone. The stratum reticularis, which is thought to be mainly concerned with the production of this hormone, was absent. On the other hand, they considered that the steroids regulating anabolic activity and nitrogen retention (Allbright's "N" Hormone) were being satisfactorily produced during periods when the patient was not subjected to stress, and indeed accounted for a marked acceleration of growth which occurred at these periods. It was also of particular interest that this case exhibited no craving for salt, and a disturbance of blood electrolytes was only detectable during periods of stress. These clinical and biochemical findings were supported by the detection of a definite stratum glomerulosa at post-mortem. The conclusion was that the "salt and water" hormone was present in satisfactory amounts in "non-stress" periods. Consequently it was found that adequate treatment with full suprarenal extract (Eschatin), which contains the glycogenic hormone, gave the best therapeutic result.

This case report again emphasizes the concept of fractional disturbances of adrenal function which can occur in infancy, and these authors conclude that:—

"Only the introduction of the most fascinating chapter on adrenal cortical insufficiency has yet been written. The incidence of proved cases of adrenal insufficiency is very low. There is much circumstantial evidence available to support the hypothesis that although proved cases of adrenal insufficiency are rare, functional adrenal insufficiency may be a common clinical problem (Selye, 1946). Future clinical and experimental investigation may provide a more firm foundation for the brilliant theories advanced."

ACKNOWLEDGMENT.

I should like to place on record my appreciation of the help, interest and assistance of the Honorary and Resident Medical Staffs, and the Nursing Staff of this hospital. It has been a privilege to work in the same wards where the late Dr. H. Swift, who first drew the attention of the world to this disease, worked as an Honorary Physician.

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PINK DISEASE: THE MANIFESTATION IN OLDER CHILDREN AND THE ESTIMATION OF THE BLOOD ADRENALINE CONTENT.

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THE EXTRARENAL BASIS OF ALKALOSIS IN POTASSIUM DEFICIENCY*

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The function of the kidney in the regulation of the pH of the extracellular fluid has been studied intensively (1,2) and alterations in muscle composition accompanying disturbances in acid base balance have been pointed out (3). The determination of the precise role of the kidney and of the muscle cell in modifying extracellular composition has been handicapped by the difficulty in designing experiments which separate the influence of different organs and systems. The need for a "body-less kidney" or a "kidney-less body", in the study of the problem, has been emphasized by Darrow (4).

The physiological basis for metabolic alkalosis, associated with potassium deficiency, has not been satisfactorily determined. On the basis of changes in renal potassium excretion during administration of the carbonic anhydrase inhibitor, 6063, Berliner (5) has postulated that alkalosis in potassium deficiency results from the excessive substitution of hydrogen and ammonium ions for sodium in the distal tubule of the kidney. In this way chloride is excreted without sodium, and hypochloremic alkalosis of the extracellular fluid results.

This paper presents the findings in experiments designed to test this hypothesis by simultaneous measurement of renal excretion and analysis of serum and muscle of rats during recovery from potassium deficiency alkalosis.

Plan of Experiments and Methods

Male albino rats, weighing 250-300 grams, were made alkalotic and potassium deficient in the course of three and one half weeks by the feeding of a potassium deficient diet* and the intraperitoneal injection of 2mg. of D.C.A.** daily. A solution of sodium chloride and sodium acetate with a sodium to chloride ratio of 1.5/1.0 was substituted for drinking water and was the sole source of sodium and chloride.

Animals were then divided into two groups of nine animals each and transferred to individual metabolism cages. Distilled water was substituted for the salt solution and D.C.A. injections were discontinued. After three days a control urine collection for 24 hours was obtained.

Group K then received twice daily intraperitoneal injections of isotonic potassium chloride for six days (6mM of KCl per Kg. of body weight per day). Complete urine collections were obtained and analysed each day. Group Na received twice daily intraperitoneal injections of the equivalent amount of chloride as Group K, in the form of isotonic sodium chloride, and all urine was likewise collected and analyzed.

* Constituents per Kg. of Diet - dextrin 550g., hydrogenated vegetable oil (Swiftning) 150g., raw casein 100g., vitaminized casein 100g., corn oil 100g., vitaminized corn oil 100g., basic salts 5g.(including Calcium, phosphorus, iron, zinc, copper, manganese, magnesium).

** Percorten, supplied by Ciba Inc., Summit, N.J. whose generosity made possible these studies.

All cages, screens and funnels were treated with a silicone to permit rapid and complete drainage. All urine was collected under toluene and mineral oil. Feces were excluded from urine but were not analyzed. Other animals were treated similarly except that urine was not collected. These animals were sacrificed at the beginning of, and at appropriate intervals throughout the experiment. Analyses of blood drawn from the abdominal aorta during ether anesthesia, and of muscle after exsanguination, were obtained on these animals as well as those studied in metabolism cages.

The methods of analysis are the same as described earlier by this laboratory (7) with the exception that sodium and potassium were determined by a Barclay internal standard flame photometer. The micro-diffusion method of Conway (8) was used for analysis of ammonia in urine. The Beckman pH meter was used for the determination of pH and titratable acidity by titration with N/100 NaOh to pH 7.40.

Results

There was essentially no difference in mean renal excretion between Groups K and Na during the control period. There was a marked difference in composition of urine between Groups K and Na after injections of potassium and sodium chloride were begun.

Group K excreted urine of lower pH and bicarbonate content, and higher titratable acidity, than Group Na. These findings became more marked as the experiment progressed (Fig. I,II,III). The ammonia excretion was somewhat greater in Group K than in Group Na (Fig. IV). In Group K there was marked increase in the excretion of sodium after the injection of 6mM

of potassium per kilogram of body weight, although no sodium had been taken in for five days (Fig. V). This loss of sodium diminished after 18-24 mM per kilogram of potassium had been administered. In those animals receiving sodium chloride, the excretion of sodium was almost equivalent to the administered sodium from the first day of the experiment.(Fig. V).

The excretion of potassium by Group K did not increase significantly until 12mM/Kg. of potassium had been injected, (Fig. VI) and it was not until 24mM/Kg. had been administered that excretion of potassium approximated the intake. There was essentially no increase in potassium excretion in those animals given sodium chloride (Fig. VI).

There was a fall in phosphorous excretion after the administration of 6mM of potassium but there was no consistent difference in the excretion of phosphorous between the two groups of animals (Fig. VII). There was no uniform difference in Cl excretion between the two groups (Fig. VIII).

The average cumulative balance for potassium, sodium and chloride was calculated for each group of animals. The intake of each ion was that administered by injection. The loss of these ions in the feces was not included in the calculation of outgo and probably produces a small but significant error in the potassium balance only. In the potassium chloride treated animals, the retention of potassium amounted to 20.2mM/Kg. of body weight over six days, and the loss of sodium amounted to 10.8mM/Kg. of body weight. The retention of chloride was less than the sum of the

retentions of sodium and potassium. This difference was more marked in the animals receiving potassium chloride than in those receiving sodium chloride (Fig. IX and X).

Analysis of serum during, and at the end of the experiment, revealed complete correction of the extracellular hypochloremic alkalosis, in those animals receiving potassium chloride (initial serum CO_2 content = 30.0mM/L final serum CO_2 content = 23.7mM/L). By contrast, those animals receiving sodium chloride were more alkalotic at the end, than at the beginning of the experiment (Table I).

Analysis of muscle at the beginning of the experiment revealed the characteristic changes in composition noted previously in potassium deficiency.(3). There was a marked increase in muscle sodium and a decrease in muscle potassium (Table II). The increase in intracellular sodium amounted to about two thirds of the decrease in intracellular potassium, as described earlier (3,8) ($Na_i = - 2/3\ K_i$). In the Group K, analysis of muscle during, and at the end of the experiment, revealed a gradual return toward normal composition (Table II). The fall in intracellular sodium during repair, lagged somewhat behind the rise in intracellular potassium.

Discussion

The results of this experiment indicate that the kidney has played a minor role in the correction of extracellular alkalosis; and that transfer of ions from muscle cell, is of cardinal importance in this adjustment. From these data, it is possible to formulate a general theory of extrarenal modification of pH of the extracellular fluids. It is postulated

that there is an equilibrium between cell and extracellular fluid in which there is exchange of potassium, and to a less degree sodium from without, for hydrogen ions constantly formed from metabolic processes within the cell. This has been surmised by Conway with respect to yeast (9). As a result of the net loss of potassium from the body, by any means whatever, extracellular potassium concentration is reduced, and the equilibrium is disturbed. The transport of hydrogen ion outward is reduced, and the content of potassium within the cell falls and intracellular sodium rises. The increment in intracellular sodium (Na_i), is about two thirds of the decrement in intracellular potassium (K_i), - the difference representing the net reduction in hydrogen ion transferred outward. The reduced transfer of hydrogen ion from cell to extracellular fluid, leads to a rise in the bicarbonate concentration and the pH of the extracellular fluid. A new equilibrium is established. In the presence of an added load of sodium, in excess of chloride from the diet, the kidney is unable to correct completely the alkalosis resulting from cell transfer, so that chloride is lost from the body, thereby leading to hypochloremic alkalosis.

