STUDIES ON RENAL FUNCTION IN THE COW WITH PARTICULAR REFERENCE TO SODIUM AND POTASSIUM EXCRETION

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PREFACE

The kidneys are commonly described as excretory organs, but few physiologists would accept this as an adequate description. In distinguishing between the external environment of an organism and the immediate environment of the component cells of that organism, Claude Bernard established more than 100 years ago a unifying concept in physiology within which the interrelation of the activities of the body came increasingly to be appreciated. A wide variety of physiological devices and processes operate to preserve and stabilise the physical properties and chemical constitution of this internal environment and the resulting constancy, as Bernard pointed out, liberates the organism from immediate and continuous dependency on the conditions which obtain in the external world. The kidneys are therefore more properly regarded as an important executive of the processes which regulate the volume and the composition of this internal fluid environment; the excretory function of the kidneys is incidental to this regulatory function.

Most experimental investigations of mammalian kidney function have been carried out on dogs, in which the renal responses to experimental procedures are in general closely similar to those of man. In addition the dog may conveniently be studied using the facilities available in most physiology laboratories. Herbivores have been used much less frequently since the findings of such investigations clearly may less readily be applied to man, because the major dietary differences must give rise to differences in homeostatic requirements. Also the accommodation and restraint of large domestic herbivores in laboratories

designed for work on co-operative human volunteers, or on experimental animals no larger than the dog, present considerable practical difficulties.

The ruminant herbivore has nevertheless attracted considerable attention from physiologists; but this has been directed mainly towards the alimentary tract, on which much information has been gained from applying techniques originally developed in the dog and in man. The normal diet of herbivores, however, differs from the normal diet of man and the dog, not only in its greater content of material indigestable by mammalian gastrointestinal enzymes, but also in its mineral content. In particular it is well known that the normal diet of grazing animals is high in potassium and low in sodium content, so that the excretion of these ions must involve differences in emphasis of the renal processes which have been described in man and in the dog.

The following study of kidney function in the cow was therefore undertaken to investigate these differences and so to establish more clearly some details of the homeostatic role of the kidney in this animal. It is hoped that the findings will contribute to our general understanding of mammalian kidney function and also aid in furthering our understanding of disease conditions in cattle which involve disturbances of the balance or the distribution of water and electrolytes in the animal body.

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GENERAL INTRODUCTION

Artistotle did not regard the kidneys to be necessary for life. He believed that urine was formed in the bladder and that the kidneys served only to supply the body with a "greater finish and perfection." This gross error, however, was corrected by Galen, who recognised that the ureters served to conduct the urine from the kidneys, in which it was made, to the bladder, from which it was evacuated at varying intervals (Smith, 1959). It is of interest that the first report, by Bellini in 1662, ascribing a tubular structure to the kidney substance was based on a study of the kidneys of a ruminant (a deer). Bellini's observations were chiefly concerned with the gross anatomy of the renal pelvis, but he did demonstrate that the inner or papillary part of the kidney was composed, not of solid fibrous strands as previously supposed, but of minute hollow channels (Bellini's ducts) draining into the pelvis (Jones and Rewell, 1954). Four years later the first description of many details of structure, which lay beyond the range of unaided vision, was forthcoming, following the application of the Galileo microscope to the study of the structure of animal organs (Malpighi, 1666).

Malpighi's description of the kidney marks the beginning of an accurate knowledge of this organ. Where other anatomists had considered the outer part or cortex to be coagulated blood, Malpighi discovered that it was composed of many minute tortuously twisted tubes (the renal tubules) and, though he was unable to demonstrate the point, he correctly inferred that the tubules were connected with the much larger ducts of the papilla. Among the tubules he found many spherical, translucent bodies resembling fish eggs (Malpighian corpuscles) which were attached to the blood vessels like apples to a tree and which he thought corresponded in number to the tubules of which the mass of the kidney was

composed. He failed to observe that each of these spherical bodies, which he called "glands", consisted of an elaborate tuft of capillaries, but by injecting the kidney with coloured fluids he was able to demonstrate that the "glands" were connected with the arteries and veins. He inferred that urine is formed in these innumerable "glands" during the passage of blood through them, and drained through the attached tubules to the larger ducts, from which it escaped through the pores in the papilla into the renal pelvis, and thence, by way of the ureters, into the bladder.

After Malpighi's treatise of 1666 it is remarkable that further progress towards an understanding of the anatomy and physiology of the kidneys was not achieved until the publication, in the mid-nineteenth century, of Bowman's precise morphological study (Bowman, 1842). The most notable contributions of this work included an accurate description of the glomerular capillary tuft and its relations to the afferent and efferent arterioles; a description of the renal tubule as a single layer of epithelial cells resting on a basement membrane; and a description of the capsule investing the glomerular capillaries (Bowman's capsule) as an expansion of this tubular basement membrane into a sphere, firmly sealed to the afferent and efferent arterioles at the vascular pole of the glomerulus. Bowman emphasized that this capsule had an open connexion with the tubule at the urinary pole, so that any fluid escaping from the capillary tuft could drain freely into the tubule. He concluded correctly that each tubule was connected with a glomerulus, and he inferred, though, like Malpighi, he could not prove the point, that each tubule continued as a single conduit until it joined the collecting system of (Bellini's) ducts. Lastly, Bowman's use of arterial

and venous injection methods enabled him to describe how the efferent arteriole broke up into a second capillary plexus closely applied to the basement membrane around the tubules, and he concluded, again correctly, that all the blood from the renal artery, with the exception of a small quantity distributed to the renal capsule, the surrounding fat and the walls of the larger blood vessels, entered the capillary tufts of the Malphighian bodies. Thence it passed into the capillary plexus surrounding the uriniferous tubules before finally leaving the organ through the branches of the renal veins.

Regarding the mechanism of urine formation, Bowman conceived that the capillary tuft of the glomerulus was ideally suited for the separation of water from the blood, a process which he attributed to retardation of the flow of blood in the tuft; and he proposed that this glomerular water served to dissolve, and to wash down the tubule, urinary solutes, all or most of which were excreted by the tubular epithelium. This latter part of the hypothesis was in keeping with the widespread belief then current that secretion involved actual rupture of secretory cells with discharge of part or all of the cellular contents.

In advancing this concept of how urine was formed Bowman acted as an early advocate of what became established as the vitalistic theory of urine formation. With our present knowledge, his failure to sense that the separation of water in the glomeruli might also entail the separation of salts and other substances in solution seems surprising. However, Bowman's paper was scarcely in circulation before a physical theory of urine formation was proposed by Ludwig (1844), who, without knowledge of Bowman's work, had carried out extensive studies

of the anatomy of the kidney and had arrived at the same views on all important points. Ludwig's hypothesis was, first, that urine formation began with the physical separation in the glomeruli, under the hydrostatic pressure of the blood, of a protein-free ultrafiltrate sufficient in volume to contain all of the urinary constituents; and, second, that this filtrate was reduced in volume, with proportional concentration of the urinary constituents, by the tubular reabsorption of a large fraction of this glomerular filtrate. This hypothesis dispensed with any vital activity on the part of the glomerular capillaries, to which Ludwig attributed only such semipermeability as would permit water and all solutes of small molecular weight to pass into the ultrafiltrate, whilst restraining the plasma proteins, lipids and formed elements. It also disposed of any secretory activity on the part of the renal tubules as proposed by Bowman, because the volume of the filtrate was supposedly large enough to account for the excretion of all the urinary constituents.

Ludwig recognised that blood pressure in the peritubular capillaries must be too low to favour filtration in this site and conceived that the blood was somehow "concentrated" by the filtration which had occurred in the glomeruli. Consequently he postulated that in the peritubular capillaries this "concentrated" blood would draw water (and some solutes) from the relatively "dilute" tubular urine by a process of "endosmosis". He did not make clear what was concentrated in the glomerulus and rediluted in the capillaries but emphasised that this reabsorption did not involve any "vital" activity on the part of the tubular epithelium. His description of "endosmosis" as "chemical" was apparently adopted to distinguish it from the mechanical (hydrostatic) filtration occurring in the glomeruli.

Present day concepts do not support "endosmotic" reabsorption as described by Ludwig, but his physical theory of glomerular filtration proved to be true, and remains probably the most brilliant generalisation in the history of renal physiology. Ironically it was on this cardinal principle that Ludwig's hypothesis was first challenged. Heidenhain (1883) pointed out that, contrary to Bowman's description of the glomerular capillaries as "naked", in his view these vessels were more or less completely covered by a continuous layer of "dense" epithelium, similar to that of secretory glands. He suggested that this layer would oppose filtration and that, as in other glands, it could have a secretory function. He calculated that to explain the excretion of urea in man by "filtration" some 70 litres of filtrate would have to be formed per day, of which 68 litres or more must be reabsorbed, which seemed like impossible figures. It was argued that the effects on urine flow described in experiments involving interference with the arterial or venous blood supply of the kidney pointed to the conclusion that urine formation was related to renal blood flow rather than to glomerular pressure. When combined with Heidenhain's own observations on the tubular secretion of indigo carmine, and with those of Nussbaum (1878), these arguments seemed very convincing.

Heidenhain therefore concluded that, in the formation of urine, water, primarily, was secreted by the glomerular epithelium, and other urinary constituents (and possibly some water) were secreted by the tubules. It should be noted that vitalism was not invoked with reference to secretion. Heidenhain spoke of water and other secretory products as being propelled by the cell's "active force", as to-day one would speak of "active transport", in contradistinction to filtration or passive diffusion; and he

recognised that the activity of a secretory cell was completely obscure, but added that functions in the living cell were subject everywhere and without exception to exploration "by means of physics and chemistry".

Thus began the controversy between, on the one hand, those who supported Ludwig's filtration-reabsorption hypothesis and, on the other, those who supported the so-called Heidenhain-Bowman secretory hypothesis. No indubitable proof of either interpretation was advanced as the literature increased in volume (Smith, 1959). Both points of view failed to agree on the significance to be attached to results of procedures involving, for example, changes in blood pressure and blood flow, changes in the composition of the blood, and the presence or absence of dyes in Bowman's capsule or in the tubule cells. The vitalistic school referred essentially all physiological phenomena to the "vital force" of the living cell, a force which purposefully, albeit mysteriously, directed all cellular activities to an Aristotleian "final end". Supporters of the filtration-reabsorption hypothesis, on the other hand, so completely abandoned the teleological implication of mystic cellular forces in favour of mechanistic interpretations, that "vital activity", even in the modern sense of "metabolic activity" or "active transport", was not acceptable.

The first monograph to be devoted exclusively to the physiology of the kidney was published early in the present century (Cushny, 1917), by which time physiology as a whole had acquired a strong mechanistic cast. In reviewing "a mass of printed matter of over 6000 pages" Cushny stated the different views, then, somewhat arbitrarily, rejected that secretion played any part in urine formation. This was not because the filtration-reabsorption hypothesis was proved (it was not), nor because the arguments

against secretion were convincing (they were not), but because, to Cushny, secretion was synonymous with vitalism. He pointed out that the Bowman-Heidenhain theory was mainly remarkable for its defensive strength, in that every possible experimental finding could be attributed to some special activity of unspecified cells, whilst, in reality, it explained nothing, and offered no point from which advance could be made. In this broad condemnation, however, Cushny ignored the possibility that tubular secretion, "vitalistic" or otherwise, might supplement the process of filtration and reabsorption which he advocated.

In formulating what he called "The Modern Theory" Cushny accepted as the initial step Ludwig's process of glomerular filtration, but rejected "endosmotic reabsorption". The facts that glucose and amino acids, which he conceived to be filterable, are usually wholly absent from the urine, and that some substances such as urea, sulphate and chloride may be present in greater concentration than in the blood, and yet not concentrated to the extent required by the filtration-reabsorption theory, forced him to accept that these substances were reabsorbed so long as the plasma concentration was below a critical value. Such substances were designated "threshold" substances to distinguish them from waste products such as creatinine, which were rejected by the tubules regardless of plasma concentration and were designated "no-threshold" substances. He therefore completed the "Modern Theory" by the supposition that the tubules reabsorbed a "perfected Locke's fluid" containing glucose, amino-acids, salts, urea etc. in approximately the proportions in which they were "best adapted to the tissues":

"The formation of the glomerular filtrate is due to a

blind force. The absorption in the tubules is equally independent of any discrimination, forthe fluid absorbed is always the same, whatever the needs of the organism at the moment." (Cushny, 1917, p.48).

Cushny was unable to propose any mechanism for the re-absorption of this "perfected fluid" and was forced to admit that this reabsorption depended on "the vital activity of the tubular epithelium". Invoking "vital activity" in this context, after an earlier dismissal of the term as "nebulous" and "uncompromising" when used by those advocating tubular secretory processes in urine formation, presumably was regarded as acceptable since the vital activity had been reduced to the constant and supposedly definable dimensions of a "perfected Locke's fluid".

Cushny's endorsing of Ludwig's theory of filtration has been upheld by more recent work, although his theory of tubular reabsorption of a "perfected fluid" has not, and his rejection of tubular excretion or secretion has proved unwarranted. However, Cushny's stated purpose that his monograph might serve "as an advanced post from which others may issue against the last ramparts of vitalism" was achieved, since the book provided a valuable summary of, and guide to, the complex literature, and a powerful stimulus to the critical evaluation of experimental findings. In addition the monograph pointed the need for quantitative rather than qualitative studies on renal function, and from 1920 onwards were developed investigational techniques by which many of the uncertainties of the older theories of kidney function have been resolved.

A.N. Richards and his colleagues at the University of Pennsylvania applied microdissection techniques to the study of individual nephrons and developed the technique of micropuncture which supplied the first definitive evidence of the role of the glomeruli in urine

formation. The issue was settled in favour of Ludwig's hypothesis by the demonstration that glomerular filtrate, collected by direct micropuncture of Bowman's capsule in the frog, was virtually protein-free (down to the lower limit of the analytical method) and that it contained glucose and chloride when the bladder urine contained virtually none of these substances (Wearn and Richards, 1924). Subsequent development of new microanalytical methods applied to the examination of capsular fluid of the frog, the mud puppy (Necturus), and in a few instances, to the snake, demonstrated that this fluid had the composition with respect to osmotic pressure, electrical conductivity, glucose, pH (and by inference, bicarbonate), chloride (and by inference, sodium), potassium, phosphate, urea, creatinine and uric acid to be expected of a protein-free filtrate. By puncturing tubules at different points, subsequently identified anatomically, or by perfusing a segment of tubule blocked at each end by droplets of mercury, important differences in function of the proximal and distal segments of the nephron were identified (Richards, 1929, 1934, 1939). Application of the micropuncture technique to the study of the mammalian kidney is technically more difficult than in the amphibian because of differences in renal anatomy, but Walker, Bott, Oliver and MacDowell (1941) obtained results from the guinea pig, white rat and opossum which were wholly consistent with those obtained in the amphibia.

At about the same time that Wearn and Richards (1924) provided definitive evidence supporting Ludwig's filtration hypothesis, Marshall and Vickers (1923) showed that the dye-stuff phenolsulphonphthalein (phenol red) accumulated in high concentrations in the renal cortex of hypotensive dogs in which no urine was being formed. This accumulation apparently was not the result of

continued filtration and total reabsorption in the proximal tubules since, in normotensive dogs, the dye was quickly and copiously excreted in the urine, despite the fact that some 60% of the dye in the plasma was reversibly bound to plasma protein and so was not available for filtration. Although this work was not accepted entirely as proof of the secretory ability of renal tubules, Marshall and Crane (1924) later showed that the rate of excretion of phenol red in the rabbit and dog did not increase in direct proportion to the plasma concentration, as is required in theory for a substance excreted solely by filtration. At high concentrations the rate of excretion levelled off as though approaching a maximal constant value. This indicated that the tubules did participate in its excretion and that tubular transport became "saturated" at higher plasma concentrations.

In retrospect, many earlier proponents of tubular secretion had been correct, although their evidence was inconclusive and the observations of Marshall and his co-workers on phenol red provided the first unassailable evidence of tubular secretion in any animal. Striking evidence in support of this secretory ability was provided by the finding that the proximal tubule cells of the chick embryo, in <u>in vitro</u> culture, took up phenol red, chlorphenol red, cresol red and other sulphonphthalein indicators and excreted them into the tubular lumen (Chambers and Cameron, 1932). The fact of tubular secretion was finally established beyond dispute by comparative studies, and, in particular, by those on urine formation by the aglomerular kidneys of certain marine teleost fishes (Marshall, 1934).

Further progress in the study of kidney function followed the introduction of the clearance concept and the discovery of a substance which, by undergoing neither subtraction nor addition by the renal tubules between the glomerular filtrate and the final urine,

provided a standard of reference for the identification and measurement of tubular participation in the net excretion of other substances. The degree of concentration of such a standard, at any point along the tubule or in the final urine, depends only on water reabsorption. If the degree of concentration of any other substance simultaneously present in an ultrafilterable state in the blood be greater or less than that of the standard of reference, tubular secretion or tubular reabsorption, respectively, of this other substance must have occurred; also, application of the clearance concept, first introduced by Möller, MacIntosh and Van Slyke (1929), allows calculation of the rate of filtration and the rate of tubular reabsorption or secretion.