On the other hand, when potassium chloride restores tissue composition in alkalosis, due to potassium deficiency, essentially the reverse processes occur. Potassium is transported into the cell, and hydrogen and sodium outward, while chloride remains extracellular. The hydrogen ion combines with extracellular bicarbonate to form carbon dioxide and water. The fall in bicarbonate concentration and pH of the extracellular fluid is a result of

this altered equilibrium, (Fig. XII) and not a result of renal adjustment to the alkalosis.

It must be kept in mind however, that extracellular bicarbonate concentration will not decrease, unless potassium is administered along with a fixed anion. If potassium bicarbonate or acetate were administered, exchange of hydrogen for potassium would simply result in the production of carbon dioxide - without change in extracellular bicarbonate concentration.

The evidence of this theory has been obtained mainly from the study of the second half of this process, namely, the correction of alkalosis associated with potassium deficiency; but it is likely that the mechanism for the development of alkalosis in potassium deficiency is analogous to its repair. Analysis of urine indicates that there was no specific renal correction of the hypochloremic alkalosis during repair with potassium chloride. There actually occurred a greater excretion of titratable acid and ammonia, and less bicarbonate, in the animals receiving potassium chloride than in those receiving sodium chloride. Furthermore, the excretion of chloride in excess of sodium and potassium was greater in the potassium treated animals. These renal adjustments should have exacerbated, rather than corrected the extracellular alkalosis. This indicates an excessive transfer of hydrogen from the cells to the extracellular fluid. Although the renal excretion of potassium, in the potassium chloride treated animals, increased considerably in the course of the experiment, the output of hydrogen ions in the urine increased in an almost parallel fashion. Whereas in the sodium chloride treated group, hydrogen ion output

changed relatively little (Fig. XIII). The hypothesis of Berliner (6) that hydrogen and potassium secretion are reciprocally related, is not consistent with these data.

Muscle analysis further supports the theory presented in this paper. Darrow et al (3) originally, and Muntwyler and Griffin (8) subsequently, demonstrated that in potassium deficiency with alkalosis, the rise in intracellular sodium amounted to approximately two thirds of the fall in intracellular potassium. If electrical neutrality is to be maintained, some cation other than sodium must have exchanged for potassium. The data of Cotlove et al (11) indicate that the cation is not calcium or magnesium. By exclusion hydrogen ion exchange seems almost mandatory.

It is probable that part of the potassium leaves the cell during the development of deficiency, accompanied by an anion such as lactate or bicarbonate; but this would be equivalent to an exchange for hydrogen ions from the extracellular fluid. This hypothesis is suggested from the fact that the difference between the sum of the serum sodium and potassium concentrations, and the sum of chloride and bicarbonate, is 24.3 mEq./l in the alkalotic animals. By comparison this difference is 18.4 mEq./l in the corrected animals. These findings are consistent with the data of Browne and Vineberg (12) who described a rise in serum lactate concentration of 5 mEq./l during respiratory alkalosis.

Muscle analysis, obtained during the repair of potassium deficiency in these experiments, suggests some lag in the excretion of sodium from the cells, data consistent with those of Muntwyler et al (8). The slow rise in the renal excretion of sodium parallels these findings (Fig.XIV).

Furthermore, the rapid fall in extracellular bicarbonate concentration (TableI), before replacement of more than a part of the deficit of potassium, suggests that preferentially hydrogen ion is exchanged for potassium during repair, with subsequent transfer of sodium out of the cells. If three quite probable assumptions are made, namely that muscle makes up 50 per cent, and extracellular fluid 20 per cent, of the body weight, and also that the fat free solids (FFS) of muscle remain constant over a short period of time; then the actual body balance of chloride, sodium and potassium, after 18 mM per kilogram of potassium chloride have been injected (3 days), is of the same order of magnitude as the theoretical balance calculated from observed changes in muscle composition during the same period of study. The balances are compared for this period of study, rather than at the end of one week, to avoid cumulative errors as undetermined losses, and to emphasize the large changes in body composition which occur rapidly. It is assumed that the determined balance of chloride, 3.7mM/Kg., remains in the extracellular fluid. This balance of extracellular chloride is used to calculate the change in extracellular volume, according to the method of Darrow (6).

Calculations:* per Kg. of body weight

$$(Cl)_2 = (H_2O)_{e2} \times [Cl]_2$$

$$= .200 \times 119 = 23.8 \text{ mM}$$

$$(Cl)_1 = (Cl)_2 - (Cl)$$

$$= 23.8 - 3.7 = 20.1 \text{ mM}$$

$$(H_2O)_{e_1} = \frac{(Cl)_1}{[Cl]_1}$$

$$= \frac{20.1}{104.9} = .192 \text{ Kg.}$$

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*( )	refers to total amount in mM or Kg.
[ ]	refers to concentration in ultrafiltrate of serum in mM per kilogram of water.
(H_2O)	refers to volume of fluid in Kg.
FFS	refers to fat-free solids of muscle in gm. and equaled 120 g./Kg. of rat.
Subscripts e and i	refer to extracellular and intracellular respectively.
Subscripts 1 and 2	refer to values before and after correction respectively.

$(Na)_2 = (H_2O)_{e2} \times [Na]_2 + FFS \times (Na)_i$ 2/100g.FFS

$= .200 \times 144.0 + 120 \times 5.7$

$= 35.6$ mM

$(Na)_i = (H_2O_e)_1 \times [Na]_1 + FFS \times Na_i$ 1/100g.FFS

$= .192 \times 147.4 + 120 \times 11.9$

$= 42.6$ mM

$\Delta (Na) = (Na)_2 - (Na)_1 = -7.0$ mM

Likewise

$(K) = (K)_2 - (K)_1 = +14.4$ mM

Theoretical Cl Balance = +3.7mM/Kg. Actual Cl Balance = + 3.7mM/Kg.
Theoretical Na Balance = -7.0mM/Kg. Actual Na Balance = - 8.2mM/Kg.
Theoretical K Balance = +14.4mM/Kg. Actual K Balance = + 14.3mM/Kg.

If the difference between the decrease in sodium within muscle ($(Na)_i$), and the increase in potassium ($(K)_i$) during repair with potassium chloride, is assumed to be mainly hydrogen ion transferred from cells to extracellular fluid, it is possible to calculate the theoretical decrease in the amount of extracellular bicarbonate (theoretical $(HCO_3)_e$) which results from extrarenal correction. The normogram of Singer and Hastings (12) is also used to correct for the buffering capacity of whole blood with the observed change in pH.

Calculation: per Kg. of body weight

$(HCO_3)_{e_2} = (H_2O)_{e_2} \times [HCO_3]_{e_2}$

$= .200 \times 27.3 = 5.46$ mM

$(HCO_3)_{e_1} = (H_2O)_{e_1} \times [HCO_3]_{e_1}$

$= .192 \times 33.3 = 6.40$ mM

$(HCO_3)_e = (HCO_3)_{e_2} - (HCO_3)_{e_1} = -0.9$ mM

Buffer base (Calculated) $= -0.3$ mM

Actual $(HCO_3)_e$ $= -1.2$ mM/Kg.

Calculation of theoretical $(HCO_3)_e$ from H transport.

$(Na)_{i_2} = FFS \times Na_i$ 2/100g.FFS

$= 120 \times 5.7 = 6.8$ mM

$(Na)_{i_1} = FFS \times (Na)_{i_1}$ 1/100g.FFS

$= 120 \times 11.9 = 14.3$ mM

$(Na)_i = (Na)_{i_2} - (Na)_{i_1} = -7.5$ mM

Likewise

$K_i = (K)_{i_2} - (K)_{i_1} = 14.0$ mM

Theoretical $(HCO_3)_e = -Na_i - K_i$

$= -6.5$ mM/Kg.

Theoretical $(HCO_3) = -6.5$ mM/Kg. Actual $(HCO_3)_e = -1.2$ mM/Kg.