The events which led to the discovery and adoption of inulin as the reference substance of choice in such studies have been described by Smith (1943), and the criteria on which inulin clearance is accepted as a measure of glomerular filtration rate have been defined by the same author (Smith, 1951). The clearance concept has also provided a means of measuring renal plasma flow in intact animals, following the discovery that some substances such as diodrast and hippuric acid derivatives were almost completely removed from the blood in a single passage through the kidney. More detailed reviews of the measurement of glomerular filtration rate and renal plasma flow are given in later sections.

From clearance techniques what goes on between the two ends of the renal tubule is purely inferential, so that, while clearance procedures have become established as essential tools in quantitative investigations of kidney function, additional and more sophisticated procedures have been developed in recent years to study mechanisms of renal tubular transport, and to localise the sites along the renal tubules where particular adjustments of the composition of the

tubular urine occur.

Active reabsorption of sodium by the cells of the proximal convoluted tubules was first strongly suggested by experiments in which dogs were infused with hypertonic mannitol to establish a profound osmotic diuresis (Wesson and Anslow, 1948). When 60% of the filtered water was excreted, only 33% of the filtered chloride, 28% of the filtered sodium and 10% of the filtered bicarbonate were excreted, indicating the occurrence of solute reabsorption against a concentration gradient. Such reabsorption presumably involved active cellular transport of one or more of these ions. Since the urine remained isosmotic with plasma (the reduction in the urinary concentration of ions was exactly balanced by an increase in the concentration of mannitol), it probably represented proximal tubular fluid relatively unmodified during its rapid transit through the more distal parts of the nephron.

Micropuncture studies have confirmed that active transport of sodium chloride occurs in the proximal convoluted tubule. Experiments using a method of stop-flow perfusion of the Necturus tubule (Shipp, Hanenson, Windhager, Schatzmann, Whittembury, Yoshimura and Solomon, 1958) showed in this animal that sodium chloride was reabsorbed from a solution containing more than 66 m-equiv/l. into plasma containing 100 m-equiv/l. and water followed in proportion to the salt reabsorbed. (Windhager, Whittembury, Oken, Schatzmann and Solomon, 1959). However, if the concentration gradient was greater than 34 m-equiv/l., sodium chloride diffused into the tubule along with sufficient water to re-establish this limiting gradient. Similar perfusion experiments in the rat have shown the limiting gradient for sodium chloride reabsorption by the proximal tubules of this species to be 50-60 m-equiv/l. between the tubular contents and the blood plasma (Windhager and Giebisch, 1961).

Electrophysiological studies of the nephron indicate that renal tubule cells share the characteristics of numerous other epithelial structures in that they are electrically asymmetrical. This implies that the cell membrane bounding the luminal and peritubular cell borders have different properties so that the tubular lumen is on average 20 mV negative to the peritubular fluid (Solomon, 1957; Giebisch, 1958).

Such studies have established that the reabsorption of sodium is active and occurs extensively along almost the entire nephron (Windhager and Giebisch, 1965). In the proximal tubule the reabsorptive process occurs, normally, without the establishment of detectable concentration gradients, but it occurs against an electrical gradient of 20 mV, and despite the demonstrated inadequacy of other possible driving forces such as the transtubular colloid osmotic pressure gradient. In the distal tubules, sodium transport is characterised by net reabsorption against steeper electrochemical gradients than those found in proximal convoluted tubules. On the other hand, the orientation of the transtubular potential difference favours the reabsorption of negatively charged ions, and, since the flux asymmetry or net reabsorption of chloride is adequately accounted for by this electrical gradient, it is concluded that reabsorption of this ion is passive: no work normally need be performed on the chloride ion to move it from lumen to peritubular fluid (Windhager and Giebisch, 1965).

The urinary excretion of sodium may be regarded as a two-component system which involves filtration of large quantities at the glomeruli followed by tubular reabsorption, so that only a small fraction of the quantity filtered escapes in the urine. In the case of potassium, a similar two-component system might be assumed to operate since, in man and the dog, the quantity of

potassium excreted is normally only a fraction of that presented to the tubules in the glomerular filtrate. However, as has been pointed out earlier, tubular secretion is known to contribute to the excretion of some substances and, in the case of potassium, evidence has accumulated which warrants the addition of the process of tubular secretion to those of filtration and reabsorption, so that the urinary excretion of this element is now believed to involve a three-component system.

McCance and Widdowson (1937) reported potassium clearance to be greater than inulin clearance in a patient with dehydration and alkalosis, so that potassium excretion could not be quantitatively accounted for, in this case, by filtration alone. Similar findings were reported in patients with renal failure by Leaf and Camara (1949) and by Platt (1950). Since glomerular filtration rate was low in these patients the possibility remained that potassium secretion was an emergency mechanism invoked to compensate for grossly diminished filtration. However, Franglen, McGarry and Spencer (1953) showed that potassium clearance exceeded the normal inulin clearance values of healthy subjects in experiments which combined oral potassium loading with hyperventilation alkalosis. These authors also showed, in anaesthetized dogs, that potassium clearance exceeded inulin clearance during acute alkalosis produced either by intravenous infusion of sodium bicarbonate or by peritoneal dialysis with solutions of sodium bicarbonate. The authors concluded that the renal excretion of potassium was largely controlled by alterations in tubular activity correlated with changes in the blood pH and with the availability of potassium for tubular secretion.

Renal tubular secretion of potassium in the dog had been demonstrated earlier using clearance techniques by Berliner, Kennedy and

Hilton (1950) and by Mudge, Ames, Foulks and Gilman (1950) during intravenous infusion of potassium salts after prior administration of potassium salts orally (10g per day for 1 - 2 weeks). Potassium clearances greater than the filtration rate were also reported in dogs during extreme cellular dehydration produced by the intravenous infusion of hypertonic sodium chloride (Mudge, Foulks and Gilman, 1950), and following the administration of the diuretic acetazolamide (Berliner, Kennedy and Orloff, 1951). Mudge, Ames et al.(1950) noted that mercurial diuretics exerted a consistent action on potassium excretion. During water diuresis they promoted marked increases in potassium excretion, but when administered during intravenous infusion of potassium chloride they greatly decreased potassium excretion. These effects were taken to indicate that mercurial diuretics depressed both tubular reabsorption and tubular secretion of potassium, and to provide evidence of the magnitude of the secretory process. The authors cautiously concluded that the renal excretion of potassium might be accomplished primarily by a secretory process, after the tubular reabsorption of most of the potassium present in the glomerular filtrate.

Analyses of proximal tubular fluid obtained by direct micropuncture have confirmed that potassium is reabsorbed from the glomerular filtrate in Necturus (Bott, 1954) and in the rat (Wirz and Bott, 1954; Lichfield and Bott, 1962). The reabsorption was not complete, but a higher concentration ratio (potassium : inulin) was observed in the final urine than in fluid from the end of the proximal tubule (Bott, 1954), which indicated that potassium secretion had occurred in the distal tubule. Stop-flow studies on dogs have confirmed that secretion of potassium occurs in a distal tubular site (Pitts, Gurd, Kessler and Hierholzer, 1958; Sullivan, Wilde, Malvin and Vander, 1958), and analyses of urine samples

collected by catheterization of the ducts of Bellini in the golden hamster indicated that potassium secretion occurs in the collecting ducts (Ullrich, 1960).

Expdriments using radio-active potassium (^{42}K) have provided additional evidence of tubular secretion in animals not subjected to procedures such as potassium loading or experimental alkalosis. Morel (1955) and Morel and Guinnebault (1956) concluded from the time course of specific activity of plasma and urine samples following injection of ^{42}K in rabbits, that urinary potassium was derived largely from a distal tubular cellular "compartment" rather than from the glomerular filtrate. Black and Emery (1957) reviewed the findings of similar experiments on human subjects and reached similar conclusions. Whilst the occurrence of tubular secretion of potassium is clearly established no evidence justifies the assumption that reabsorption of filtered potassium is complete. Koch, Brazeau and Gilman (1956) concluded from experiments on potassium -depleted and potassium-fed dogs, in which sodium and potassium excretion were measured during induced changes of chloride excretion, that the renal tubules made no distinction between these two cations in reabsorbing sodium and potassium in conjunction with anions. Thus the contribution of filtered potassium to urinary excretion was calculated as the product of the rate of excretion of sodium and the ratio of the plasma concentrations (K : Na); any potassium which appeared in the urine in excess of this amount was presumed to be the result of tubular secretion.

In a review of electrophysiological studies on individual nephrons Windhager and Giebisch (1965) support, in general, these conclusions. These authors state that in mammals, in a wide variety of metabolic situations, some 70% of the filtered potassium is reabsorbed before the filtrate leaves the proximal convoluted tubule,

and the reabsorption is by an active transport mechanism since the net movement of potassium occurs against an electrochemical gradient. In the distal convoluted tubule it was shown that potassium reabsorption may continue, in situations where the rate of urinary excretion was a very small fraction of the filtered load, but varying degrees of net secretion occurred when excretion rates were higher. However, the measured net entry of potassium into the lumen of the distal convoluted tubule could be adequately accounted for by the transtubular potential difference, so that tubular secretion of potassium at this site can be explained on the basis of passive diffusion, with net reabsorption, when present, constituting active transport. Unfortunately no information was available relating net movements of ions to electrical driving forces and concentration gradients in the collecting ducts, and an exact evaluation of the nature of potassium transfer across the collecting duct epithelium must await a more precise knowledge of the electrochemical potential gradient between the collecting duct lumen and vasa recta plasma.

It is now generally accepted that the reabsorption of bicarbonate and acidification of urine are accomplished by the tubular secretion of hydrogen ions in exchange for reabsorbed sodium (Pitts, 1963), and accumulated evidence suggests that coupled exchanges are also involved in the secretion of potassium (Berliner, 1960). In this the renal tubules show similarities with other biological systems, for such exchanges have been implicated in the transport of sodium and potassium in nerve (Hodgkin and Keynes, 1955), in red blood corpuscles (Glynn, 1957) and in frog skin (Koefoed-Johnsen and Ussing, 1958).

The evidence for an ion-exchange mechanism in potassium secretion by renal tubules is based largely on two phenomena:

first, the dependence of potassium excretion on the excretion of sodium and, second, the lack of relationship between the glomerular filtration rate and the excretion of potassium when the excretion of sodium is maintained. The first phenomenon was most strikingly demonstrated in stop-flow studies by Jaenike and Berliner (1960) who showed that potassium concentrations were minimal in samples which, from their very low concentrations of sodium and chloride, were identified as having sojourned in distal convoluted tubules during the period of stopped flow. It was pointed out that samples from this site must traverse the remainder of the tubule system before they are collected and that more distal parts of the tubule can and do secrete potassium (Pitts et al., 1958; Sullivan et al., 1958). The low concentrations of potassium were therefore interpreted as being due to failure to secrete potassium into these particular samples since they contained virtually no sodium to be exchanged for potassium. This explanation was strongly supported by the findings of Walker and Cooke (1960) in experiments using a modified stop-flow technique in which the total period of stopped flow (8 min) was interrupted (after 4 min) to permit samples from one tubular site to move down to another. Interruption of the period of stopped flow allowed the urine which had sojourned in the most distal regions of the nephrons to escape. These samples showed the typical peak in potassium concentration which is taken to indicate the occurrence of potassium secretion at this site, but during the second stop-flow period this most distal region of the tubules was occupied by urine which had become low in sodium and potassium concentration, during the initial period of stopped flow, and had then moved down the tubule. When the concentration pattern from this second occlusion was examined, high concentrations of potassium were no longer found in the samples from the most distal

regions of the nephron.

The lack of a relationship between the glomerular filtration rate and potassium excretion when sodium excretion is maintained was demonstrated by Davidson, Levinsky and Berliner (1958) in clearance experiments on unanaesthetized dogs in which provision for the separate collection of urine from each kidney had been made by prior surgical division of the bladder. One kidney was used to give control values for comparison with those obtained from the other kidney when subject to experimental reductions in filtration rate. Reduction of the filtration rate of the experimental kidney, by inflation of a cuff previously placed around the renal artery and connected to the exterior by a fine polyethylene catheter, resulted in a sharp drop in sodium excretion accompanied by a marked, though smaller, drop in potassium excretion. When attempts were made to maintain sodium excretion by the administration of the mercurial diuretic salyrgan, or by the carbonic anhydrase inhibitor acetazolamide, or by infusions of sodium sulphate, reduction of the glomerular filtration rate by up to 35% was entirely without effect on potassium excretion, although sodium excretion still fell to an extent equal to or greater than the reduction in glomerular filtration rate. These results were consistent with the interpretation that the effect of reductions in glomerular filtration rate on potassium excretion were not due primarily to reductions in the quantity of potassium filtered but to the decrease in the sodium available for exchange with potassium in more distal parts of the nephron. Attempts to maintain sodium excretion during imposed reductions in filtration rate were unsuccessful, but maintained potassium excretion since these procedures presumably made an increased proportion of the filtered sodium available for exchange with potassium. Thus potassium excretion was maintained but sodium

excretion was, in consequence, still depressed.

The hypothesis that potassium excretion is accomplished largely by a process of tubular secretion involving concurrent reabsorption of sodium has required modification to account for the relationship existing between the excretion of potassium and of hydrogen ions. Berliner, Kennedy and Orloff (1951) made the important observation that when the availability of hydrogen ions for excretion was decreased, by acetazolamide, potassium excretion increased. This increase in the excretion of potassium was apparently the result of an active secretory process since it was inhibited by mercurial diuretics. A similar response to acetazolamide administration has also been reported in man (Counihan, Evans and Milne, 1954). Berliner et al. (1951) therefore suggested that in distal regions of the nephron potassium ions and hydrogen ions compete for secretion in exchange for reabsorbed sodium. This addition to the simple ion-exchange hypothesis has proved valuable in the interpretation of a number of observations. For example, it has long been known that oral administration of potassium salts, in addition to increasing potassium excretion, also increases the excretion of bicarbonate, the reabsorption of which is dependent on the tubular secretion of hydrogen ions. Fuller, MacLeod and Pitts (1955) have, accordingly, shown that intravenous infusion of potassium salts in the dog reduces the rate of reabsorption of bicarbonate. Similarly Mills and Stanbury (1954) have presented evidence that in the diurnal rhythm of electrolyte excretion in man there are converse changes in the output of potassium and hydrogen ions, and Black and Mills (1954), in a study of nocturnal excretion in man after small doses of sodium and potassium chloride and bicarbonate also found that the observed changes were consistent with this hypothesis.

Experimental investigation of renal processes involved in the

excretion of sodium and potassium have mainly been conducted on dogs, although findings using human subjects, laboratory animals and amphibia have also made important contributions. This aspect of kidney function may be broadly summarised as the regulation of urinary losses of sodium and potassium in relation to their intake, and to the body's requirements. The emphasis of renal mechanisms which have been elucidated using one species may therefore be different in others which show differences in the dietary intake of sodium and potassium, or differences in metabolic activity. Lactation and pregnancy clearly will give rise to differences in metabolic requirements between individuals of the same species, which may well be reflected in the urinary excretion of water and electrolytes. However, by restricting the comparison to non-pregnant and non-lactating animals any differences in the urinary excretion of sodium and potassium presumably will be the result of differences in dietary intake.

The normal diet of grazing animals compared with that of man and the dog is high in potassium and low in sodium content (Morrison, 1951). Thompson (1960) gives figures of analyses of various grasses, legumes and herbs which show ratios (potassium:sodium) of 15.3, 34.4 and 20.5 respectively. Feeding prepared concentrate mixtures, mineral supplements, or provision of salt licks, in many cases will increase the sodium intake and reduce the potassium:sodium ratio of the total food intake, but, with very few exceptions, foliage, whether grazed, fed as hay, silage, or after other treatments, provides the major dietary component of cattle. The feeding of concentrates, moreover, even to high-producing animals, is reduced in those seasons when grass is rapidly growing and more readily meets the nutritional requirement of the animal. At these times ratios of the potassium: sodium content of the herbage are widest, and are sustained by the modern practice of promoting and maintaining rapid growth of pastures

by treatment with nitrogen and potash-containing fertilisers (Hemingway, 1961).

The efficient absorption of dietary sodium must therefore be regarded as an important function of the alimentary tract of herbivores, and in cattle Van Weerden (1961) has shown that in the lower part of the small intestine and in the large intestine sodium absorption occurs against a considerable concentration gradient so that relatively little sodium is lost in the faeces. This work indicated that the cow can subsist on surprisingly small quantities of sodium: less than 0.1% of the dry matter of the feed. There is evidence also that the intestine contributes to the regulation of sodium excretion and retention. Sodium concentrations in ultrafiltrates of faecal juice from cattle fed a diet with a low sodium content were significantly increased when sodium chloride was added to the diet, and mean values of faecal excretion of sodium rose from 196 m-equiv/day to 752 m-equiv/day (Renkema, Senshu, Gaillard and Brouwer, 1962). These authors also noted that potassium concentrations in ultra filtrates of faeces were distinctly higher when the animals did not receive the dietary supplement of sodium. Similar results were reported from studies in which cation exchange resins were fed, in man (Emerson, Kahn and Jenkins, 1954; Spencer, Ross and Lloyd-Thomas, 1954), in the dog (Field, Dailey, Boyd and Swell, 1954; Field, Swell, Dailey, Trout and Boyd, 1955) and in the rat (Ross and Spencer, 1954).