As can be seen from the above calculations, the fall in extracellular bicarbonate calculated from change in muscle composition is considerably greater than that actually measured. The reason for the discrepancy is obvious, in view of the urinary findings during repair with potassium chloride. The presence of a low urine pH, a high titratable acid, and ammonia excretion as well as a low bicarbonate output, indicates some renal compensation to prevent extracellular acidosis which results from the transfer of large quantities of hydrogen from the cells. The hydrogen ion excreted as titratable acid, and the difference in ammonia excretion between the two groups of animals, may be considered equivalent to potential extracellular bicarbonate (a probably valid assumption). The actual change in extracellular bicarbonate plus the bicarbonate conserved by the kidney, (the sum = Effective $(HCO_3)_e$) is of the same order of magnitude as the theoretical fall in extracellular bicarbonate as derived from muscle analysis.

Actual	$(HCO_3)_e$	=	- 1.2
+	Tit. Ac	=	- 1.0
+	NH_3	=	- 2.5
Effective	$(HCO_3)_e$	=	- 4.7 mM/K
Theoretical	$(HCO_3)_e$	=	- 6.5 mM/K

No conclusions can be drawn as yet from these experiments as to the effects of alkalosis on the transport of potassium out of muscle cells. However, the presence of intracellular acidosis with concomitant extracellular alkalosis is consistant with the findings of Wallace and Hastings (13).

Summary

Alkalosis, associated with potassium deficiency, was produced in rats by injection of desoxycorticosterone acetate, while on a diet deficient in potassium and containing an excess of sodium over chloride. Over a period of six days one group of these animals received injections of 6mM of potassium chloride daily, while another group received 6mM of sodium chloride daily.

The group receiving potassium chloride restored serum and muscle composition to normal over a period of three to six days. This repair was accompanied by an increase in the urinary titratable acidity and in ammonia content. The sodium chloride group showed no correction of serum or muscle composition, and there was no change in urinary titratable acidity and ammonia, over this period.

It is pointed out that the repair of alkalosis in the rats receiving potassium chloride is accounted for by the transport of electrolyte between muscle and extracellular fluids. Since the gain of potassium by the cells is greater than the loss of sodium from the cells, hydrogen ion must be exchanged to preserve electrical neutrality. The calculated hydrogen ion transport from cell to extracellular fluid is sufficient to account for the reduction in serum bicarbonate concentration, and the increase in urinary titratable acidity and ammonia.

A general theory of extrarenal modification of acid-base balance is formulated from these findings.

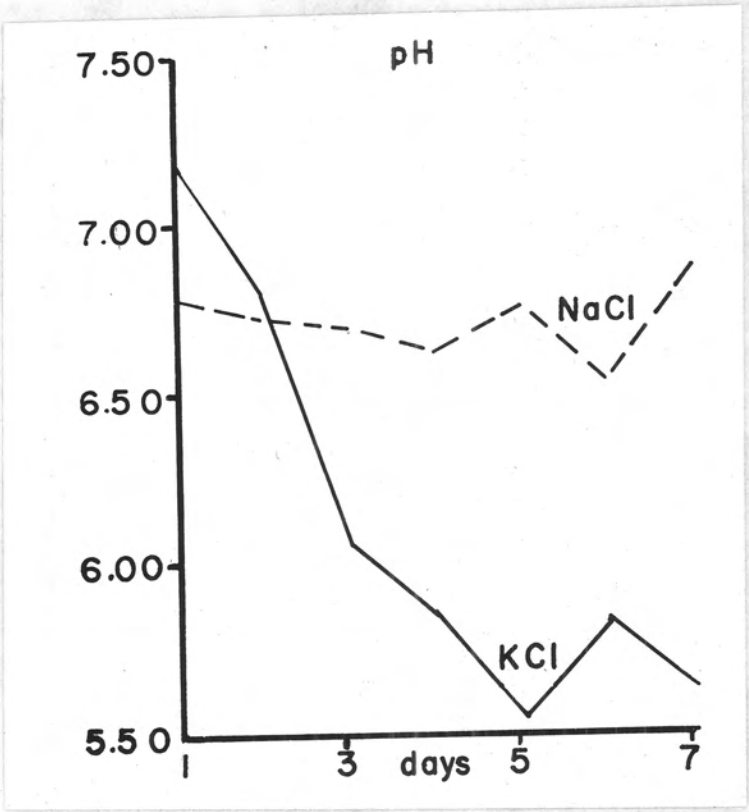

Fig. I. Average Urinary pH of Groups K and Na.

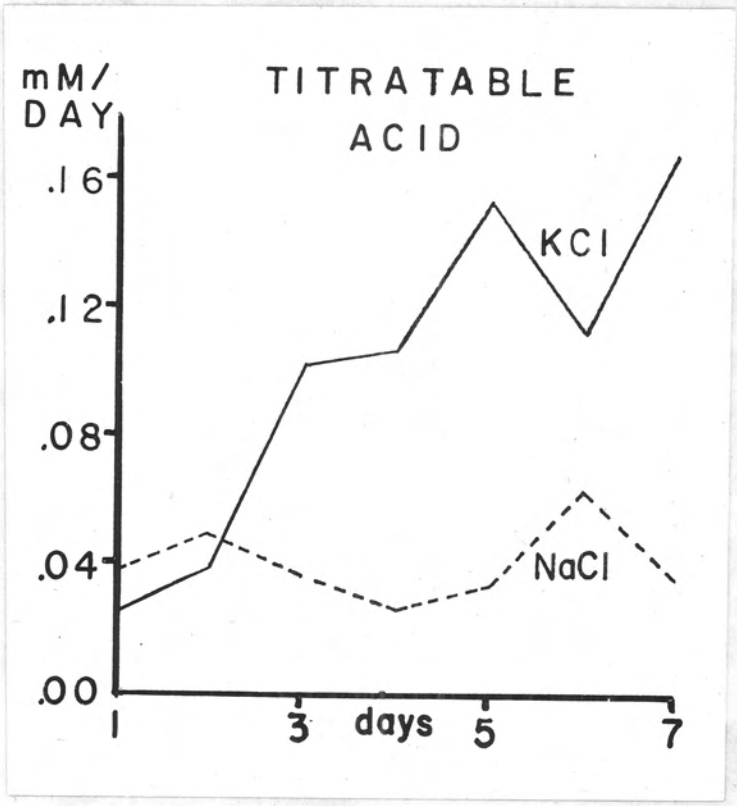

Fig. II. Average Titratable Acid Excretion in Groups K and Na.

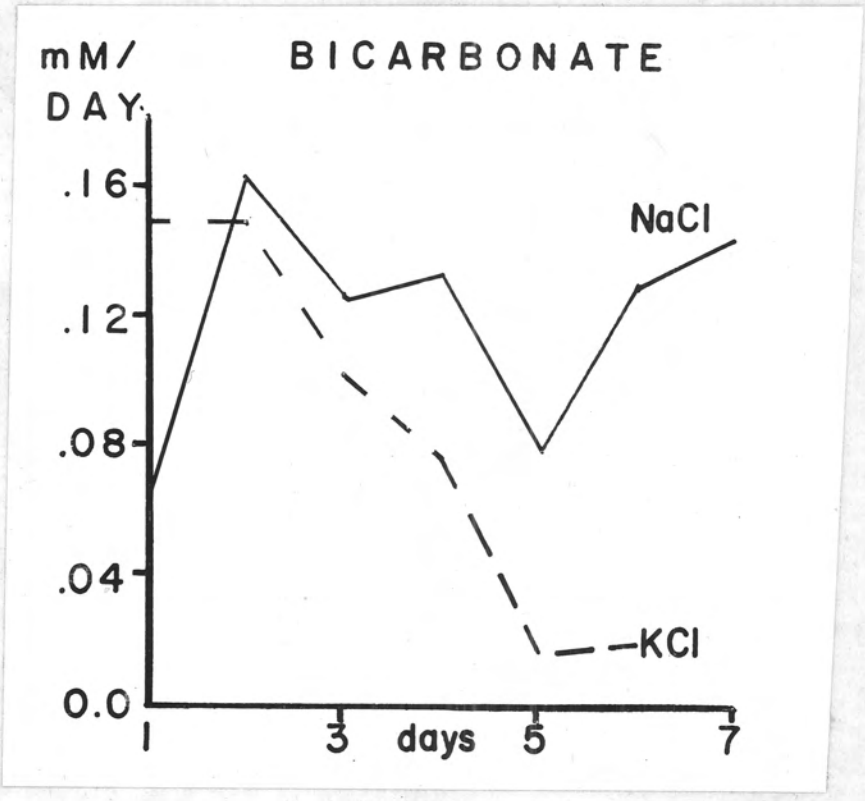

Fig. III. Average Urinary Bicarbonate Excretion in Groups K and Na.