However, there is evidence that the alimentary tract of the cow makes no comparable contribution to the regulation of potassium excretion and retention. Absorption of this ion from the alimentary tract apparently is not reduced when quantities of potassium are ingested far in excess of nutritional requirements. In balance experiments, Van der Horst (1960) showed that when dairy cows were

changed from hay to grass-feeding the potassium intake increased greatly and faecal losses of potassium in fact were slightly decreased. There was a large increase in the urinary excretion of potassium and it was concluded that the excretion of potassium in urine was approximately proportional to the intake. A time-lag in this process is suggested by the observation of de Groot (1962) that the sudden increase in the potassium intake, which occurred when cows started to graze lush spring grass, increased the serum potassium concentrations for a few days; it was suggested that the potassium content of the cells might also be increased in these circumstances.

Apart from balance studies there are few reports of investigations of the renal regulation of sodium and potassium excretion in the cow. Sellers and Roepke (1951) showed that potassium excretion was several times greater than sodium in cows in mid-lactation, and in late pregnancy, and also when non-pregnant and non-lactating. Water diuresis had no effect on potassium excretion but resulted in increased urinary losses of sodium. Sellers, Gitis and Roepke (1951) showed in five lactating animals, four of which were pregnant, that dosing by stomach tube with 0.5 g KCl/lb body weight, in approximately 10 gallons of water, resulted in increases in potassium excretion ranging from 1.2 to 6.6 times the pre-dosing rate, and increases in sodium excretion which ranged from 2.7 to more than 1200 times the pre-dosing rate. This very large percentage increase in sodium excretion occurred in one animal in which the pre-dosing rate of excretion of this ion was very low. This animal was also given a larger dose of potassium chloride (0.65 g/lb body wt.). In all the animals a rise in plasma concentrations of both potassium and sodium was noted after dosing. Similar dosing with sodium chloride in two animals resulted in large increases in sodium excre-

tion and smaller increases in potassium excretion, accompanied by increases in the plasma sodium concentration. A slight rise in plasma potassium concentration was also noted.

The measurement of glomerular filtration rate in the cow using an inulin clearance procedure was first described by Poulsen (1957). Vogel (1959) noted that potassium clearance values in cattle were similar to inulin clearance values, and during intravenous infusion of potassium chloride consistently exceeded inulin clearance values. He also reported that sodium excretion was not affected but plasma potassium concentrations rose during the infusion to a maximum of 7.1 m-equiv/l. Ketz (1960) in a study of the urinary excretion of electrolytes in calves and cows also recorded potassium clearances which approached values obtained for inulin clearance. The response to an oral water load showed the expected inverse relationship between the rate of urine flow and urine osmolarity, but the parallel increases in the clearances of endogenous creatinine and potassium noted during the diuresis were probably due to improved efficiency of urine collection at high rates of urine flow. Water diuresis has been reported elsewhere to have no effect on potassium excretion (Sellers and Roepke, 1951) and creatinine excretion similarly is believed to be independent of the rate of urine flow in the cow (deGroot and Aafjes, 1960).

Knudsen (1960) studied electrolyte excretion in the cow during variations in the urine flow produced by oral water-loading and by administration of the diuretics mersalyl and chlorothiazide. The clearance of endogenous creatinine was determined as a measure of glomerular filtration rate. His findings during water diuresis were in agreement with those of Sellers and Roepke (1951). Mersalyl (a mercurial diuretic) increased the rate of excretion of sodium and chloride but had no effect on potassium excretion. It was also

noted that two of the animals developed acute nephrosis after the infusion of mersalyl. After chlorothiazide the excretion rate of both sodium and chloride showed pronounced increases and there was a moderate increase in potassium excretion. Potassium clearances rose to exceed creatinine clearance after administration of chlorthiazide, a finding which was attributed partly to the increased rate of excretion of potassium, but mainly to the fall in plasma potassium concentration which was consistently observed. No reduction in creatinine clearance was seen after the drug and in two supplementary experiments, in which a small dose of chlorothiazide was given, smaller increases in urine flow occurred which were unaccompanied by increases in potassium excretion. A fall in plasma potassium concentrations was recorded, however, and potassium clearance increased in parallel with urine flow from 60% to 80% of the creatinine clearance.

These findings suggest that chlorothiazide promoted the disappearance of potassium from the plasma to some less accessible site within the body, rather than to the urine, but no attempt was made, in those experiments in which kaliuresis was recorded, to assess quantitatively the relative contributions of increased urinary losses and undefined extrarenal effects to the observed reductions in plasma potassium, and no explanation was offered for this effect of the drug.

Knudsen (1960) also pointed out that the animals showed varying degrees of excitement on introduction to the restraining stall in which experiments were carried out, and this was associated with high rates of urine flow and electrolyte excretion at the outset of his experiments. The transient diuresis described by Anderson (1961) in response to, or in anticipation of, the painful stimulus of brachial artery puncture confirmed that physical or mental stress

can influence kidney function in the cow, so that these factors should not be overlooked in any study in which conscious animals are used.

The present study was undertaken to confirm and extend some findings of earlier workers on bovine kidney function. It has involved the application and assessment of techniques for investigating electrolyte excretion, with particular reference to sodium and potassium. The findings are compared with those of other workers and any differences in the renal responses of the cow from those described in man and the dog are discussed in relation to the different excretory requirements of these species.

METHODS

CHEMICAL ANALYSES

Sodium and potassium estimations

Flame photometry provides a simple and rapid method for the estimation of certain elements. It is particularly suitable for the alkali metals, for which alternative methods of estimation may be difficult and time-consuming. This fact has led to the widespread use of flame photometry in biological analyses, and especially for the estimation of sodium and potassium in body fluids.

Many papers on flame photometric applications and technique have appeared over the past twenty-five years. The literature has been extensively reviewed by Burriel-Marti and Ramirez-Munoz (1957), and Wynn, Simon, Morris, McDonald and Denton (1950) give a detailed practical description of the use of flame photometry in the analysis of body fluids. It is clear that flame photometry, whilst basically simple, can give rise to errors and inaccuracies necessitating a variety of precautions before reasonable accuracy is achieved. As in other analytic procedures, overall accuracy in flame photometry is influenced by the accuracy of the preparatory procedures involved, in addition to the determinative procedure itself. If adequate care be observed in the initial preparation and handling of samples for analysis, the sources of error in flame photometry fall into four main categories (Wynn et al., 1950):

(1) Variations in flame temperature during the course of analyses. These errors become negligible if the composition and pressure of the combustive gases are strictly controlled.

(2) Viscosity differences between the unknown and standard solutions which affect the rate of delivery of the solutions to the burner. Wynn et al (1950) state that this source of error is overcome in analyses of biological fluids if all solutions are analyzed at high dilution.

(3) Superimposition of the emission energy of other substances on the emission energy of the test substance. It is generally held in the case of sodium and potassium that the wavelengths of their respective emissions are sufficiently far apart for efficient separation to be effected by filters and monochromators.

(4) Direct depression or elevation of the emission energy of the test substance by other ions or solutes present in the test solution (interference).

The EEL flame photometer was used throughout the present work for the estimation of sodium and potassium concentrations in blood plasma and urine. The performance of the instrument was investigated and a routine analytic procedure was developed and tested. Various modifications of the manufacturers' operating instructions were introduced in order to minimise innacuracies arising in the determinative procedure.

Method

General

The instrument was sited in a small, windowless room in the veterinary school, Buccleuch Street, for the first half of the work

involved in this thesis, and in a laboratory at the veterinary hospital for the remainder. A mixture of mains coal gas and air was used for the flame. In neither situation was special provision made for removal of exhaust gases when the instrument was in use. An extractor fan provided reasonable ventilation in the former site, and ventilation without draughts was possible at the veterinary hospital by opening wondows on the opposite side of the laboratory. The instrument was mounted on slabs of foam rubber to reduce transmission of mechanical shocks and vibrations from the bench to the galvanometer suspension.

Air pressure was adjusted by means of the needle valve on the instrument to 12 lb/sq. in. and was carefully maintained at this pressure whenever the instrument was in use.

Variation in the pressure of the mains gas supply was noted using a water manometer attached to a tap adjacent to the flame photometer, and a Jeavons 'J55' gas governor (supplied by the regional gas board) was fitted in the supply line to the machine. The governor was set to regulate gas pressure to the machine at 3 in. water and the pressure was recorded continuously over 24 hr. to demonstrate its efficiency. By means of water manometers, the pressure from a side arm of the machine supply was recorded on a kymograph simultaneously with the pressure of the supply to a Bunsen burner from an adjacent tap with no governor fitted. A section of the pressure trace when fluctuations in gas pressure were maximal is shown in Fig. 1 and illustrate that the governor was effective in eliminating these fluctuations.

The manufacturers of the flame photometer later pointed out that a gas governor was incorporated in the instrument which was

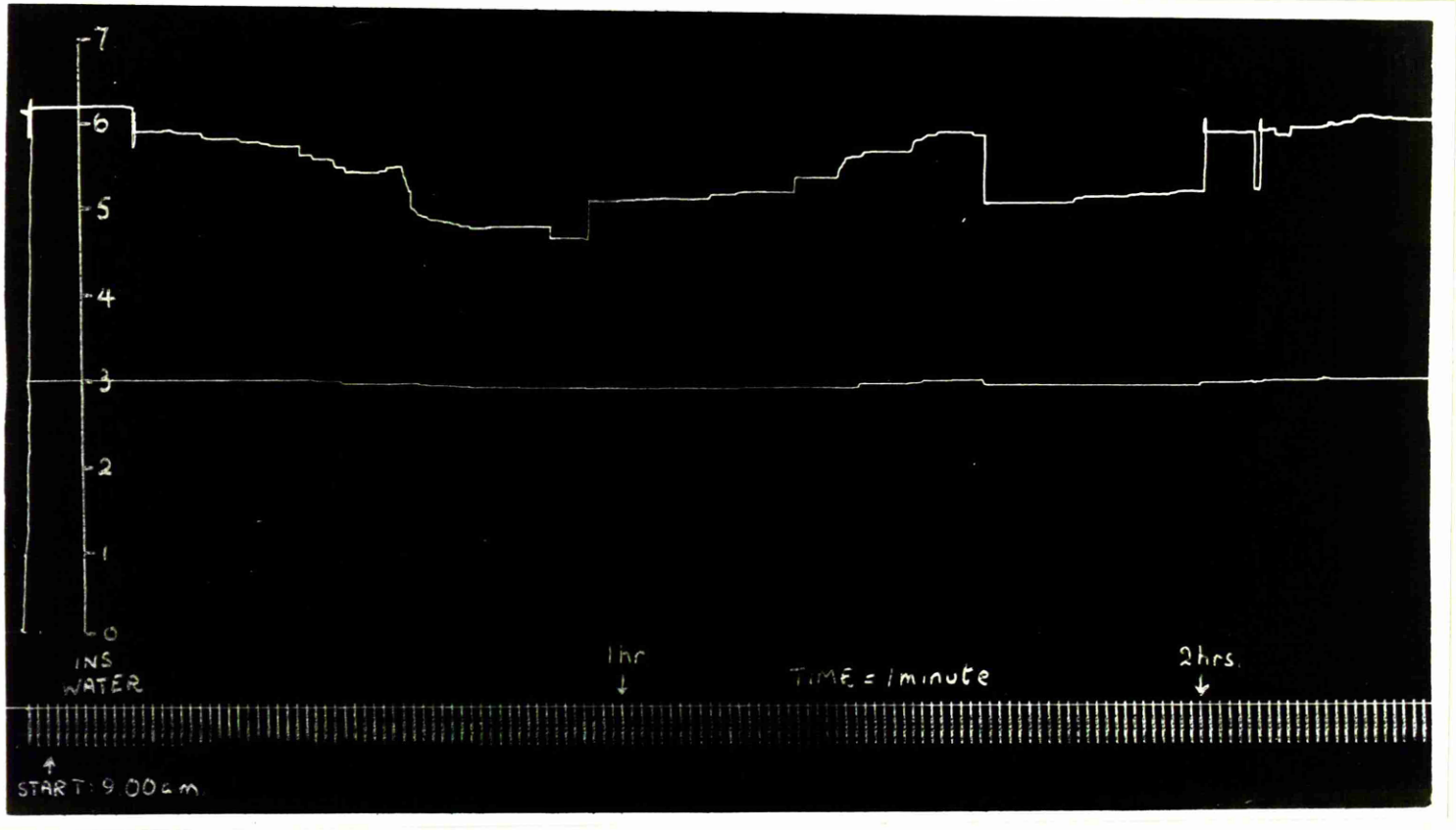

Fig. 1. Record of mains gas pressure. The lower trace was recorded from a tap fitted with a gas pressure governor.

set to stabilize the pressure of the supply to the machine at 2 in. water. Having demonstrated that mains gas pressure was subject to sudden changes of pressure of 1-2 in. water, the external governor was retained as additional insurance against these changes affecting the flame.

Drainage. The drainage system was modified to improve stability of the instrument by leading the drain tube vertically down through the instrument casing and the bench. Provision for continuous drainage of the atomizer chamber was made by fixing the tip of the drain tube in a small crucible supported over a large (3 l.) beaker. With this arrangement, waste dripped from the crucible as drops trickled down the drain tube so that back pressure from waste accumulating in the tubing was avoided, whilst escape of gas from the atomizer chamber was prevented.

Under these conditions, the stability of the galvanometer spot allowed readings to be made accurately to 0.5 scale divisions.

Glassware and standard solutions

All glassware used was of Pyrex or borosilicate glass and after washing was rinsed at least three times with deionized water (Elgastat, Elga Products Ltd.) of resistance greater than 10^6 ohms. Care was taken to avoid contact with the fingers of interior surfaces of all containers. Smoking was not allowed in the vicinity of the instrument, and contamination with dust particles from the atmosphere was avoided as far as possible by covering all unstoppered containers when not in use.

Standard solutions were prepared using 'Analar' NaCl and KCl and deionized water. The reagents were dried by heating in an oven at 140°C. and cooling in a desiccator to constant weight.

In the early stages of the work a series of standards from 20 m-equiv/l. to 200 m-equiv/l. were prepared at 20 m-equiv/l. intervals by weighing out accurately the calculated amounts of the salts. Later it was found more convenient to make up a single standard of 50 m-equiv/l. from which a range of dilutions equivalent to a chosen range of standards at a dilution of 1:1000 was readily prepared.

Operative procedure

A length (25 cm) of fine polythene tubing was attached to the tip of the atomizer capillary tube for convenience in feeding samples to the machine and to minimise handling of the small cups containing diluted solutions for analysis. Analyses were carried out in batches of twelve samples, carried in the trays provided with the instrument, by dipping the end of the polythene tube into individual containers. In order to reduce the possibility of blockage, the atomizer system was never allowed to dry out. Deionized water was sprayed when not reading dilutions of standard or unknown solutions, and the tip of the extension to the atomizer capillary tube was kept immersed in deionized water whenever the machine was not in use.

The sensitivity of the instrument was maintained constant by adjusting the potentiometer setting so that a 1:250 dilution of a 50 m-equiv/l. solution of NaCl or KCl gave full scale deflection of the galvanometer for Na and K readings respectively. Zero was set using deionized water, and checks on sensitivity and zero setting were made as a routine after every six readings. Readings between 20 and 80 per cent of the scale length were obtained for samples undergoing analysis by making appropriate dilutions of the samples, and not by varying the instrument sensitivity.

Duplicate readings were made on each series of twelve samples, in reverse order to the initial series of readings, in an attempt to avoid 'observer bias' (q.v.). The readings were noted after the test solution had been passing into the machine for 20-30 sec, the operator observing a stop clock (not the galvanometer spot) until the time to note the reading. The procedure was repeated if necessary until agreement within 0.5 scale divisions between successive readings was achieved.

Influence of 'observer bias' in analysis of replicates

The possibility was considered that less variation might arise between replicated measurements on a solution taken from the same bottle than between measurements on the same solution taken from different bottles, through a subconscious bias on the part of the operator. Readings might preferentially be noted when the galvanometer spot reached a value previously noted for the same solution, regardless of subsequent shifts of the galvanometer spot. Thus, close agreement between duplicate readings might not reflect the true repeatability of the procedure.

The present procedure in which readings were taken on a time schedule was designed to avoid this tendency. It was tested by noting duplicate readings for a range of standards from 40 - 160 m-equiv/l. diluted 1:1000. Potassium was added to the sodium standards, before dilution, to give seven series of solutions with Na:K concentration ratios of 1:0, 1:1, 1:2, 1:3, 1:4, 1:8 and 1:16, and sodium was added to the potassium standards to give a similar series for potassium estimations. The bottles were numbered in random fashion so that the concentrations of the test element and the Na:K concentration in each solution were unknown to the operator. One series of duplicate readings was taken, then the numbers on the bottles were altered and a second

series of duplicate readings obtained.

The results are shown in Tables 1 and 2. The close agreement apparent between the two series of readings for each bottle showed that 'observer bias' had no demonstrable influence on duplicate agreement. The technique of noting readings to a time schedule proved also to be less fatiguing to the operator when performing a long series of analyses, since his attention was not continually focussed on the movements of the galvanometer spot.

Effect of sensitivity setting on the shape of the calibration cuurve

Readings were obtained for a range of standards of pure NaCl and KCl from 50 - 200 m-equiv/l. at dilutions of 1:100, 1:250, 1:500 and 1:1000. Zero was set using deionized water and sensitivity was adjusted by means of the potentiometer so that the 200 m-equiv/l. standard for each series of dilutions gave full scale deflection of the galvanometer. The readings obtained are plotted in Figs. 2 and 3. It is clear that the calibration curve for both sodium and potassium became less curvilinear at higher dilutions. Strict linearity was not obtained by further increase in dilution of this range of concentrations since dilutions greater than 1:1000 approached the limits of sensitivity of the instrument. Instability of the galvanometer spot then gave rise to difficulties in taking accurate readings. The standard sensitivity setting as described under 'Operative Procedure' was therefore adopted in all analyses.