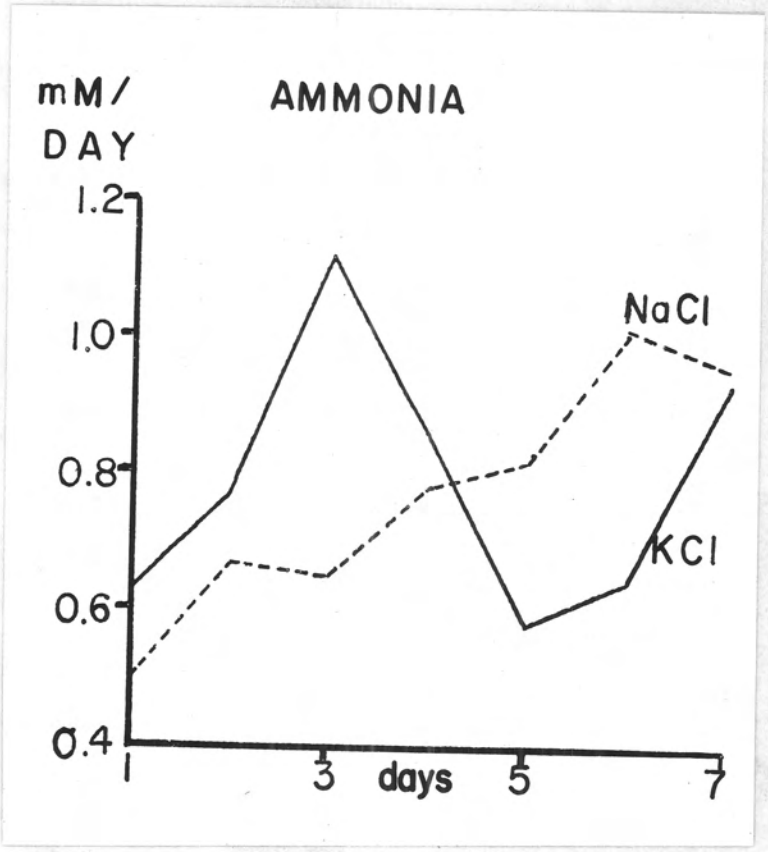

Fig. IV. Average Urinary Ammonia Excretion in Groups K and Na.

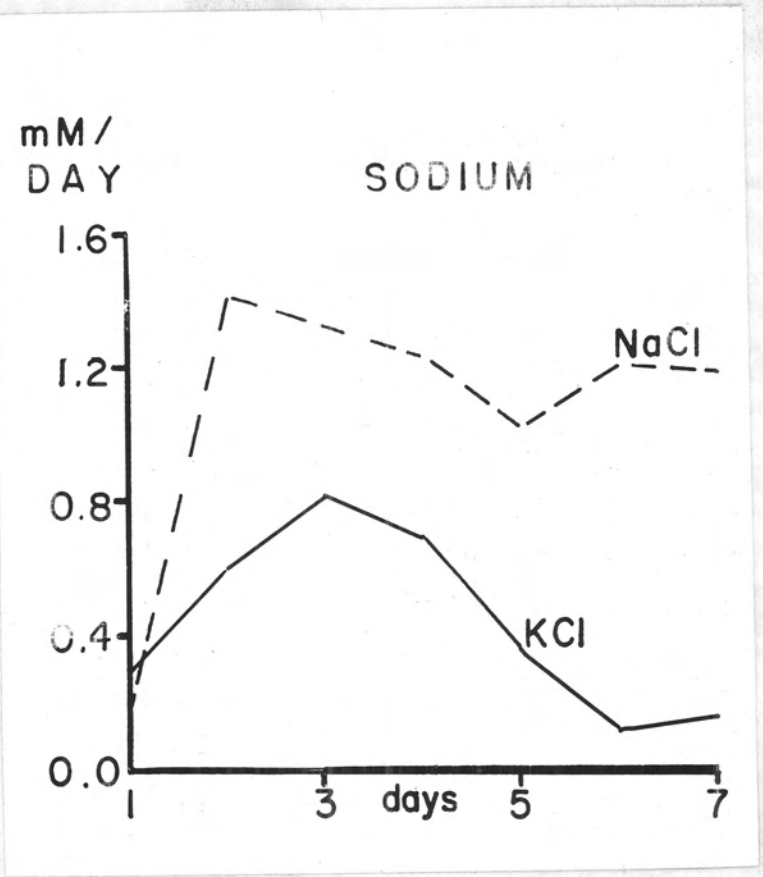

Fig. V. Average Urinary Sodium Excretion in Groups K and Na.

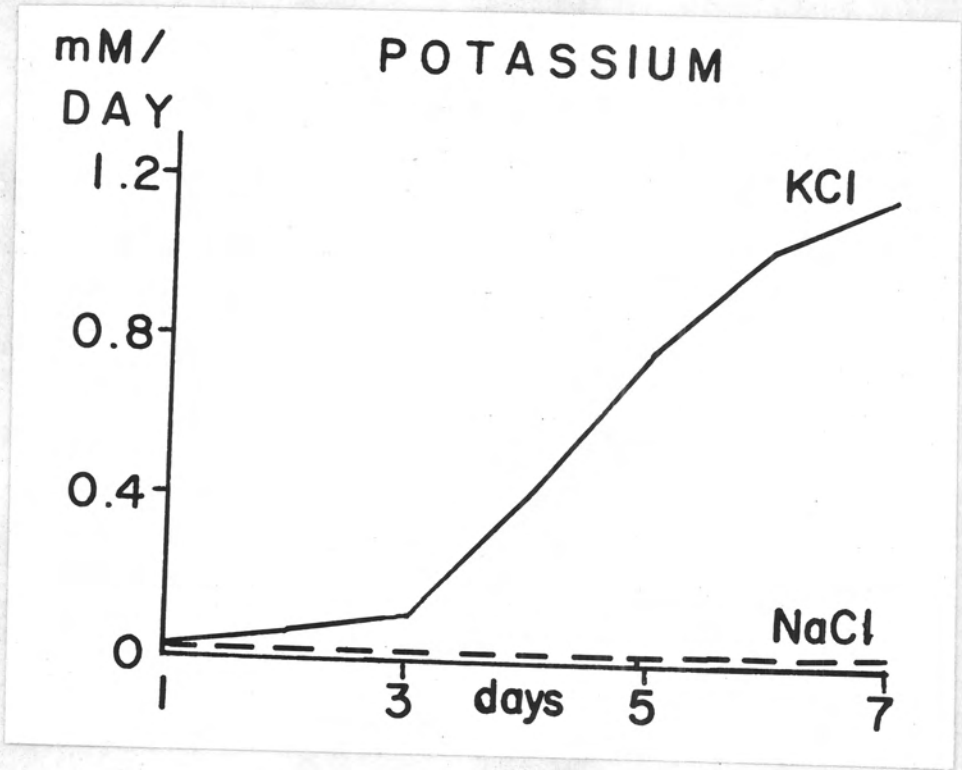

Fig. VI. Average Urinary Potassium Excretion in Groups K and Na.

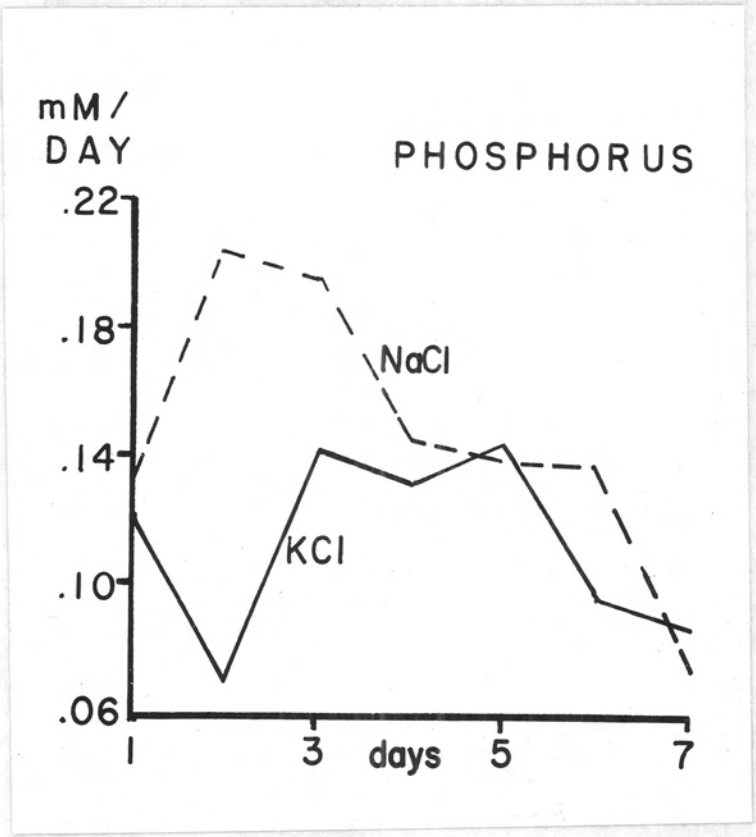

Fig. VII. Average Urinary Phosphorus Excretion in Groups K and Na.