Interference effects on sodium and potassium estimations

'Interference' is used throughout this discussion to describe direct depression or elevation of the emission energy of the test substance by other ions or solutes present in the test solution. Such effects on the emission of either sodium or potassium are not

TABLE 1

Investigation of possible 'observer bias' in replicate analyses: Sodium readings

Ratio K : Na	Na conc. (m-equiv/l.)	Bottle No. (1st series)	Bottle No. (2nd series)	Instrument readings 1st series	Instrument readings 2nd series
0:1	40	27	1	21 , 21	21 , 21
	80	21	27	41.5, 42	41.5 , 42
	120	5	2	62.5, 62	62.5 , 62.5
	160	13	26	82 , 82	81.5 , 81.5
1:1	40	20	4	21.5, 22	23 , 23
	80	4	25	44 , 44	43.5 , 44
	120	12	6	65 , 65	64.5 , 64
	160	19	28	83 , 83.5	83.5 , 84
2:1	40	3	8	23 , 23	23 , 22.5
	80	11	17	44 , 44.5	48 , 45.5
	120	18	10	66 , 66	65.5 , 66
	160	2	24	84.5, 85	84.5 , 84
3:1	40	10	12	22.5, 22	22.5 , 22.5
	80	17	22	44.5, 45	45 , 44.5
	120	1	14	66.5, 66.5	65 , 65.5
	160	22	23	84 , 84	85 , 84.5
4:1	40	26	3	22.5, 23	23.5 , 23.5
	80	6	20	45.5, 46	45.5 , 45
	120	23	5	66 , 65.6	65.5 , 65.5
	160	14	18	85 , 85.5	86 , 86
8:1	40	7	7	23 , 23	23.5 , 23
	80	24	19	44.5, 45	45 , 45.5
	120	15	9	66, 66	66 , 66
	160	8	16	84.5, 85	85 , 85
16:1	40	28	11	23.5, 23.5	23.5, 23.5
	80	16	21	46.5, 47	46.5, 46
	120	9	13	66 , 66	66.5, 66.5
	160	25	15	85.5, 86	85.5, 85.5

TABLE 2

Investigation of 'observer bias' in replicate analyses: Potassium readings

Ratio Na : K	K conc. (m-equiv/l.)	Bottle No. (1st series)	Bottle No. (2nd series)	Instrument readings 1st series	Instrument readings 2nd series
0:1	40	27	18	21.5, 21.5	21.5, 21.5
	80	23	17	42.5, 42	42.5, 42.5
	120	14	1	62 , 62	62.5, 63
	160	4	2	81.5, 81.5	81.5, 81.5
1:1	40	24	16	21.5, 21.5	22 , 22
	80	5	25	42.5, 43	43 , 43
	120	22	19	63 , 63	63.5, 63.5
	160	13	3	81.5, 82	82 , 82
2:1	40	3	4	21.5, 22	22 , 22
	80	15	15	42.5, 43	43 , 43
	120	6	5	63.5, 63	63.5, 63
	160	21	20	82 , 82	82 , 81.5
3:1	40	12	6	21.5, 21.5	21.5, 21.5
	80	2	21	43 , 43	43.5, 43
	120	16	14	63.5, 63.5	64 , 64
	160	7	7	82.5, 82.5	82.5, 82.5
4:1	40	20	24	21.5, 21.5	22 , 21.5
	80	11	28	43.5, 43.5	43 , 43.5
	120	1	8	63.5, 63.5	64 , 63.5
	160	17	13	82.5, 82.5	82.5, 82.5
8:1	40	8	9	21.5, 22	22.5, 22
	80	19	23	43.5, 43.5	43.0, 43.5
	120	10	26	63.5, 63.5	63.5, 64
	160	25	10	82.5, 83	83 , 83
16:1	40	18	12	22.5, 22	22 , 22.5
	80	9	27	43.5, 44	43.5, 43.5
	120	28	11	63.5, 64	64 , 64
	160	26	22	83.5, 83	83 , 83

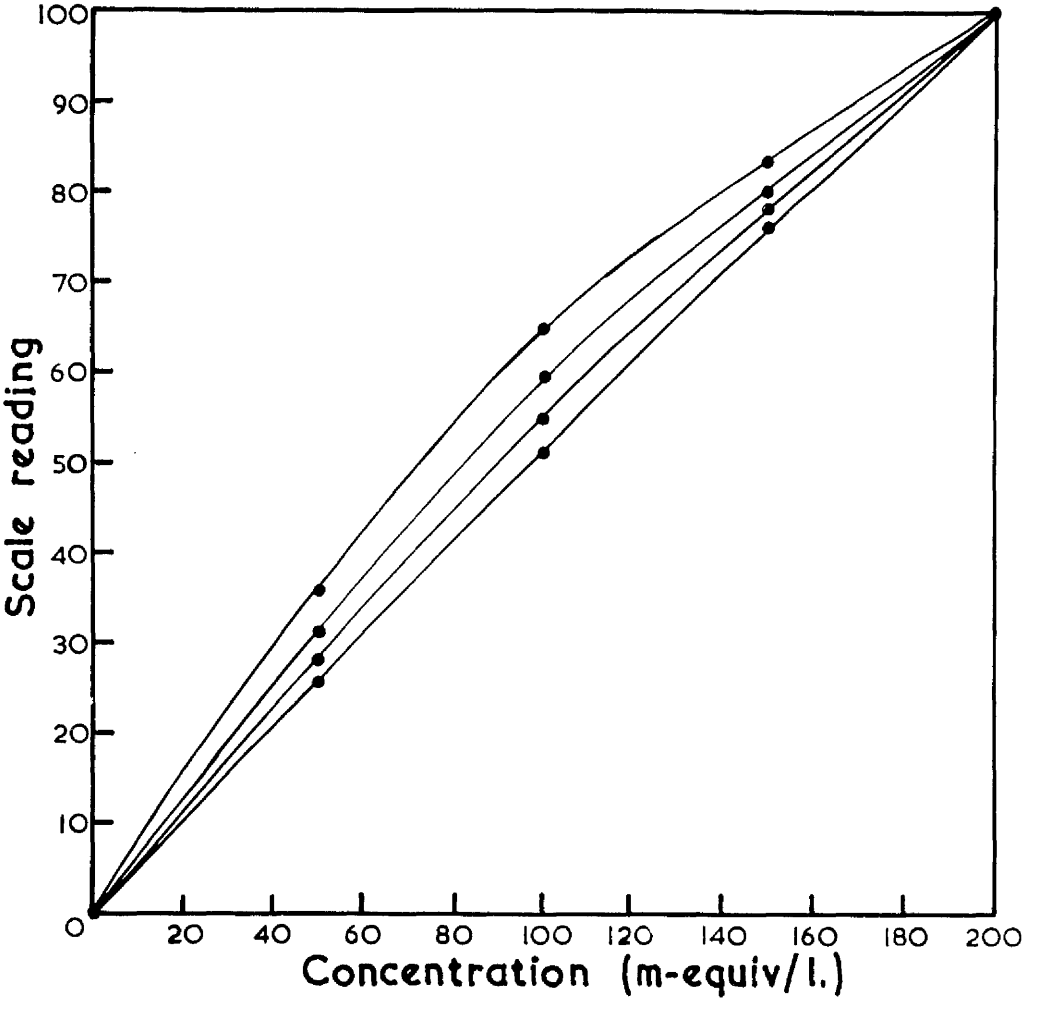

Fig. 2. Effect of sensitivity setting on calibration curve (sodium readings). The curves, from above downward, correspond to dilutions of 1:100, 1:250, 1:500 and 1:1000 respectively, over the range of concentrations shown. Zero was set using deionised water and full scale deflection was set for each series of dilutions using the 200 m-equiv/l. standard of that series.

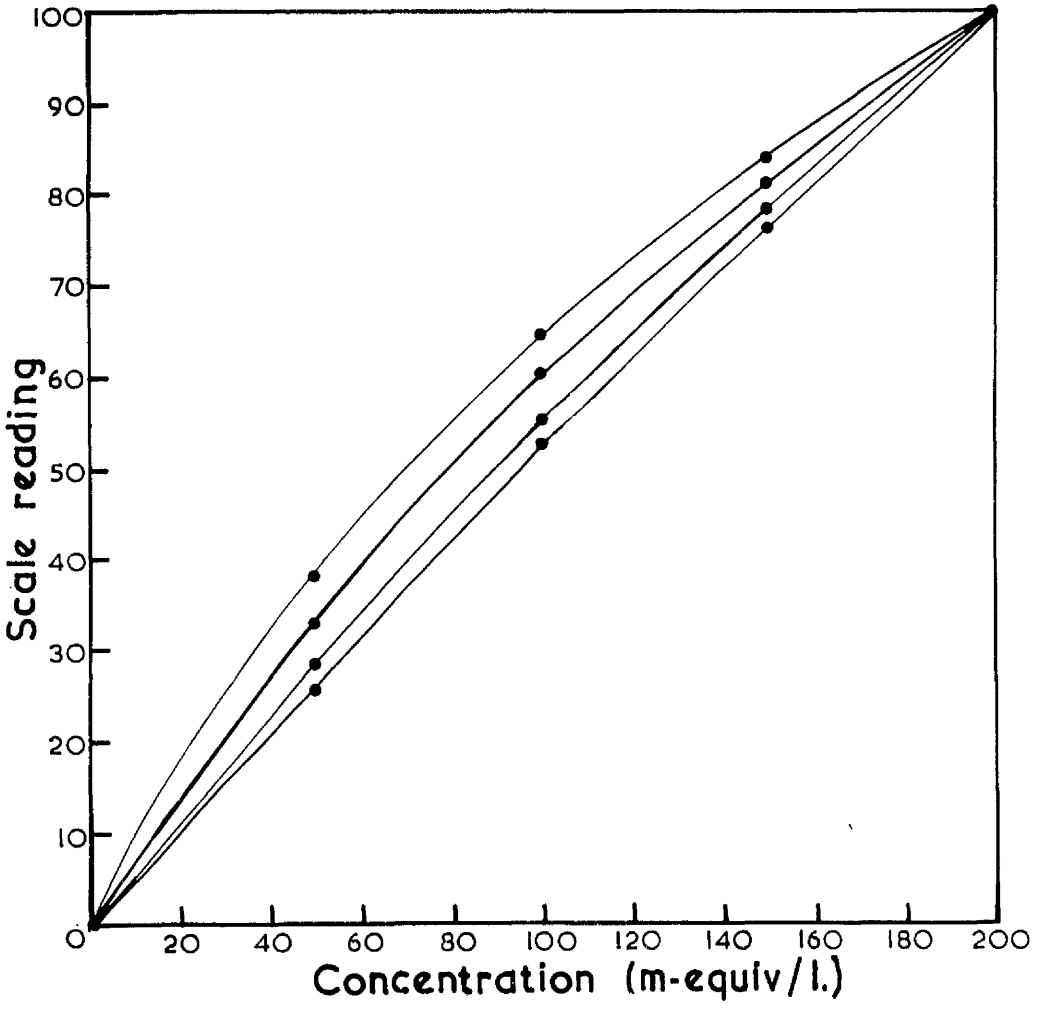

Fig. 3. Effect of sensitivity setting on calibration curve (potassium readings). The curves, from above downward, correspond to dilutions of 1:100, 1:250, 1:500 and 1:1000 respectively, over the range of concentrations shown. Zero was set using deionised water and full scale deflection was set for each series of dilutions using the 200 m-equiv/l. standard of that series.

demonstrable when calcium, organic acids (oxalic, citric and acetic), ammonia, glucose, or lipid (cholesterol) are present in concentrations approximately twice those which can be expected in biological materials at the dilutions used in flame photometric analyses (Wynn et al., 1950). Protein similarly, apart from the viscosity factor (which these authors showed could be ignored at the dilutions used), was reported to have no effect on sodium and potassium emissions.

Phosphate interference has been emphasized by some workers (Parks, Johnson and Lykken, 1948), although Shapiro and Hoagland (1948) found that removal of phosphate by precipitation did not affect significantly the values obtained for sodium and potassium concentrations in urine. Wynn et al. (1950) were unable to demonstrate any interference by phosphate on sodium determinations, although significant depression of the potassium emission was noted when increasing amounts of sodium phosphate were added to a constant amount of potassium chloride, but only after the molar ratio of phosphate to potassium exceeded unity. Collins and Polkinhorne (1952), using the EEL flame photometer, showed that phosphate depressed the values found in both sodium and potassium estimations, by 2 per cent and 11 per cent respectively. These interference effects were obtained, however, with phosphate concentrations of 1000 p.p.m., when sodium and potassium concentrations were 10 p.p.m. - a most unlikely circumstance in body fluids. The possibility of phosphate interference has, therefore, been discounted in the present work.

Mutual interference between sodium and potassium.

Burriel-Marti and Ramirez-Munoz (1957) conclude from an extensive review that, in general, the presence of both sodium and potassium in the solution undergoing analysis tends to

increase their respective light intensities. Domingo and Klyne (1949), using an air-acetylene flame, and Wynn et al. (1950), using a coal gas-oxygen flame, found that increasing amounts of sodium, when added to a constant amount of potassium, increased the photometer reading at 767 mμ (the wavelength of potassium emission). The same amounts of sodium in the absence of any potassium registered zero emission at this wavelength. Wynn et al. (1950) also showed that the interference effect depended on the ratio of the ionic concentrations and not upon the absolute amounts present, and that the interference effect of sodium on potassium readings reached a maximum for a given flame temperature when a particular concentration ratio had been reached. At higher flame temperatures the increase of potassium emission when sodium was also present was more marked, and reached maximum values at considerably higher sodium:potassium concentration ratios. Thus, when the high temperature air-acetylene flame was used, sodium interference on potassium readings did not attain a maximum value even at a molar ratio of 200:1 (Domingo and Klyne, 1949). These findings may well explain the reports that sodium does not enhance potassium emission (Berry, Chappell and Barnes, 1946; Hald, 1947; Parks et al., 1948) when gas mixtures are used which give a flame of relatively low temperature.

The interference effect of potassium on sodium readings similarly was reported to depend on the ratio of the ionic concentrations, but attained a maximum when the molar ratio, K:Na, was quite low (3.5:1). Also, and unlike the interference of sodium on potassium, the interference effect of potassium on sodium estimations showed little sensitivity to alterations of flame temperature (Wynn et al., 1950).

Mutual interference effects between potassium and sodium were demonstrated with the EEL flame photometer by plotting the readings obtained in four series of standards for each element. The series contained increasing amounts of the interfering ion to give molar ratios of 0:1, 1:1, 4:1 and 16:1. The readings for all solutions were taken with the instrument sensitivity set to give full scale deflection with a 1:250 dilution of 50 m-equiv/l. standard solutions of pure (Analar Reagent) NaCl and KCl respectively.

Progressively higher readings for the sodium standards were obtained in each series as the proportion of potassium present increased (Fig. 4). Potassium readings were similarly affected by the presence of increasing proportions of sodium in the solutions analyzed (Fig. 5). Further increases in potassium readings were not obtained after further increases in the proportion of sodium present. In Figs. 6 and 7 scale readings obtained for solutions containing the same concentration of the test element with increasing concentrations of the interfering element are plotted against the molar concentration ratio. The enhancement of potassium emission reached an apparent maximum at a concentration ratio (Na:K) of approximately 8:1 (Fig. 6), but potassium interference on sodium readings apparently had not reached a maximum at a concentration ratio (K:Na) of 20:1 (Fig. 7). This contrasts with the report that potassium interference in sodium estimations becomes maximal at a molar ratio of 3.5:1 (Wynn et al., 1950).