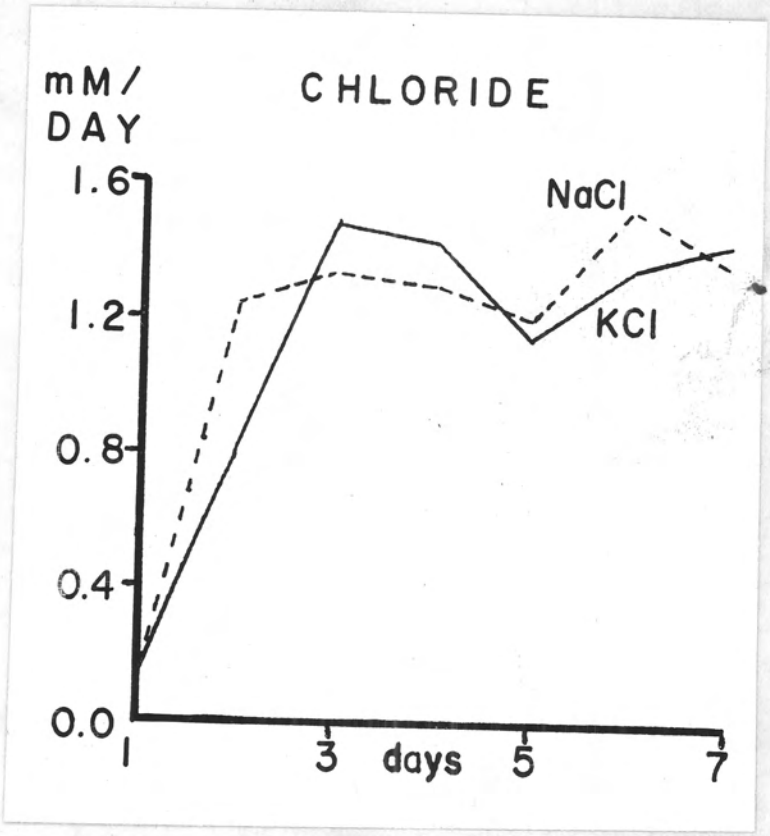

Fig. VIII. Average Urinary Chloride Excretion in Groups K and Na.

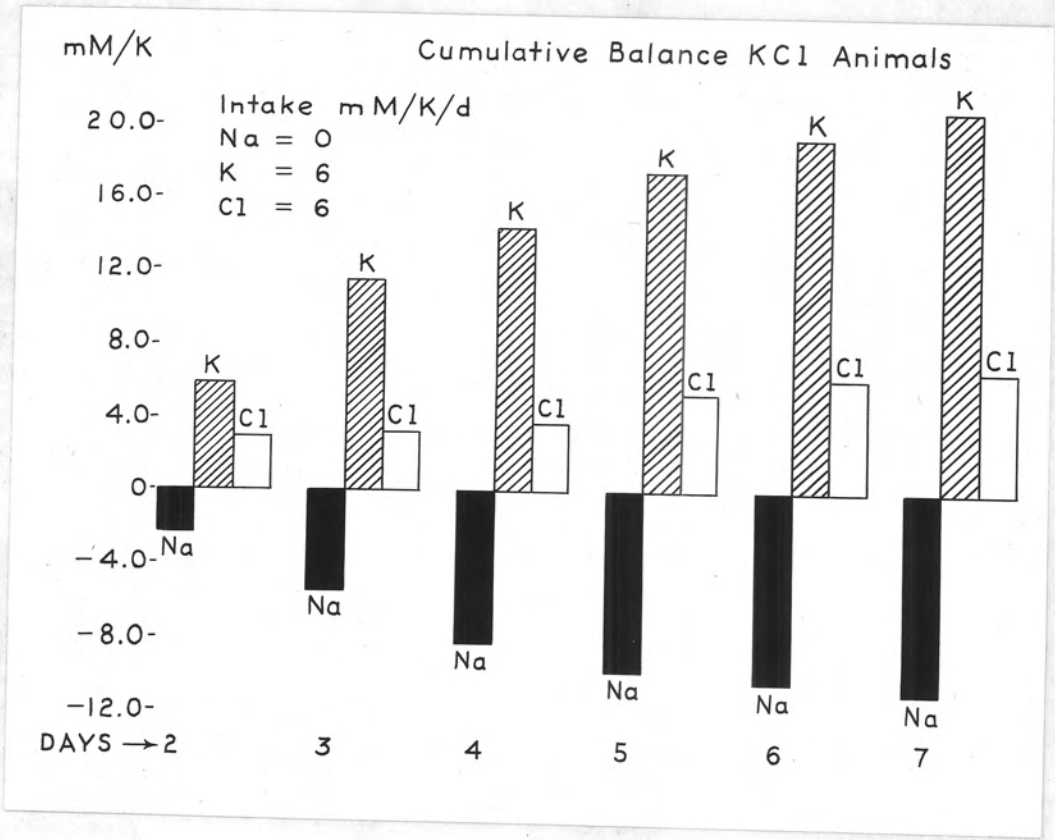

Fig. IX. Cumulative Balance of Sodium, Potassium, and Chloride during Administration of KCl.

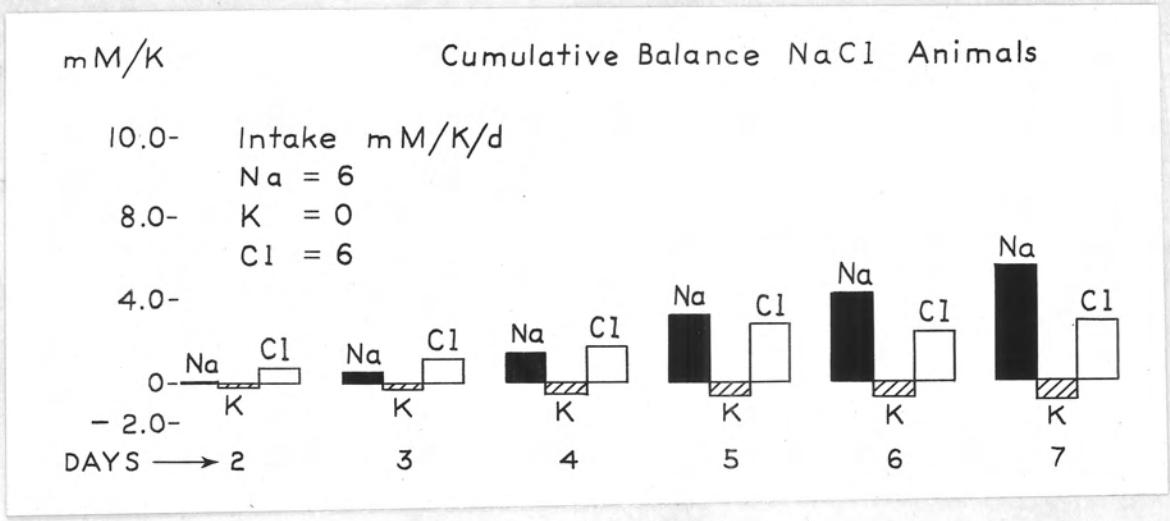

Fig. X. Cumulative Balance of Sodium, Potassium, and Chloride during Administration of NaCl.

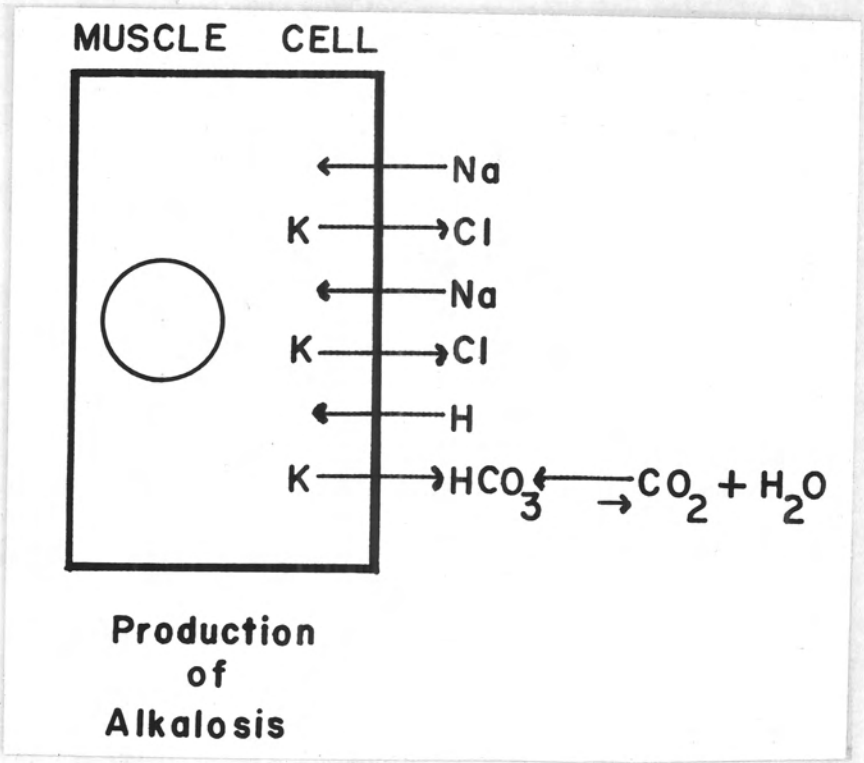

Fig. XI. Mechanism of Production of K deficiency Alkalosis

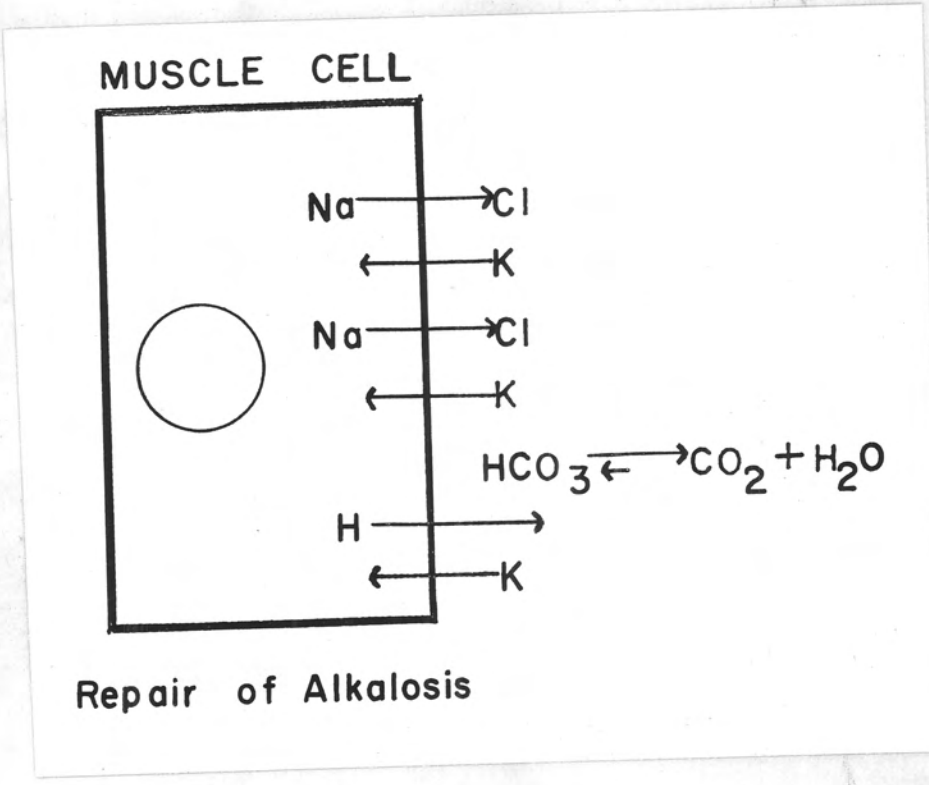

Fig. XII. Mechanism of Repair of K Deficiency Alkalosis

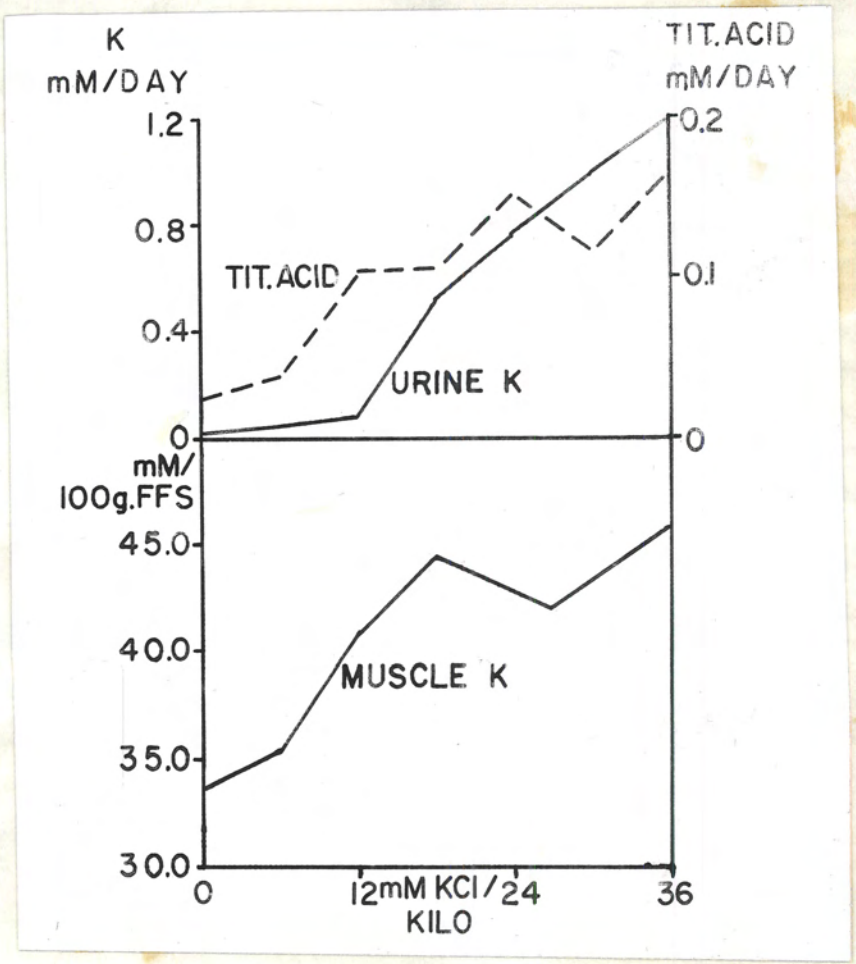

Fig. XIII. Relationship of Urinary Titratable Acidity and Potassium Excretion to Muscle Potassium Content