In the present work standard solutions of potassium were prepared from 'Analar' KCl, and this reagent may contain NaCl (maximum permitted limit: 0.2 per cent by weight). Thus, when additions of potassium were made to the standard solutions of

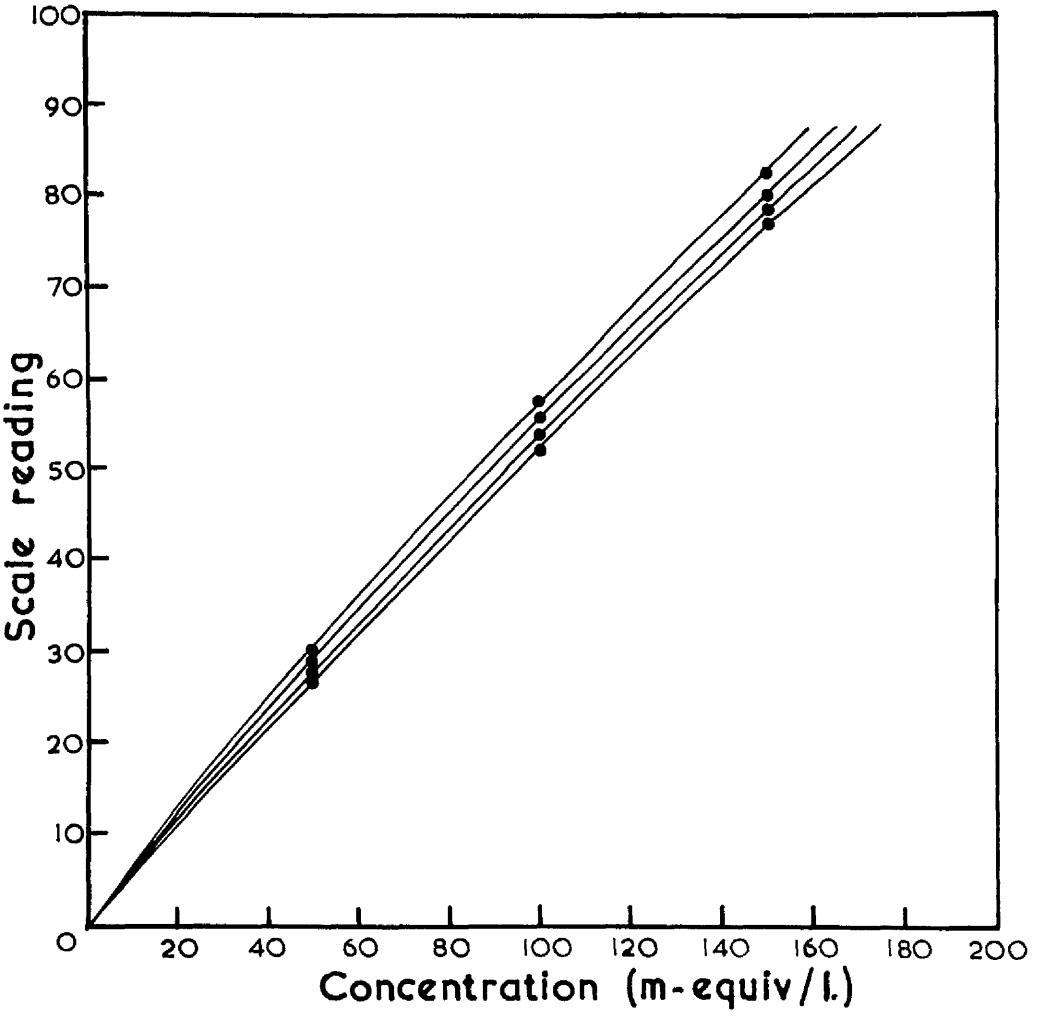

Fig. 4. Effect of potassium on sodium readings. Zero was set using deionised water, and full scale deflection using a 1:250 dilution of 50 m-equiv/l. NaCl. Over the concentration range shown, at a dilution of 1:1000, the readings plotted are, from above downward, for mixtures of sodium and potassium standards with molar ratios (K : Na) of 16:1, 4:1, 1:1 and 0:1 respectively.

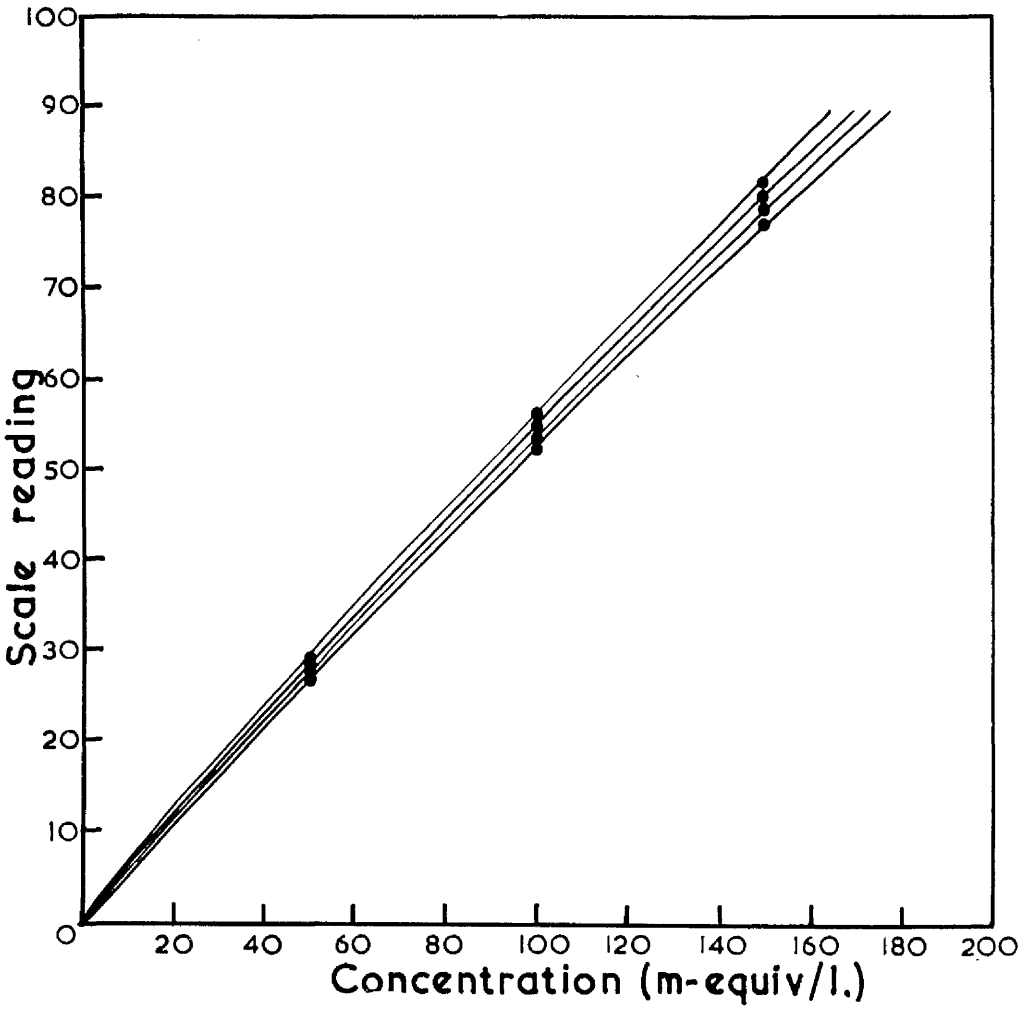

Fig. 5. Effect of sodium on potassium readings. Zero was set using deionised water and full scale deflection using a 1:250 dilution of 50 m-equiv/l. KCl. Over the concentration range shown, at a dilution of 1:1000, the readings plotted are, from above downward, for mixtures of potassium and sodium standards with molar ratios (Na : K) or 16:1, 4:1, 1:1 and 0:1 respectively.

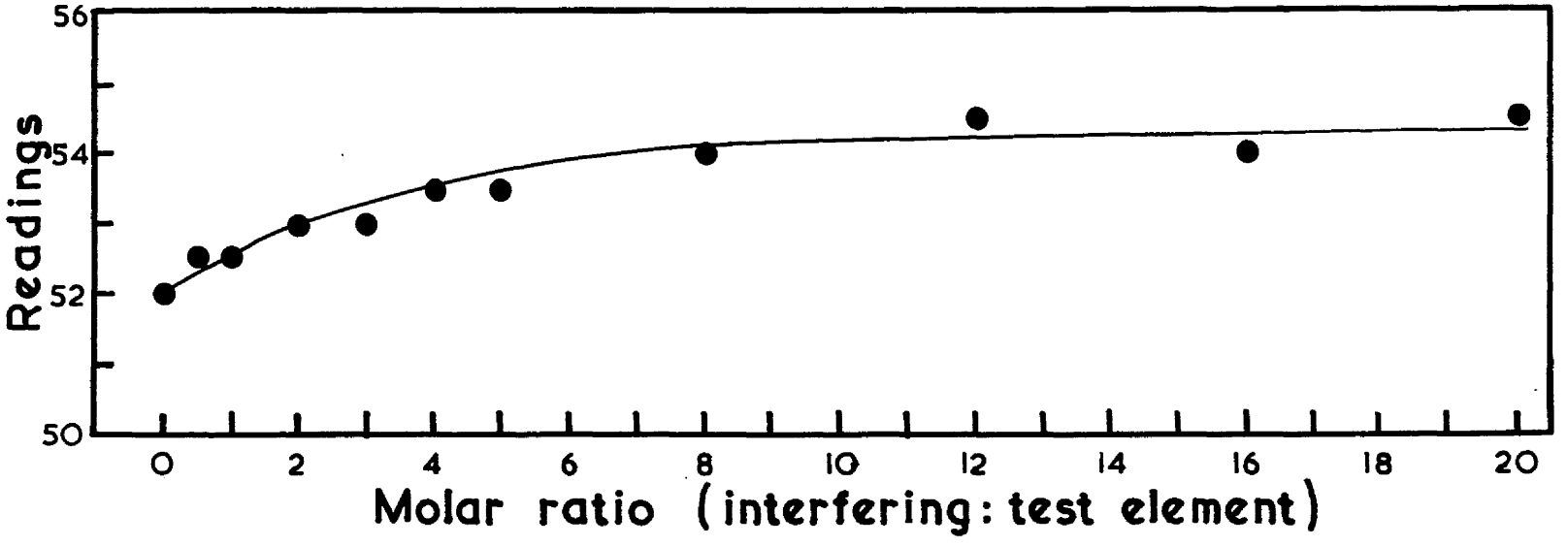

Fig. 6. Sodium interference on readings of a potassium standard of 100 m-equiv/l. diluted 1:1000. Zero was set using deionised water and full scale deflection using a 1:250 dilution of 50 m-equiv/l. KCl.

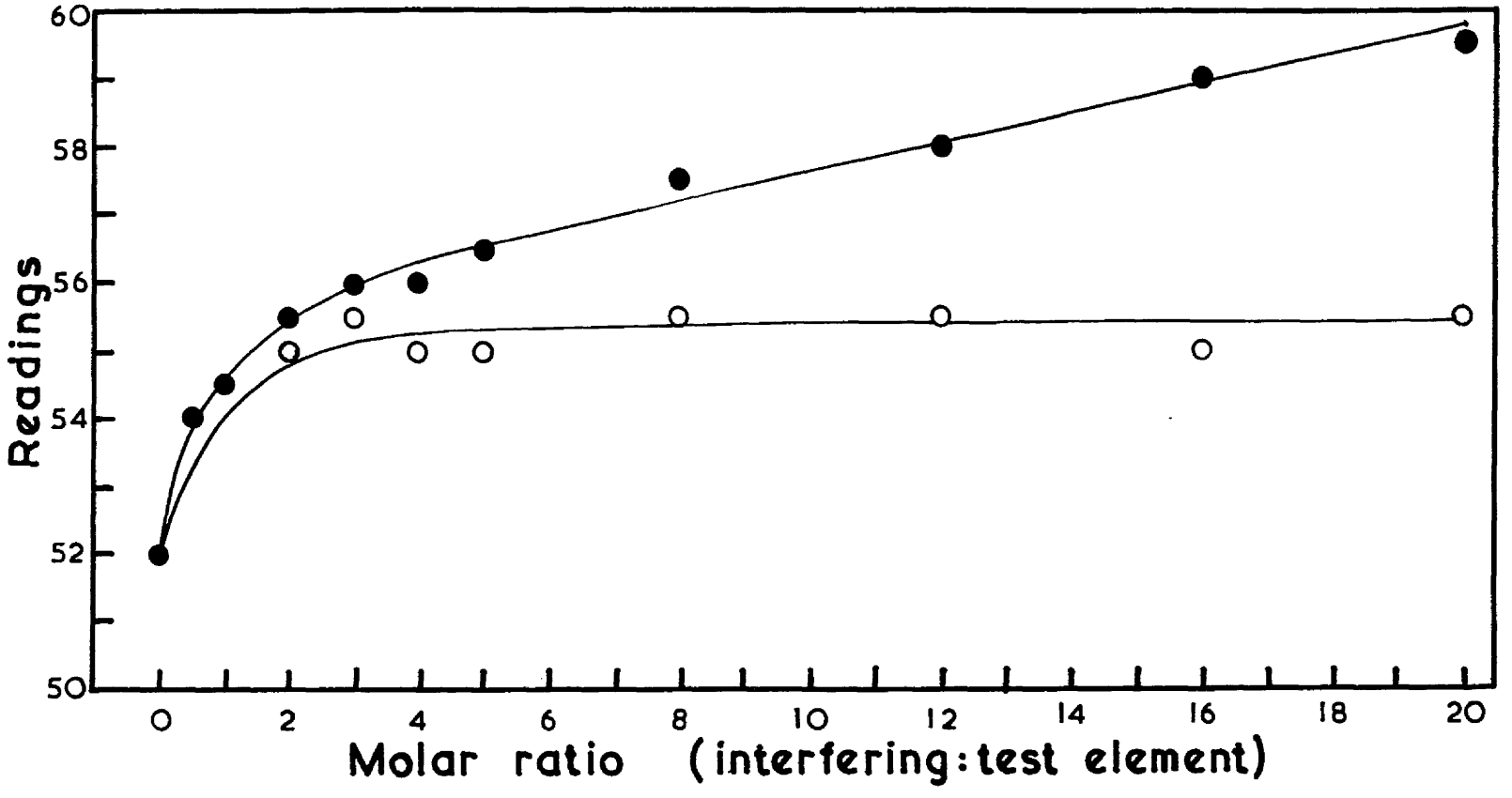

Fig. 7. Potassium interference on readings of a sodium standard of 100 m-equiv/l. diluted 1:1000. Open circles are readings after correction for sodium impurity present in potassium standard used (see text). Zero was set using deionised water and full scale deflection using a 1:250 dilution of 50 m-equiv/l. NaCl.

sodium, observed increases in sodium readings probably reflected interference effects due to potassium, and, also, a direct contribution from small amounts of sodium present as an impurity in the KCl solution added.

The presence of sodium in the potassium standard was confirmed by observing the yellow colour of the flame when the potassium standard alone was fed into the machine. The quantitative contributions of this contaminant to the sodium readings plotted in Fig. 7 were measured by recording the sodium emission of the pptassium standard alone at concentrations which had been used in constructing the interference curve. Sodium contamination was not detected at the two lowest concentrations, but became increasingly apparent as the concentration of the potassium standard increased. By subtracting these readings from those plotted on the interference curve for sodium, a corrected interference curve was constructed (lower line in Fig. 7) which was not distorted by the emission of sodium introduced as a contaminant of potassium added. It was then apparent that enhancement of sodium readings by potassium reached a true maximum at a molar ratio (K:Na) of approximately 4:1, in agreement with the findings of Wynn et al. (1950).

No potassium contamination of the NaCl used in the construction of the potassium interference curve was detected, and potassium does not appear in the list of permitted impurities of 'Analar' NaCl.

Correction of mutual interference effects between sodium and potassium

Errors arising from interference effects may be eliminated by using standard solutions which approximate to the test solution in the ratio of the interfering and test ions. This method is

applicable particularly to determinations on blood plasma, but is unsatisfactory for estimations on urine since the composition of urine is extremely variable and the approximate ratio of interfering and test ions in any sample cannot be predicted.

Alternatively, an excess of the interfering element may be added to both the test sample and the standards, during the preparatory dilution procedure, so that the degree of interference is constant, as demonstrated by the flattening of the interference curves. This method was suggested by Domingo and Klyne (1949) and is applicable to urine analyses, but has the disadvantage that analysis for both sodium and potassium cannot be performed using the same diluted sample. The possibility of introducing additional amounts of the test element to the sample as a contaminant of the excess of interfering element added is an additional disadvantage.

Wynn et al. (1950) point out that since the degree of mutual interference between sodium and potassium is a function of their concentration ratio until the interference effect becomes maximal, it is possible to correct the observed emission of the test element if the concentration ratio of the interfering element to test element is known. For a given concentration ratio the percentage increase in the transmission energy of the test substance above its theoretical value may be calculated from the interference curve. It is assumed that the transmission energy of the test substance in the unknown sample is increased proportionately by the presence of the interfering element.

This method of correcting for mutual interference effects entails a preliminary estimation of the apparent concentrations of sodium and potassium in the sample. From the concentration

ratio found the theoretical transmission value of the test substance is then calculated as follows:

Let A = theoretical emission value of test substance,

B = found emission value of test substance,

x = percentage increase in A for the known concentration ratio of interfering and test element, as calculated from the interference curve.

Then $A + \frac{x}{100}A = B$

and $A = \frac{100B}{100 + x}$

The concentration ratio of interfering and test element need not be accurately determined: the correction factor is for practical purposes the same whether the ratio is 2:1 or 2.2:1.

Duthie and McDonald (1960), using the EEL flame photometer, describe a similar system for correcting interference effects in the analyses of sodium, potassium and calcium in biological materials. Algebraic corrections of the instrument readings were obtained from regression equations relating the extent of interference to the concentrations of the interfering element. Preliminary determinations of the apparent concentrations of interfering and test elements were again necessary.

Allowance for mutual interference effects were made in the present study by using calibration curves which had been plotted from several series of standard solutions of the test element in the presence of increasing quantities of the interfering element.

Sodium estimations. Three series of diluted standard were prepared, each covering an effective concentration range of 0-200 m-equiv/l. at a dilution of 1:1000. Appropriate quantities of potassium standard were added to each series before dilution to

give molar ratios (K:Na) of 0:1, 1:1 and 4:1 respectively. The flame photometer readings obtained were plotted as in Fig. 4. At the 4:1 ratio, true potassium interference on sodium readings is maximal (as shown in Fig. 7), and any increase of the sodium readings due to sodium present as a contaminant in the potassium added is only slight.