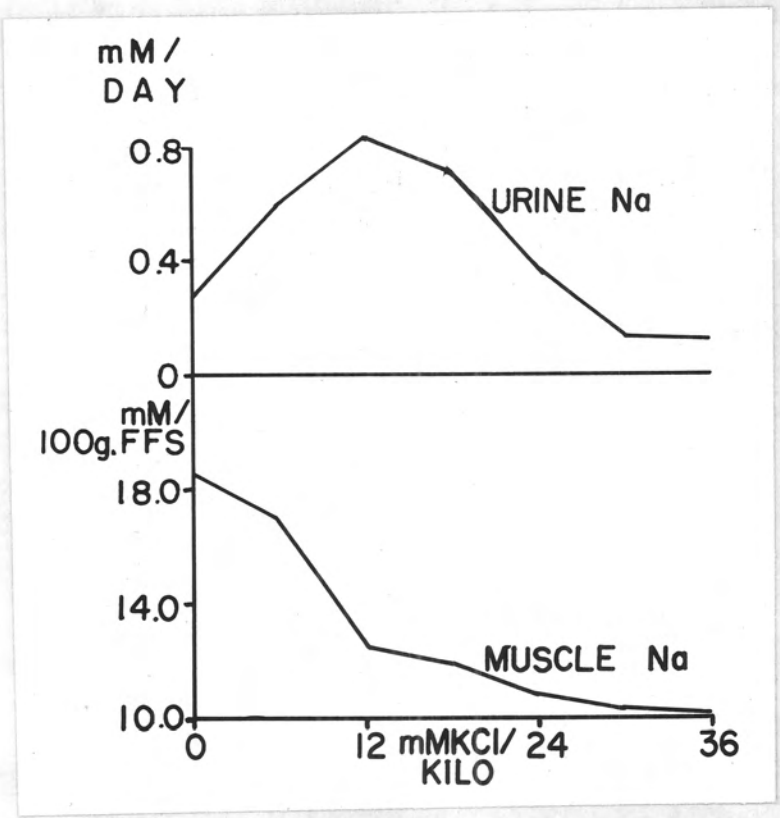

Fig. XIV. Relationship of Urinary Sodium to Muscle Sodium content

TABLE I

Average Serum Analyses

Group	No.	pH	H_2O	Na	K	Cl	HCO_3	[Na]	[K]	[Cl]	$[HCO_3]$
			g/l	mM/l	mM/l	mM/l	mM/l	mM/Kg of water in ultrafiltrate of serum*			
Normal (10)	-	7.28	935	143	5.4	103	21	145	5.4	115	23
Deficient	6	7.50	935	145	2.7	93	30	147	2.7	105	33
NaCl 36mM/Kg	8	7.46	935	143	2.9	96	30	145	2.9	108	34
KCl 6mM/Kg	3	7.56	935	144	3.1	95	27	146	3.1	107	31
KCl 12mM/Kg	2	7.45	935	143	4.0	107	21	145	4.0	120	23
KCl 18mM/Kg	3	7.37	935	142	4.8	106	24	144	4.8	110	27
KCl 27mM/Kg	4	7.43	935	141	4.4	101	24	143	4.4	114	27
KCl 36mM/Kg	2	7.45	935	141	4.6	105	23	143	4.6	118	25

* Donnac factor 0.95

TABLE II

Average Muscle Composition per 100g. Fat-free solids

Group	No.	H_2O	Cl	Na	K	Phos.	Na_i
Normal (10)	-	340	7.1	10.0	48.9	32.4	2.4
Deficient	6	334	5.71	18.5	34.1	34.2	11.9
NaCl 36mM/Kg	8	332	4.81	18.1	33.0	35.5	13.0
KCl 6mM/Kg	3	336	6.72	16.8	35.5	36.0	9.0
KCl 12mM/Kg	2	334	5.62	12.5	40.4	34.9	6.9
KCl 18mM/Kg	3	319	6.17	11.9	44.4	35.1	5.7
KCl 27mM/Kg	4	322	5.97	10.7	42.0	35.9	4.3
KCl 36mM/Kg	2	317	4.89	10.1	45.7	- - -	5.3

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A METHOD FOR THE ESTIMATION OF THE BROMIDE SPACE

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Within recent years the need for investigation of the behaviour of extracellular body water has become increasingly apparent. Dissatisfaction of the present day methods, as applied to edematous conditions, has of late been expressed (1). It seems that most substances are reliable only in non edematous states.

Complex polysaccharides have a large molecular weight and size, and evidence is accumulating that these substances do not readily enter connective tissues (2). The urinary losses through the kidney provide an additional hazzard. Inulin (3), mannitol (4), and sucrose (5) have been utilized. A study of the behaviour of radio-sodium (6) and radio-bromide (7) has been carried out, but elaborate equipment and speed (in the latter two cases) are necessary.for these determinations.

More recently the thiosulphate ion has been employed (8). This ion enters the red blood cells and some is metabolized in the liver. It seems that is not appreciably bound by non diffusable protein components of human serum (8).

Obviously, with the ideal substance, there should be no excretion, metabolism, sequestration, or permeation of the cells.

The use of the bromine ion (9) provides some advantages. Besides its relatively small size, and its presumed entrance into connective tissue, less than 1 per cent is excreted in the urine over a 5 hour period (9). This latter error is very small. However, there is some

sequestration of bromine in the central nervous system, which is apparent after a 24 hour period (10). The work of Wallace and Brodie (10) shows that in dogs, after 3 hours, the spinal fluid level is some 25 per cent of the bromine present in the blood serum. After 24 hours this value increases to about 50 per cent. About 1/3 of the spinal fluid bromine (milli-equivalents per liter) enters into a kilogram of nervous tissue. This error is not remarkable and the bromine ion reaches equilibrium, outside the nervous system in a very short time (about 2-1/2 hours).

The bromide space is thought to be the same as the chloride space (11), (12), and this is an index of extracellular fluid volume. We do not as yet know exactly how much of the bromine ion enters the cells of man, and this provides the main source of error and difference between the bromide space and the true extracellular space.

Possibly technical difficulties encountered in the estimation of blood bromide levels have been a deterring factor in the exploitation of this ion. The method described below, which is a modification of that of Conway (13), is offered as an easy and conveniently accurate assay.

TABLE III. (PATHOLOGICAL CASES)

Patient	Age in Years	Weight in Kilograms	Remarks	Bromine given in mgs.	Serum* Bromine mg%	Hours following dose	Extracellular Water Index (litres)	Bromide Space (% Body Weight)+
D.A. (M)	31	61.6	Cirrhosis of Liver, Ascites (retaining chlorides) no edema obvious.	2350	12.20	5	19.25	28.2
					12.07	9	19.45	28.4
					12.00	22	19.60	28.6
					11.86	30	19.80	28.9
M.H. (F)	50	72	Slight edema of legs (mild cardiac failure) and rheumatoid arthritis	2350	12.41	4-1/2	26.30	23.7
					11.40	9	28.6	25.7
					11.02	24	29.6	26.6
					11.02	28	29.6	26.6
O.S. (M)	60	58	Definite cardiac failure. Carcinomatosis. Generalized edema.	2013	8.28	5	24.2	37.0
					7.11	8	28.3	43.0
					7.20	23	28.0	43.9
E.V. (F)	50	132	Exceptional obesity. ? Acromegaly	3355	15.62	2-1/2	21.45	14.5
					14.37	4-1/2	23.32	15.9
					15.62	19	21.45	14.5

* Corrected for Donnan factor and for serum water.

\+ Corrected for red cell Bromine Content (10% - 13%)

Experimental

Determination of serum water.

1 c.c. of serum to be assayed is placed in a 15 c.c. centrifuge tube and weighed. About 1 c.c. of distilled water is added and the serum is dried in an oven at 95° C. The dry weight of the serum is noted and the serum water is calculated.

Determination of Bromine.

5 c.c. of distilled water are added to the tube containing dried serum, and this is allowed to stand for about 30 minutes. The tube is gently agitated once or twice, and the mouth can be covered with paraffin paper. A 4 c.c. aliquot is taken and this is transferred to the outer chamber of the porcelain conway unit (13). The unit is placed in the 95° C oven and the aliquot is evapourated to dryness. The unit is then transferred to a muffle furnace at 380-400° C. for 18 to 30 minutes (20 minutes was used, as a rule, by us). After cooling on asbestos, the procedure is the same as that described by Conway (13). Roughly 0.2 of a gram of powdered potassium dichromate is placed into the outer chamber, and 1 c.c. of 20 per cent potassium iodide into the center chamber. Approximately 1 c.c. of distilled water is run into the outer chamber and the ground glass lid, which is coated with a suitable mixture of paraffin and mineral oil (3:1) is almost completely placed in position. Approximately 1 c.c. of 45 per cent pure sulphuric acid is gently and quickly run into the outer chamber with a graduated eye dropper, and the unit is then completely sealed. The sulphuric acid reacts with the

dichromate and free bromine is liberated which displaces an equivalent amount of iodine. The iodine liberated in the center chamber is titrated after 2 hours with $\frac{N}{100}$ sodium Thiosulphate, using a few drops of 0.2 per cent soluble starch as an indicator. A microburette is necessary, and the one used by these authors was devised from a micrometer screw guage as devised by Scholander (14). The volume of our burette is 0.937 c.c. and this is conveniently divided into 1000 divisions. The whole procedure is done in duplicate or triplicate. With respect to the investigations presented below, the determinations were done in quadruplicate.

Calculations.

As the bromide liberated from the outer chamber displaces the equivalent amount of iodine the concentration of bromine in micrograms per c.c. is as follows:

$$\text{No. of Divisions on the Microburette} \div 1000 \times (0.937 \times 79.9 \times 1.07 \times 5/4) \times \frac{1000}{100}$$

0.937 = volume of burette in cubic centimeters

79.9 = equivalent weight of bromine

1.07 = back diffusion factor

5/4 = correction for aliquot taken (4 c.c. of 5 c.c.)

This expression in brackets = 100 (approx.)

The factor 1.07 is the back diffusion factor which arises from the slight back diffusion of the liberated iodine from the center chamber. This was determined by using standard iodine solutions in the center chamber, other reagents being as usually employed. This factor applies at least

for levels of bromine between 80-200 micrograms per c.c. . It slightly diminishes with higher levels.