After noting the sodium and potassium readings of a test sample the approximate molar ratio (K:Na) of the sample was calculated, and the sodium concentration of the sample, corrected for the interference effect of potassium, was then obtained by reading off from the pencil of calibration curves at a position appropriate for the K:Na ratio found. Whilst this involved some approximation, the need for mathematical treatment as in the methods of correction described by Wynn et al. (1950) and by Duthie and McDonald (1960) was eliminated. Another advantage of this procedure was that the preliminary determination of concentration ratio was possible by direct comparison of the instrument readings, adjusted to equal dilution where necessary, since the calibration curves for potassium and sodium are closely similar (Figs. 1 and 2), and full scale deflection was set for both sodium and potassium readings using standard solutions of the same concentration (50 m-equiv/l.) at the same dilution (1:250).

Potassium estimations. Four series of potassium standards were made up each covering an effective concentration range of 0 - 200 m-equiv/l. at a dilution of 1:1000. Appropriate quantities of sodium standard were added to each series before dilution to give molar ratios (Na:K) of 0:1, 1:1, 4:1 and 8:1. The instrument readings were plotted as in Fig. 5. The series of standards with the Na:K concentration ratio of 8:1 was necessary since it had been shown that sodium interference did not

become maximal until this ratio was reached (Fig. 6).

The concentration of potassium in a test sample was then obtained from observed readings using this pencil of curves as described for sodium estimations.

Since the operating conditions of the instrument were maintained uniform throughout all analyses, the same calibration curves were used from day to day. However, when fresh standard solutions were made up, for setting instrument sensitivity (full scale deflection), new calibration curves were plotted from readings obtained using diluted mixtures of the new standard solutions.

Assessment of accuracy of method

Plasma estimations. Six samples of freeze-dried serum of known composition (C. Davis Keeler, London) were reconstituted to the supplier's instructions and analyzed for sodium and potassium. Sodium concentrations were read off the calibration curve for pure sodium chloride (concentration ratio K:Na = 0:1), and potassium concentrations were read off the potassium calibration curve allowing for maximal interference from sodium (concentration ratio Na:K = 8.1). Samples were diluted 1:50 for potassium readings and 1:2,500 for sodium readings. The concentrations found agreed on average within 1% of the values stated by the supplier (Table 3).

Urine estimations. The accuracy of estimations on bovine urine was assessed by recovery of known increments of sodium and potassium added to samples of bovine urine. Preliminary analyses were carried out to determine the approximate molar ratio of sodium or potassium in each sample. A volume of sodium or potassium standard was then added to measured volumes of the samples, before dilution, to increase the concentration of the test element

TABLE 3

Analysis of serum of known composition

Sodium : 145 m-equiv/l. Potassium : 5.1 m-equiv/l.

Concn. found (m-equiv/l.)	Error (%)	Concn. found (m-equiv/l.)	Error (%)
146	0.7	5.1	0
145	0	5.1	0
143	1.4	5.0	2
145	0	5.1	0
147	1.4	5.2	2
144	0.7	5.0	2
Mean error	0.7%	Mean error	1.0%

by exactly 50 m-equiv/l. Appropriate amounts of the interfering element were also added, to maintain the original concentration ratios of interfering and test elements in the samples.

The results are shown in Tables 4 and 5. An average error of 6.1% was found for sodium recoveries when the concentrations were read off the calibration curve for pure sodium. This error was reduced to 1.2% when the sodium concentrations were obtained from the pencil of calibration curves according to the known K:Na concentration ratios of the samples. The potassium recoveries did not demonstrate that interference gave rise to so large an error. This was expected since the Na:K concentration ratio in all the samples was less than unity. In samples containing as much or more sodium than potassium, interference effects presumably would give rise to a greater error in uncorrected potassium estimations.

Comparison of sodium and potassium concentrations in bovine and human urine

As an exercise in flame photometric technique, and to demonstrate the existence of species differences in urinary composition similar to those described by previous workers (Spector, 1956), sodium and potassium concentrations were determined in urine samples collected at mid-day from twenty male students and from twenty cows of the dairy herd at the veterinary school farm (p. 55). Results of the analyses are given in Table 6.

The concentrations found varied widely both between and within groups, presumably because of individual variations in the intake and expenditure of electrolytes and water. Details

TABLE 4

Recovery of sodium increment added to samples of bovine urine

Sample	Concn. ratio (K : Na)	Apparent* increment (m-equiv)	Error %	Corrected* increment (m-equiv)	Error %
1	2.6 : 1	54.0	8	51.5	3
2	4.4 : 1	53.5	7	51.0	2
3	5.8 : 1	53.0	6	50.5	1
4	4.4 : 1	53.0	6	50.0	0
5	3.2 : 1	52.5	5	50.0	0
6	4.2 : 1	54.0	8	51.0	2
7	2.5 : 1	52.5	5	50.0	0
8	6.3 : 1	53.0	6	50.5	1
9	7.3 : 1	53.0	6	51.0	2
10	2.7 : 1	52.0	4	49.0	2
Mean errors			6.1 %		1.3%

* Apparent values of increment were obtained when concentrations were read from calibration curve for pure sodium, corrected values when concentration ratio (K : Na) was taken into account as described in text.

TABLE 5

Recovery of potassium increment added to samples of bovine urine

Sample	Concn. ratio (Na : K)	Apparent* increment (m-equiv)	Error %	Corrected* increment (m-equiv)	Error %
1	0.4 : 1	50.5	1	49.5	1
2	0.4 : 1	50.5	1	49.5	1
3	0.2 : 1	50.0	0	50.0	0
4	0.5 : 1	51.5	3	49.5	1
5	0.4 : 1	51.0	2	50.5	1
6	0.6 : 1	52.0	4	51.0	2
7	0.2 : 1	50.0	0	50.0	0
8	0.4 : 1	50.5	1	49.5	1
9	0.5 : 1	52.0	4	51.0	2
10	0.3 : 1	50.5	1	50.0	0
Mean errors			1.7%		0.9%

* Apparent values of increment were obtained when concentrations were read from calibration curve for pure potassium, corrected values when concentration ratio (Na : K) was taken into account as described in text.

TABLE 6

Sodium and potassium concentrations in bovine and human urine.

Sample	MAN			COW		
	Na (m-equiv/l.)	K (m-equiv/l.)	Ratio K:Na	Na (m-equiv/l.)	K (m-equiv/l.)	Ratio K:Na
1	307.5	42.5	0.14	34.5	87.0	2.52
2	43.5	26.8	0.62	94.0	241.3	2.57
3	268.8	53.8	0.20	122.0	307.5	2.52
4	322.5	104.4	0.32	18.5	385.0	20.81
5	422.5	51.1	0.12	63.5	280.0	4.41
6	196.3	85.0	0.43	114.0	238.8	2.10
7	181.3	103.7	0.57	116.0	266.3	2.30
8	133.8	113.8	0.85	64.0	367.5	5.75
9	160.6	41.5	0.26	80.0	348.8	4.36
10	191.3	36.0	0.19	136.5	225.0	1.65
11	178.8	155.0	0.87	28.0	187.5	6.70
12	211.3	110.6	0.52	93.0	300.0	3.23
13	233.8	69.4	0.30	91.5	383.8	4.22
14	268.8	93.8	0.35	88.5	220.0	2.49
15	225.0	94.4	0.42	63.5	400.0	6.30
16	252.5	88.8	0.35	45.5	332.5	7.31
17	243.8	108.8	0.45	24.5	323.8	13.22
18	165.6	88.8	0.54	33.0	353.8	10.72
19	210.0	97.5	0.46	95.0	185.0	1.95
20	133.1	80.6	0.61	81.5	222.5	2.73
Mean ± S.D			0.43 ± 0.21			5.39 ± 4.75

of the total intake and losses of sodium and potassium of the subjects were not available but the mean value of the molar concentration ratio, potassium to sodium, in bovine urine (5.39 ± 4.75 (S.D.)) was over twelve times greater than that found in human urine (0.43 ± 0.21 (S.D.)). This difference was statistically highly significant ($P < 0.001$) and reflected differences in the intake of sodium and potassium in these species as suggested from differences in the mineral content of human and bovine foodstuffs (Morrison, 1951).

pH, total CO_2 and bicarbonate estimations

Techniques for the measurement of the pH and the total CO_2 content of bovine urine and plasma had been studied previously in our laboratory by Anderson, and the methods he described (Anderson, 1964) were adopted in the present work.

Anaerobic treatment of samples

Arterial blood was taken into an oiled, heparinized, 10 ml. syringe from the coccygeal artery as described elsewhere (p. 68), and the syringe nozzle closed with a rubber cap. Within 5 min of withdrawal the syringe was carried to the laboratory and the pH of the sample measured. The remainder was then centrifuged under oil in stoppered tubes and the plasma separated into small stoppered tubes and stored in a refrigerator for determination of total CO_2 content on the following day.

Urine samples were taken at the mid-point of 15 min clearance periods into oiled 20 ml. syringes and were retained in the syringes with the nozzles occluded by rubber caps. The pH value of the samples were measured before some of the volume remaining was transferred, under oil, to small stoppered tubes for storage in the refrigerator. Total CO_2 content of the samples was measured on the following day.

Measurement of pH at 39°C

A 'Vibron' electrometer (Eledtronic Instruments Ltd., model 33B) with a pH measuring unit (Electronic Instruments Ltd., model C-33B) was used in conjunction with a capillary glass electrode system enclosed in a water jacket through which water was pumped

at a thermostatically controlled temperature of 39°C (Electronic instruments Ltd., Replaceable Capillary Glass Electrode System, Model SHH33). Readings were set using the procedure described by Anderson (1964), with buffers prepared as described by Bates and Acree (1945), Hamer, Pinching and Acree (1946) and Manov, De Lollis and Acree (1946). The buffers used to standardise the instrument initially were 0.05 molal potassium hydrogen phthalate (pH = 4.027 at 39°C) and a mixed phosphate buffer of 0.025 molal disodium hydrogen phosphate and 0.025 molal potassium dihydrogen phosphate (pH = 6.838 at 39°C). For daily use the meter was standardised on the phosphate buffer only.

Samples were introduced into the electrode system without exposure to the air via a fine polyethylene tube attached to the capillary electrode. Suction was applied to the system from a filter pump.

Urine. Measurements were made at least twice and were considered acceptable if within 0.005 pH units of each other.

Arterial blood. Difficulty was encountered in obtaining close agreement between duplicate determinations. Repeated measurements were possible because of the small volume of blood required by the capillary glass electrode and were made until agreement between successive readings of $\pm$ 0.01 pH units was achieved.

Measurement of total CO_2

Total CO_2 in samples of urine and arterial plasma was estimated using the Van Slyke manometric apparatus by the methods described by Peters and Van Slyke (1932). Because of the high concentration of bicarbonate in bovine urine the modification described by Anderson (1964) was adopted, namely, replacement of

0.2 ml. Van Slyke pipette used for addition of a measured volume of the sample, by an 'Agla' micrometer syringe of 0.5 ml. capacity capable of delivering to an accuracy of 0.0002 ml. Determinations on each sample were repeated until replicate agreement within $\pm$ 1% was obtained.

Calculation of HCO_3 concentration

The bicarbonate concentration in samples was calculated from the values found for pH and total CO_2 using the Henderson-Hasselbalch equation arranged in the following form (Peters and Van Slyke, 1932) :

$$\left[BHCO_3\right] = \left[\text{Total } CO_2\right] \frac{1}{1 + \frac{\left[H^+\right]}{K'}}$$

$$= \frac{\left[\text{Total } CO_2\right]}{1 + \text{antilog } (pK' - pH)}$$

A value for pK' of 6.1 was assumed in plasma samples. For each urine sample the value of pK' was calculated using the formula given by Hastings and Sendroy (1925):

$$pK' = 6.33 - 0.5 \sqrt{B}$$

Where B is the total concentration of cations in the sample. Since sodium and potassium constitute at least 95% of the total cation concentration in bovine urine, the sum of their molar concentrations was substituted for B.

Chloride estimations

Chloride estimations in samples of urine and venous plasma were determined electrometrically using an EEL chloride meter. All samples were estimated in duplicate unless duplicate agreement was less than ± 1%, when a third estimation was carried out.

Inulin estimations

Inulin estimations were carried out by the direct resorcinol method without alkali treatment (Roe, Epstein and Goldstein, 1949), using a Unicam 'SP 600' spectrophotometer, and inulin standards of 1, 2 and 3 mg/100 ml. for each series of estimations. Plasma and urine samples were diluted with deionised water to give estimated inulin concentrations within this range. Plasma was diluted 1:10 and urine between 1:100 and 1:1000 according to the rate of urine flow. Urine dilutions as low as 1:100 were necessary in some experiments in which very high rates of urine flow occurred in response to procedures which promoted diuresis.

Inulin is a polymer consisting principally of fructose molecules and in the direct resorcinol method it is estimated as fructose, after hydrolysis. Contamination of inulin with free fructose can give rise to errors in clearance determinations since contaminating fructose is recorded as inulin in plasma analyses but does not appear in the urine becuase of its reabsorption by the renal tubules. The inulin used in our laboratory had previously been shown not to contain free fructose in measurable amounts (Anderson, 1964).

The accuracy of the above method of inulin estimation in our laboratory had been investigated previously (Anderson, 1964). The mean concentration found in ten samples of plasma to which inulin had been added 72 hours previously, to give a concentration of 15 mg/100 ml., was 14.4 $\pm$ 0.4 (S.E.= $\pm$ 0.1) mg/100 ml., when the optical density of each tube was measured as soon as possible after colour development, and 15.1 $\pm$ 0.6 (S.E. = $\pm$ 0.2) mg/100 ml. when the optical density was measured 30 min after the first

reading. This experiment also showed that inulin in solution in plasma remained fairly stable at room temperature for at least three days.

p-Amino hippurate estimations

Determinations of PAH concentrations in plasma and urine samples were carried out photometrically after diazotizing the p-amino group of PAH with nitrous acid and coupling with N-(1-naphthyl) ethylene diamine, as described by Smith, Finklestein, Aliminosa, Crawford and Graber (1945), with the modification that plasma samples were diluted 1:10 with deionized water and plasma proteins precipitated using zinc sulphate and barium hydroxide as in inulin estimations (v. s.). PAH standards of 0.1, 0.2 and 0.3 mg/100 ml. were included in each series of estimations, the urine samples being diluted with water from 1:500 to 1:2,500 to give an estimated PAH concentration within this range.

Investigation of the accuracy of PAH estimations showed an average recovery of 100.3% (range 98.3% - 102.2%) for plasma samples with PAH concentrations of 1.8 - 5.4 mg/100 ml. (Table 7). These plasma solutions were prepared by adding known volumes of a standard solution of PAH to measured volumes of bovine plasma.

T A B L E 7

Recovery of PAH added to samples of bovine plasma

Sample	PAH conen. (mg/100 ml.)	PAH conen. found (mg/100 ml.)	Recovery (%)
1	1.80	1.77	93.3
2	1.80	1.84	102.2
3	3.60	3.64	101.1
4	3.60	3.62	100.6
5	5.40	5.36	99.3
6	5.40	5.42	100.4
		Mean :	100.3%

EXPERIMENTAL TECHNIQUES

Management of experimental animals

Experiments involving clearance procedures were carried out on healthy, non-pregnant, non-lactating Ayrshire cows. In the summer months the animals were kept at grass without supplementary feeding, and from late autumn to spring were housed and fed hay (20-30 lb, 9.1 - 13.6 Kg) and a commercial concentrate mixture (7 lb, 3.2 Kg) daily, in two feeds. Water was available ad libitum. Preliminary hydration of experimental subjects, as is common practice in renal investigations on man and the dog, was not carried out before experiments. The experiments lasted 4-6 hr and water, but not food, was available during this period.

Because of the risk of damage to equipment and personnel, dehorned animals, judged to be of placid temperament were purchased for this work. Recently dehorned animals often showed resentment when the head and neck were manipulated so the early practice of dehorning experimental animals in the hospital was abandoned because of the long delay before such animals were suitable for use.

In addition to the inconvenience of an animal's struggling during an experiment, any emotional disturbance of the animal associated with the experimental procedures may complicate the experimental findings. Knudsen (1960), in experiments on cows, noted that the rate of excretion of sodium declined in successive clearance periods and attributed the high initial rates of excretion to the initial excitement associated with introduction to the experimental room, and the disturbance of urethral

catheterization. Miles and De Wardener (1953) described an emtional diuresis with increased salt excretion in women which occurred on bladder catheterization or resulted from apprehension of a surgical procedure, and Anderson (1961) has described a similar phenomenon in cattle: a marked, transient diuresis seen in response to the painful stimulus of brachial arterial puncture. After repeated experiments on the same animal, a similar response was also seen during the preliminary procedure of clipping, swabbing and anaesthetizing the site for puncture. In the present study, therefore, the animals were handled quietly at all times and pain or discomfort during experiments was minimised wherever possible by the use of local or regional anaesthesia. Newly purchased animals were allowed a period of at least a fortnight to become accustomed to their new location. Dairy cows are accustomed to close contact with man so that, with care in handling, most animals became readily amenable to experimentation. On two occasions newly purchased animals became fractious in the experimental room and were replaced, but other animals became fully accustomed to the experimental procedures; they ruminated frequently and rarely showed signs of distress or discomfort.

On the morning of an experiment the animal was brought in from the field or byre and placed in a restraining stocks or crush. The stocks consisted of four stout corner posts of metal piping carrying lateral rails of timber and two removable bars of timber which slotted respectively across the front and back ends of the animal. The crush was of tubular and sheet metal construction of a design commonly used on farms. It had a removable bar at the back and a half door at the front over which the head of the animal could be restrained by means of a yoke in the shape of an inverted Y. The yoke was brought down on a ratchet by means of

a lever; it was occasionally used during the initial preparation of the animals but was seldom necessary.

Cannulation of the jugular veins was carried out under local anaesthesia with the animal firmly restrained by the nose. Catheterization of the bladder was carried out under posterior epidural anaesthesia and required no special restraint of the animal. After cannulation of the jugular, therefore, restraint was reduced to a minimum with the stocks or crush preventing excessive movement while the animal's head was held loosely by a halter. Detailed description of these preliminary procedures is given in later sections.

An early survey of the rate of excretion of sodium and potassium in urine samples collected serially under epidural anaesthesia over periods of 3-4 hr was carried out on a cow standing in a byre at the veterinary hospital with no other restraint. Urine samples for investigation of flame photometric technique and for comparison of sodium and potassium concentrations in human and bovine urine were collected during normal micturition from male student volunteers and from cows of the dairy herd of the veterinary school farm. These animals were healthy and in various stages of pregnancy and/or lactation. They were under an indoor system of management, fed on turnips, silage and hay for maintenance requirements with additional concentrates according to milk yield.

Measurement of the rate of urine flow

In studies on renal function it is generally assumed that no alteration in volume or composition of the urine occurs during passage along the urinary tract. Although there is little evidence that the urine formed by the kidneys is modified during ureteral passage, or during storage in the bladder, Garby, Risholm, Thoren and Ulfendahl (1957) showed, in man, a statistically significant tendency for concentrations of urinary solutes to be lower at the lower end of the ureter than at the upper end or at the renal pelvis; and Rapoport, Nicholson and Yendt (1960) state that in some circumstances the composition of bladder urine need not be identical with that of urine as it leaves the kidneys. These latter authors measured changes in the concentrations of electrolytes in solutions introduced into the bladder of dogs. The magnitude of the changes depended on the concentration gradient across the bladder wall and was influenced also by the pH of the solution, the time the solution remained within the bladder, and, probably, by the volume of the test solution. On the other hand, studies in dogs on the permeability of the bladder wall to water (D_2O) by Johnson, Cavert, Lifson and Visscher (1951) showed that when the bladder was moderately distended a considerable exchange of water molecules took place across the mucosa, but that net volume changes were barely demonstrable.

In the present study, urine was collected by an indwelling urethral catheter. The urine collected was assumed to have been unaltered by passage down the ureters and, since the urine was collected continuously, any exchange of water and electrolytes across the bladder wall was assumed to be negligible.

The techniques used for urine collection in investigations

of kidney function in cattle have not been extensively described. Assessment of the accuracy of collection over short periods of time is generally lacking and any resentment shown by the experimental animal is only briefly mentioned. Sellers and Roepke (1951) do not give details of the catheter which they used during two hour collection periods. Cunningham, Frederick and Brisson (1955) describe a self-retaining urethral catheter with an inflatable bulb which they employed for the continuous collection of urine over long periods (up to three weeks) and they report that some cows showed slight discomfort when first catheterized. Sellers, Pritchard, Weber and Sautter (1958) mention a soft rubber catheter secured to webbing straps for urine collection during clearance periods of 15-30 min duration. Poulsen (1957), Vogel (1959), Ketz (1960) and Knudsen (1960) describe 'balloon' catheters similar to that used by Cunningham et al. (1955) which were used in clearance experiments involving collection periods ranging from 10 min to 2 hr. De Groot and Aafjes (1960) report inconsistent results when they used urethral catheters in adult cattle and they adopted the special apparatus described by Van Es and Vogt (1959) in which catheterization was avoided. This apparatus did not measure continuously the production of urine and would seem to have no application when 15-30 min collection periods are employed. None of these workers mention the use of any form of anaesthesia. Knudsen (1960), however, emphasised the excitement and struggling which occurred at the outset of his experiments, and described associated variations in electrolyte excretion.

Accuracy in the measurement of the renal excretion of any substance is largely determined by the efficiency of the technique used for urine collection. Winton (1956) stated that renal

clearance measurements under good steady-state conditions, involved uncertainties of 5-10%, and Smith (1951) ascribed such uncertainties to the difficulty of obtaining complete bladder drainage. Such inaccuracies are exaggerated as the period of collection is shortened. In the cow a possible residual volume of 50 ml.is a small proportion of the average volume of urine (3 l.) voided during normal micturition (Katongole, 1964) and is insignificant in proportion to an estimated daily output of 15-20 l., but a residual volume of 50 ml. represents a large error in a 15 min collection period. In addition, physical and psychical disturbances associated with the collection procedure must be minimal when conscious subjects are used in investigations of renal physiology, since these factors have been shown to affect the rate of urine flow and the rate of excretion of electrolytes in cattle (Knudsen, 1960; Anderson, 1961).

In our laboratory difficulties were encountered in early attempts to collect bovine urine continuously using modifications of the Foley catheter (Warne, 26F.G. : 100 ml. bulb capacity) (Anderson and Pickering, 1961). Catheterization without any form of anaesthesia was never accomplished without manifestations of resentment, and, although gross struggling subsided once the catheter was in position, animals continued to show signs of discomfort and agitation. These varied from frequent shifting of the hind feet with twitching of the tail and lowing, to repeated or even continuous attempts to micturate. Determined attempts to micturate commonly resulted in some loss of urine around the catheter and occasionally in forceful expulsion of the catheter. The animals also defaecated frequently, soiling the catheter and collection tubing. Whilst urine collection in cattle by urethral catheter without anaesthesia may be satisfactory over long periods,

when animals might become accustomed to the presence of the catheter, the resentment shown by our animals to this procedure indicated that it was unsuitable for the physiological studies proposed.

Additional difficulties encountered in urine collection were, first, an entry of air into the bladder during catheterization so that the siphon initially established to allow continuous collection could not be maintained, and, second, an irregularly intermittent flow during continuous collection which was attributed to occlusion of the perforations on the catheter by folds of bladder mucous membrane. That these occasional stoppages were not reflexions of the actual rate of urine formation was supported by the observation that on some occasions, after clamping the collecting tubing for 30 min, an immediate flow of urine was not seen on release of the clamp.

Entry of air into the bladder during catheterization occurred because of the subatmospheric pressure which normally obtains within the bovine bladder (Anderson and Pickering, 1961). Most previous workers do not mention this phenomenon and it has not been extensively studied. However it does not indicate that the physiology of the bovine bladder differs from that described in other species. Katongole (1964) showed by simultaneous recording of intraabdominal and intracystic pressures that both these values were subatmospheric in the standing cow. Intraabdominal pressure was recorded by needle puncture of the abdominal wall in the sublumbar fossae. Increases of intraabdominal pressure produced by inflating the abdominal cavity with oxygen were faithfully reflected by the intracystic pressure, and both rose above atmospheric pressure when the animals were tilted into lateral recumbency. Bovine cystometrograms showed the typical features of those described in other species except that values of intracystic

pressure in the cow remained subatmospheric until micturition occurred. It was concluded that the pressure within the bladder was probably a reflexion of the pressure which obtained in dorsal regions of the abdominal cavity, and this pressure was subatmospheric in the standing animal because of the downward drag of the viscera on the abdominal wall. Although cystometrograms demonstrated the relationships of intracystic pressure to bladder volume, the absolute value of intracystic pressure was largely determined by the intraabdominal (extracystic) pressure.

In the present work, therefore, catheterization and urine collection were carried out under posterior epidural anaesthesia. The alternative procedure of inducing local anaesthesia by topical application of amethocaine to the mucous membrane of the vulva, vagina and terminal urethra was not adopted since Anderson (1964) had shown the effect of this procedure to be inconsistent and variable in duration. When introducing the catheter, precautions were taken to prevent the entry of air into the bladder and a catheter with a coiled, multiperforate tip was used so that some perforations were shielded from occlusion.

Method

Epidural anaesthesia

The skin over the sacro-coccygeal space was clipped and swabbed with Tincture of Cetrimide B.P. (I.C.I., 'Tincture of Cetavlon'). A no. 0 hypodermic needle (20 SWG x 1.5 in) was then inserted through the skin into the epidural space and 4 ml. of 5% $^{W}/_{V}$ procaine hydrochloride with adrenaline (May and Baker, 'Planocaine') was injected, observing the criteria for proper location of the needle described by Wright and Hall (1961).

Flaccidity of the tail developed almost immediately and loss of sensation in the vulva developed during the following 5-10 min. Absence of micturition, defaecation and motor function of the tail then persisted for 2-3 hr. Early signs of returning sensation were tail twitching and defaecation and if the experiment was not complete at this time the anaesthesia was reinforced by a further injection of 3 ml. of anaesthetic into the sacro-coccygeal space. If this procedure was not followed, recovery from the initial dose of anaesthetic continued and was marked by straining and increasing restlessness in most subjects with, usually, an increase in the rate of urine flow.

Using the method described, sensation from the vagina and urethra was never completely abolished. The animals always showed slight reaction during catheterization and to the stimulus of pulling on the catheter when <u>in situ</u>. However, if the catheter was left undisturbed once it had been introduced the animal showed no reaction to its presence until the effect of the anaesthetic began to wear off. In addition, the anaesthesia of the tail resulting from this procedure allowed sampling of arterial blood, from the coccygeal artery, without disturbing the animal. In some cases slight motor paralysis of the hind limbs was noted when animals were led out from the experimental room before the effects of the epidural anaesthetic had worn off, and during many experiments the animals were seen to rest their hindquarters on the transverse bar at the back of the restraining crush.

Most animals were subjected to epidural anaesthesia on at least ten occasions without difficulty or apparent ill effect. It was noted, however, that after repeated epidural anaesthesia on the same animal induction sometimes became progressively more difficult. In such cases it was probable that fibrosis resulting

from repeated puncture of the epidural space limited the distribution of the anaesthetic.

Catheterization

After administration of the anaesthetic the tail was tied to one side, the vulva, anus and perineal area were washed with soap and water and the lips of the vulva swabbed with antiseptic solution (Reckit and Colman; 'Dettol'). A catheter of moulded rubber with two or three additional holes cut on the inside of the coiled, multiperforate tip was used (Vicarey Davidson, Glasgow, 'Vicson Ramshorn', 14 EG) (Fig. 8). For introduction the catheter was straightened with a stainless steel stilette and lubricated with sterile, water soluble jelly (Johnson & Johnson, 'KY' lubricating jelly). The coiled tip retained the catheter in situ when the stilette was withdrawn. During catheterization a tightly fitting rubber cuff around the stilette plugged the open end of the catheter and prevented air from entering the bladder. The stilette was partly withdrawn, through the cuff, once the tip of the catheter was in the bladder and the catheter clamped before the stilette, and the cuff, were completely removed. With the catheter in place, polythene drainage tubing was attached and the bladder emptied by establishing a siphon. Urine was then collected continuously.

Assessment of accuracy of urine collection

A continuous flow of urine once the bladder had been emptied was a consistent feature of this technique. Two criteria were used to assess the validity of this method of urine collection: first, recovery of a known volume of sterile water injected into the bladder, and, second, comparison of the values found for

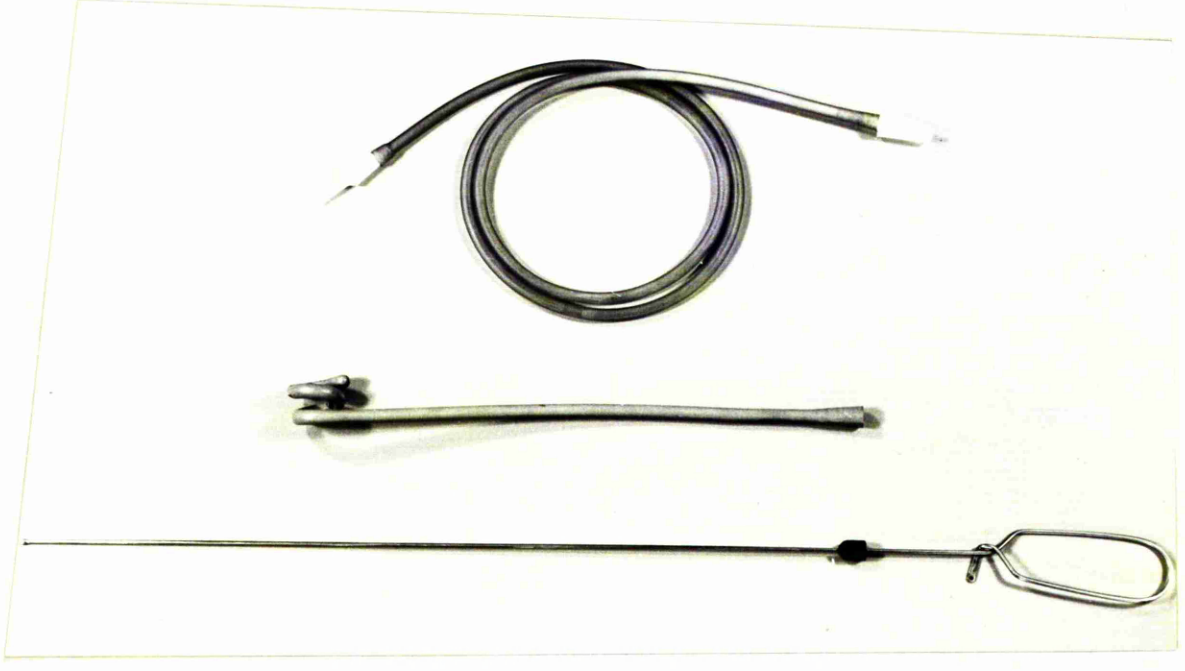

Fig. 8. The 'ramshorn' urethral catheter with introducer and bung (bottom) and polythene drainage tubing (top). With the introducer in place the tip of the catheter is straightened and its open end is plugged by the bung. The collection tubing is fitted with nylon adaptors to facilitate attachment to the catheter and aspiration by a syringe to establish a siphon.

successive determinations of inulin clearance over wide variation in urine flow.

Bladder washout. In a preliminary experiment urine was collected over a period of 50 min with the volume measured every 10 min. Five injections of 100 ml. of sterile water were then made into the bladder and the total volumes recovered in succeeding 10 min collection periods were measured (Fig. 9). The mean urine flow before washing out was 21.6 ml./min. The total volume recovered during the washout period was 718 ml. Assuming that urine flow had remained constant at 21.6 ml./min, the recovery volume expected should have been 10 x 21.6 + 500 = 716 ml. This assumption of a constant rate of urine flow is supported by the good agreement between the urine flows measured before and after washing out. The correlation between the anticipated volume (716 ml.) and the volume actually recovered (718 ml.) was taken to indicate that there was a negligible residual volume of urine in the bladder. Further experiments of this type were not carried out since no evidence of inefficient urine collection was apparent from inulin clearance determinations.

Evidence from measurements of inulin clearance. Glomerular filtration rate normally remains fairly constant despite wide variations in urine flow (Smith, 1951). If, in the measurement of glomerular filtration rate, urine collection is inefficient, aberrant values are to be expected. In the course of repeated measurements of glomerular filtration rate by the inulin clearance procedure described on p. 65, there was no evidence of errors which could be attributed to incomplete bladder drainage. Repeated measurements of glomerular filtration rate remained constant within the accepted limits of 5-10% (Winton, 1956) despite variations of 200-600% in the urine flow. Results of representative experiments on three cows are presented in Table 8.