Accuracy.

Using a standard solution of bromide, which contained 159.8 micrograms of bromine per c.c., and adding 1 c.c. of this to 1 c.c. of normal serum, complete recovery was obtained in 25 separate determinations, with a standard deviation of $\pm$ 2.6.

The Estimation of Red Cell Bromide Content.

The hematocrit of the patient is determined. The blood sample is drawn and placed into a graduated centrifuge tube. After centrifuging the sample the total volume is noted. Upon removal of the plasma or serum required for serum bromine estimation, the remaining volume is noted and recorded, and the amount of serum or plasma contaminating the packed red cells is calculated. This is allowed for when the bromine content of the red cells is estimated. The remaining sample is poured into a small beaker, and any remaining cells are washed over with distilled water. Drying is then carried out in the 95° C. oven. Before the residue is completely hard, it is broken up into small fragments with a glass rod. After drying, 10 c.c. of distilled water are added, and the beaker is sealed with paraffin paper and allowed to stand over night. Next morning, after filtering the extract, an aliquot is taken and is placed in the outer chamber of the conway unit. This is then dried, ashed, and assayed by the method already described.

Calculation of Red Cell Content.

It is assumed that 40 c.c. of red blood cells are present for each kilogram of body weight. Therefore 40 X amount of bromine per c.c. of packed red cells equals the amount of bromine in the red cell per kilogram. = value [1] The total amount of bromine administered to the individual is known, so that this value divided by the weight in kilograms, equals the bromine content per kilogram of Bromide Space. = value [2] Hence from these two values the percentage of bromine which enters into the red cell is calculated. This has been found to be about 10 - 12 per cent.

Urinary Bromide Losses

Investigation of the urinary bromide losses in 6 normal adults, using the same technique as described for blood serum, showed that for the first 6 hours after the initial dose, 1.95 per cent of bromine ion was lost in the urine. Values ranged from 0.92 per cent to 2.90 per cent. After 12 hours 3.80 per cent of the bromine ion administered, was excreted, and here values ranged from 2.68 per cent to 4.76 per cent. It is noteworthy that those persons excreting the most chloride also excreted the most bromide. The small bromide loss which occurs in a 3 hour period has not been allowed for.

The Estimation of the Bromide Space.

1. (Normal Adults)

Sixteen normal adults have been investigated (8 males, and 8 females) and the results are set out below (Tables I and II). Three grams of potassium bromide were given orally to normal persons above 60 kilograms in weight, and 2 grams if below. This barely raises the serum bromine level to 1 milli-equivalent. Epileptics are kept at a level of about 10 milli-equivalents per litre, while toxic manifestations are associated with values approximating 20 milli-equivalents (15).

Blood samples, as can be seen from the data presented, have been drawn at various times over a 24 or 26 hour period. For practical purposes, it seems that constancy is reached shortly after 2-1/2 hours, and is maintained for several hours. Most cases show a reduced value after 24 hours, which is mostly due to sequestration and urinary loss.

In estimating and calculating the Bromide Space, account has been taken of the Gibbs-Donnan factor, the serum water, and the entrance of bromine into the red blood cells.

2. (Pathological Cases)

Three patients with pathological edema and one with exceptional obesity have been investigated (Table III). This latter female (E.V.) showed, as one would expect, a markedly reduced bromide space in terms of body weight due to excess adipose tissue. The first case (D.A.) had no obvious edema but subsequent to this investigation 6 litres of fluid were removed from the abdomen. His chloride excretion was very low

which probably accounts for the retarded fall in Serum bromide over 30 hours. The patient (O.S.) with generalized edema showed a markedly raised bromide space. These limited investigations suggest that, for practical purposes, the bromine ion reaches equilibrium in edematous conditions in a desirably short time.

Results - Tables

The average bromide space for 8 normal males is 21 per cent of body weight, and that for 8 females is 20.4 per cent. As expected, the fattest individuals had the smallest bromide space.

Discussion.

The method, although somewhat extended in time, is itself not time consuming, and a large number of determinations can be carried out simultaneously. The method is, as stated, adapted from that of Conway (13).

We were not able to obtain complete recovery of bromine by ashing over a Bunsen flame at a dull red heat as has been suggested (13). With excess heat bromine is lost, and with incomplete ashing also, bromine is not completely recovered. This latter phenomenon may be due to the adsorption effects of carbon. The temperature time relationship, used in the muffle furnace, was worked out after many experimental trials. It is surprising that the addition of small amounts of sodium bicarbonate is not conducive towards a satisfactory ash, and interferes with the recovery of bromine, (at least for the above temperature time relationship).

The use of the Conway unit itself for ashing, instead of a crucible, overcomes the difficult task of accurately having to transfer the solute of the ash to the Conway unit.

Preliminary investigations suggest that this method is of practical use under pathological conditions. Data are presented concerning bromide loss in the urine, and this small loss is neglected for the short time required for equilibrium under normal circumstances. As most edematous patients retain chloride - no doubt the bromide loss is often conveniently small, in the presence of edema.

Summary.

A method is presented for the estimation of blood bromine. This is accurate and in comparison with other existing space determinations, is relatively simple and represents a modification of the technique of Conway. A large number of determinations can be carried out at the same time. A method for assay of the bromine entering red cells is presented. This error is allowed for. Information concerning urinary bromide loss is also demonstrated. This small error is neglected.

Sixteen normal adults have been investigated (8 males, and 8 females) and their Bromide Space calculated. The mean value shows that this space represents 21 per cent of body weight, in males and 20.4 per cent in females, which is close to what is believed to represent the true extracellular water space. Preliminary investigations (4 patients) is suggestive that this method is of practical use in edematous and pathological conditions.

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TABLE I (NORMALS)

Subject (males)	Age in Years	Height in Inches	Weight in Kilograms**	Serum Bromine* mg%	Hours following dose	Extracellular Water Index in Litres	Bromide† Space† (%Body Weight)
D.B.C.	27	64	60	8.80	2	15.25	22.8
				8.68	4	15.45	23.0
				8.57	8	15.66	23.4
				8.12	24	16.55	24.3
W.E.S.	28	71	81.8	10.70	1	18.80	
				8.98	2	22.40	24.6
				8.74	4	23.0	25.2
				9.15	7	22.0	24.2
				8.24	25-1/2	24.0	26.7
A.J.B.	29	77	74	11.20	2	18.14	22.1
				11.20	4-1/2	18.14	22.1
				10.90	8-1/2	18.50	22.5
				9.80	24-1/2	20.50	24.9
I.S.	37	67	70	13.75	2-1/2	14.65	18.5
				13.70	4-1/2	14.68	18.5
				12.60	24	15.97	20.2
P.F.	35	64	74	13.53	2-1/4	14.89	17.3
				13.48	4-1/4	14.96	17.4
				12.50	24	16.10	18.7
M.D.	25	74	84	9.74	2-1/2	20.7	21.4
				10.00	4-1/2	20.1	20.9
				8.00	24	25.2	26.1
R.E.C.	32	71	79.6	11.60	2-3/4	17.35	19.7
				11.60	5	17.35	19.7
				9.70	26	20.8	23.5
J.G.	35	72	74	11.75	3	17.1	20.4
				10.95	6	18.4	22.0
				9.74	26	20.7	24.9

** Subjects above 60 kilograms were given 2013 mgs. of Bromine - below 1343 mgs.

* Corrected for Donnan factor and for serum water.

† Corrected for red cell bromine content (10% - 13%).

TABLE II. (NORMAL FEMALES)

Subject (females)	Age in Years	Height in Inches	Weight in Kilograms**	Serum Bromine* mg%	Hours following dose	Extracellular Water Index (litres)	Bromide+ Space (%Body Weight)
F.C.	29	62	50	13.3	2-1/4	10.02	18.0
				13.05	4-1/4	10.42	18.7
				13.46	8-1/4	10.25	18.4
				12.62	24-1/4	10.64	19.1
M.B.	25	65	52.7	11.75	3	11.4	19.4
				11.10	6	12.1	20.7
				9.04	26	14.3	22.3
E.T.	20	65	55	10.68	3	12.53	20.6
S.L.	25	62	59.5	9.90	3	13.56	20.5
J.S.	27	65	59	10.12	3	13.26	20.2
T.K.	24	63	58	10.12	3	13.26	20.5
G.C.	22	63	52	11.27	3	11.9	20.6
G.N.	24	67	60	9.42	3	14.25	21.3

** Subjects were given 1343 mgs. of Bromine

* Corrected for Donnan factor and serum water.

+ Corrected for red cell Bromine Content (10% - 13%).