If the urethral catheter failed to drain a significant frac-

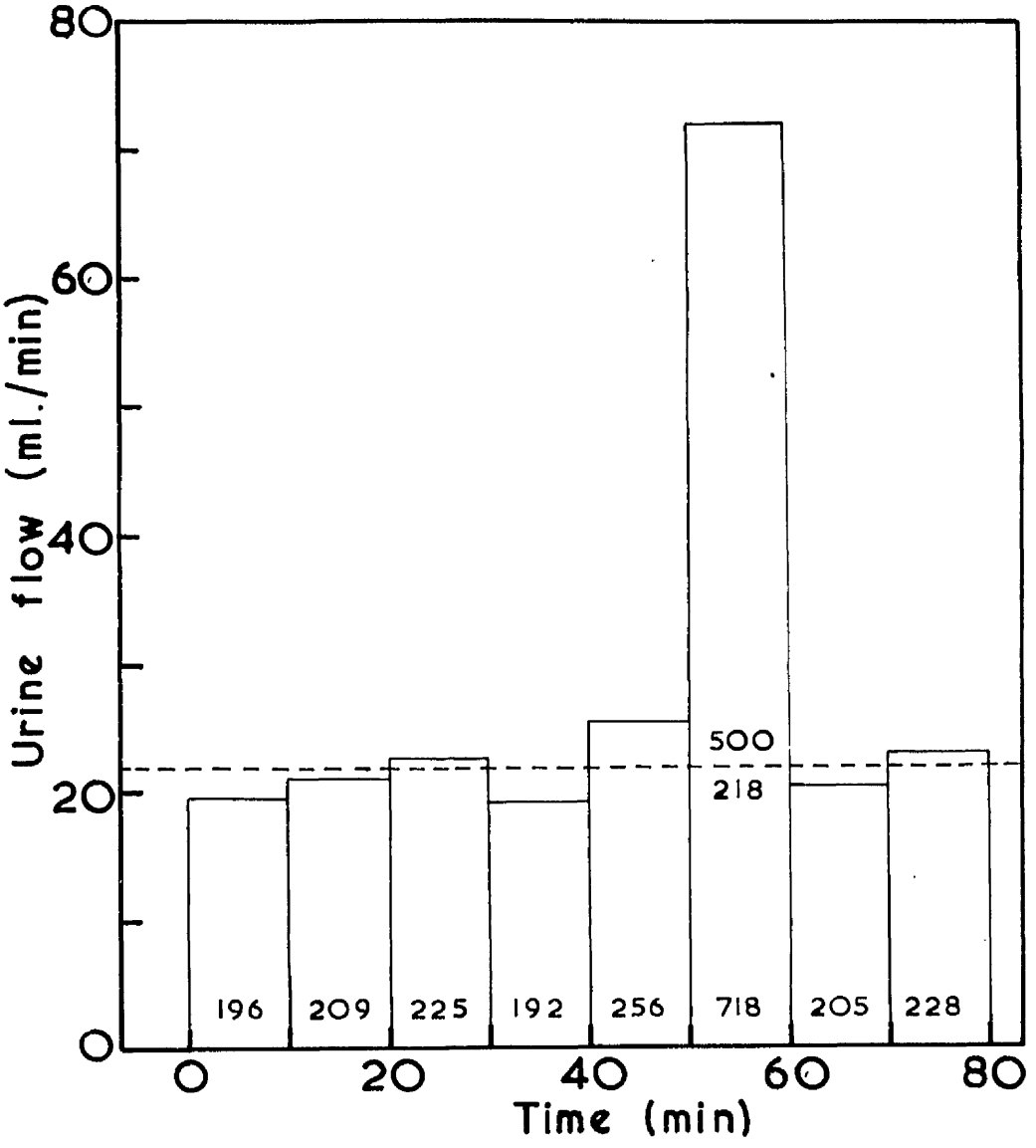

Fig. 9. Effect of bladder wash-out on urine collection. The broken line indicates the mean urine flow (21.6 ml./min) before the rapid infusion of 500 ml. of water in period 6. Figures on histogram are volumes (ml.) collected during each 10 min. period.

T A B L E 8

Inulin clearance (G.F.R.) before and during diuresis produced by intravenous infusion of potassium chloride.

Cow No.	Urine flow (ml./min)	G.F.R. (ml./min)
12916	18.8	1265
	16.1	1199
	67.5	1274
	68.3	1142
	61.7	1162
14711	15.4	918
	7.9	843
	29.9	941
	32.2	931
	27.7	894
	36.3	864
14948	18.3	958
	50.0	1003
	37.7	924
	28.2	970
	22.9	995

tion of the bladder urine, an increase in urine flow would give rise to an apparent increase in the inulin clearance because of an increase in the percentage recovery of urinary solutes. Lower values of inulin clearance were noted, however, after administration of the diuretic drug acetazolamide (p. 108). This provided further evidence of the efficiency of the method of urine collection since, despite the diuresis promoted by this drug, a fall in inulin clearance values to 50-70% of predosing values was observed similar to that noted after administration of acetazolamide in the dog (Berliner, Kennedy and Orloff, 1951). On the other hand, no alteration in glomerular filtration rate was noted during similar experiments on the same animals in which diuresis was produced by administration of hydrochlorothiazide (p. 108), a drug which has not been reported to affect the glomerular filtration rate.

Measurement of glomerular filtration rate.

The measurement of glomerular filtration rate is of crucial importance in studies on kidney function. Without this datum a quantitative assessment of the contribution of tubular reabsorption and secretion to observed rates of urinary excretion cannot be made. In intact animals the glomerular filtration rate may not be measured directly, but it is measurable using clearance procedures.

The term "clearance" was first used by Möller, MacIntosh and Van Slyke (1929) in studies on urea excretion in man, in which the urea clearance was described as a whole blood clearance. This was used as an empiric measurement of one renal function, namely, the ability of the kidney to remove urea from the blood and deliver it into the urine. Since normal renal processes operate on blood plasma, plasma clearance values are more meaningful when applying the clearance concept to the study of renal function. Any substance which appears in the urine has a clearance value, which may be defined as the volume of plasma which contains the amount of the substance appearing in the urine in unit time:

$$C = \frac{UV}{P} \quad (ml/min)$$

where: C = the clearance value,

U = urinary concentration

V = rate of urine flow (ml/min)

P = plasma concentration

That the clearance concept afforded a means of measuring the glomerular filtration rate was foreseen by Rehberg (1926). Thus, if a reference substance be freely filterable at the

glomeruli and be neither reabsorbed, excreted nor metabolized by the renal tubules, then its clearance value is equal to the glomerular filtration rate. The clearance of inulin was first described as a measure of glomerular filtration rate by Richards, Westfall and Bott (1934), in the dog, and by Shannon and Smith (1935) in man. The various criteria by which inulin clearance has come to be accepted as a measure of the glomerular filtration rate in a wide variety of vertebrates have been described by Smith (1951).

The measurement of the glomerular filtration rate in adult cows, using an inulin clearance procedure was first described and assessed by Poulsen (1957). Vogel (1959) and Kotz (1960) have also determined inulin clearance values in studies of kidney function in cattle. Sellers, Pritchard, Weber and Sautter (1958), using a constant infusion technique, showed that the clearance ratio of endogenous creatinine to inulin was close to unity in calves and heifers varying in age from 1 week to 2½ years. Clearance values of endogenous creatinine, however, were consistently, albeit slightly, greater than simultaneous determinations of inulin clearance, as had previously been noted in the sheep (Shannon, 1937). Simultaneous measurements of the clearances of inulin and exogenous creatinine in goats and sheep by Ladd, Liddle, Gagnon and Clarke (1957) showed creatinine clearance values normally to be greater than inulin clearance values at low plasma concentrations of creatinine, and that creatinine clearance was depressed, absolutely and relative to inulin clearance, by elevation of the plasma concentration of creatinine, or of p-amino hippurate, or by intravenous probenecid. In contrast, inulin clearance appeared to be independent of alterations in the plasma concentration of inulin and creatinine, and was not

affected by probenecid. The authors concluded that tubular secretion of creatinine normally occurs as an active metabolic process in these species, and that tubular reabsorption of creatinine must also be considered, as a passive process attendant upon a residual tubular permeability to creatinine relative to inulin. Simultaneous determination of the clearances of inulin and exogenous creatinine in foetal and in adult sheep by Alexander and Nixon (1964) confirmed the occurrence of tubular secretion of creatinine in this species and showed that this activity is established early in foetal life. The evidence justifies the assumption that, as in man (Smith, 1951), clearance values of inulin give the more reliable estimate in ruminants of glomerular filtration rate.

The accuracy of renal clearance measurements is dependent not only on the accuracy of the determinative, chemical, procedures for the reference substance, but also on the accuracy of recovery of the urine formed during each clearance period (see p. 55), and, in addition, involves the assumption that plasma samples truly reflect the concentration of the reference substance which obtains throughout each clearance period. In inulin clearance procedures the use of a satisfactory system for infusion will ensure the validity of this assumption. Numerous attempts have been made to avoid the use of intravenous infusions in clearance methods in man, involving either single intravenous, intramuscular, or subcutaneous injections, or combinations thereof; but efforts to replace the infusion method in this way involve assumptions one or more of which is not valid when the plasma concentration of the reference substance is changing rapidly (Smith, 1951). The inconvenience of the constant infusion procedure is clearly justifiable in seeking less precarious

estimates of glomerular filtration rate.

The clearance procedures used in the present study were developed jointly with R.S. Anderson.

Method

Each experiment occupied a period of two days. On the first day samples of urine and plasma were obtained during four to six clearance periods and urine samples were diluted to reduce the risk of inulin precipitation at the high urinary concentration. On the second day chemical analyses of the samples for inulin, and, in later experiments, for other constituents of plasma and urine, were carried out, and excretion rates and clearance values were calculated.

Cannulation of jugulars

Catheters 15-18 cm long (Portland Plastics Ltd., No. 4 nylon tubing) were introduced into each jugular vein to facilitate infusion of solutions and withdrawal of blood samples. An area of skin, approximately 3 cm square over the jugular vein on each side about 15 cm from the angle of the jaw was clipped and swabbed with Tincture of Cetrimide B.P. (I.C.I., 'Tincture of Cetavlon'). With the head of the animal held by an assistant, each site was anaesthetized by subcutaneous infiltration of 6 ml. of 2% (w/v) procaine hydrochloride with adrenaline (May and Baker, 'Planocaine'), and the underlying vein then punctured percutaneously using a 13 G x 1½ in hypodermic needle. Approximately 10 cm of a flexible stilette, 30 cm long (Portland Plastics Ltd., 1.5 mm diam. nylon rod) was then passed through the

needle into the vein towards the heart and the needle then withdrawn. The jugular catheter was then threaded on the stilette and pushed through the needle-hole in the skin into the vein, and the stilette withdrawn. The catheter was then fitted with an adapter of rubber tubing, was filled with heparinized saline, clamped with a spring clip, and fixed with adhesive plaster to a 2 in wide bandage tied round the neck of the animal. One catheter was connected through a 3-way tap to infusion tubing and the other was used for blood sampling.

Inulin infusion

Before infusion was begun, blood and urine samples were taken for inulin blank estimations. Since urine values were invariably zero, the pre-infusion urine sample was not taken in later experiments.

Solution for infusion was prepared by dissolving commercial inulin (British Drug Houses Ltd.) in sterile NaCl solution (0.9 g/100 ml.) to a concentration of 5g/100 ml. A priming dose (10 ml./50 kg body wt.) of this solution at 39°C was injected intravenously and the infusion immediately begun, at 2.5 ml./min, by gravity feed from a 2 l. aspirator bottle fitted with an air inlet tube of constant height. The infusion was led through a Murphy drip tube, previously calibrated, and the rate regulated by adjustment of the outflow tap of the aspirator bottle. The rate of flow of drops was checked at regular intervals, but further adjustment of the outflow tap was rarely necessary.

In later experiments this infusion apparatus was replaced by a pump (Distillers Co. Ltd., Micropump type 'S'), set to deliver at 2.5 ml./min. Another modification introduced in later

experiments was to halve the concentration of the inulin solution and double the volume of the priming dose whilst increasing the rate of infusion to approximately 10 ml/min. This modification was introduced to reduce the risk of precipitation of inulin from solution in the infusion reservoir. The more concentrated solution was used, with the lower rate of infusion, in experiments involving concurrent infusion of other solutions (in particular, potassium chloride) so that the total volume infused per minute was similar in all experiments.

In order to allow the plasma concentration and urinary excretion of inulin to stabilize, the first clearance period was not begun until at least 30 min after the start of infusion. Initial studies showed that plasma concentrations of inulin had stabilized within 15 min of the start of constant infusion, and it was assumed that inulin excretion had also stabilized 30 min after the start of infusion. Poulsen (1957) suggested that the main portion of an excretory load, following a single intravenous injection, appeared in the bladder after a delay of approximately twice the urinary appearance time of 2-3 min.

Plasma samples

Blood samples of 25 ml. were taken from the free jugular vein using a syringe which had been rinsed with heparinized saline. The samples were immediately transferred to 12.5 ml. centrifuge tubes containing two drops of Heparin (1000 units per ml), centrifuged without delay, and the plasma separated.

In five experiments the concentration of inulin in arterial plasma was determined for comparison with that found in venous plasma. Samples of arterial blood were taken into a heparinized syringe, by puncture of the coccygeal artery using a 17 G x 1 in needle, within 15 min of withdrawal of a venous sample. The

concentrations found in venous and arterial samples showed close agreement (Table 9) as expected when using a constant infusion procedure, and support the assumption that concentrations of inulin as measured in venous plasma were presented to the glomeruli.

In the first experiments, clearance periods were of 30 min duration, with venous blood samples taken at 0 and 30 min, the mean inulin concentration of these two samples being substituted in the clearance formula. Fluctuation of plasma inulin concentration, however, was never marked and in later work, when inulin clearance was measured simultaneously with other parameters, 15 min clearance periods were used in which a single plasma sample was obtained between the 7th and 8th minute. The adoption of 15 min clearance periods allowed investigation by repeated clearance measurements of the effects of various experimental procedures without excessive increase in the volume of analytical work.

Urine samples

Urine was collected continuously under epidural anaesthesia as described elsewhere (p. 58). The urine was collected in 250 ml. measuring cylinders and the volume noted at the end of each clearance period. The normal urine flow of the cow proved to be large enough for the catheter to achieve efficient drainage: in early experiments washing out of any uncollected urine at the end of each period with three separate volumes of 100 ml. of water or saline was not found to improve the overall accuracy of the technique. After each clearance period either the whole or an aliquot of the collected volume was transferred to a polyethylene bottle, stoppered and labelled.

TABLE 9

Inulin concentration of arterial and venous blood samples collected during intravenous infusion of inulin solution.

Inulin concentration	
Arterial blood * (mg/100 ml.)	Venous blood (mg/100 ml.)
12.6	12.7
10.4	10.2
9.7	9.3
14.3	14.3
13.1	14.9

* Arterial samples were taken from the coccygeal artery within 15 min of the venous samples

Measurement of renal plasma flow

In quantitative studies of renal function it is desirable to estimate the volume of plasma perfusing the kidneys since any alteration in glomerular filtration rate might be the result either of alteration in renal plasma flow or of alteration in the fraction of this volume which is separated as glomerular filtrate.

The Fick principle provides a well-known means of measuring the blood flow through an organ. If a substance is either taken up by or excreted from the blood perfusing an organ, the blood flow may be calculated from the equation:

$$\text{Blood flow (ml./min)} = \frac{\text{Quantity of substance taken up or excreted (per min)}}{\text{Arterio-venous concentration difference (per ml.)}}$$

This principle is readily applied to the kidney and any urinary constituent may be employed provided that it is not manufactured by the kidney. Thus:

$$\text{Renal plasma flow} = \frac{UV}{a - v} \text{ ml./min}$$

where V = rate of urine flow (ml./min)

U = urinary concentration of reference substance

and a and v = respectively, renal arterial and renal venous plasma concentrations of reference substance.

Wolf (1941) has pointed out that this simple formula is not strictly correct and should be written:

$$\text{Renal plasma flow} = \frac{V(U - v)}{a - v}$$

to allow for the fact that renal venous outflow is less than arterial inflow by the volume of the urine flow. The error involved

is small, however, if urine flow is low and if the extraction of the substance by the kidney is high.

The difficulty in applying this principle for the measurement of renal plasma flow is that of obtaining renal venous plasma. However, if extraction of the reference substance is complete, that is, if all of the substance in the blood is transferred to the urine in one passage through the kidney, then the renal venous concentration is zero and the Fick principle formula is reduced to the classical clearance formula :

$$\text{Renal plasma flow} = \frac{U\ V}{a-0}$$

If other tissues extract none of the substance from the blood, then venous blood from a superficial vein may be utilized for a clearance procedure and arterial puncture is unnecessary.

No substance which is excreted solely by filtration can be removed completely from the plasma in a single passage through the kidney. In the case of a substance which is excreted by tubular secretion, as well as by filtration, however, it is possible that the further removal achieved by tubular secretion from the postglomerular blood, as it traverses the peritubular capillaries, results in the removal of the last traces of such a substance from the renal blood before it escapes from the peritubular capillaries into the renal veins.

The high rate of excretion of phenolsulphonphthalein (phenol red) was shown by Marshall and Vickers (1923) to involve tubular secretion, in addition to glomerular filtration, and phenol red clearance has been used as an approximation of renal plasma flow. Because of grossly incomplete extraction, however, it is no longer so employed (Smith, 1951). Diodrast

(3,5-diiodo-4-pyridone-N-acetic acid), a compound originally introduced into urology for intravenous administration or retrograde ureteral injection to aid in X-ray visualization of the kidney and urinary tract, is another substance which is excreted both by filtration and by tubular secretion. It has been widely used in investigations of renal physiology since it is almost completely extracted from the renal blood and its clearance value gives a satisfactory approximation of renal plasma flow (Smith, 1951). The analytical difficulties of determining organic iodine prompted a search for other compounds having essentially complete clearances and amenable to easier analysis than diodrast (Smith, Finklestein, Aliminosa, Crawford and Graber, 1945). The study of various hippuric acid derivatives disclosed that they had the same clearance values as diodrast in both man and the dog, and from them p-amino hippuric acid (PAH) was selected for practical use (Chasis, Redish, Goldring, Ranges and Smith, 1945).

The extraction of PAH in one passage through the kidney of normal man is, on average, 90% complete (Smith, 1951), so that the PAH clearance represents only 90% of the true renal plasma flow. However, Smith (1956) points out that PAH clearance is probably more significant than a mere approximation of renal plasma flow. Some of the renal arterial blood is distributed to the perirenal fat and fibrous capsule, to renal pelves, calyces and the supportive tissue surrounding them in the sinus, and in vasa vasorum. It is unlikely that this blood can also reach tubular excretory tissue. Smith (1956) suggests that the deficit in the extraction of PAH below 100% is in part attributable to blood which passes from the renal arteries to renal veins

through these channels, and that extraction of PAH from the blood which perfuses active excretory tissue is probably very close to 100% complete. Therefore, PAH clearance may be taken to represent the volume of plasma presented for clearance to the functioning renal parenchyma, and its value may be designated as the effective renal plasma flow, or simply as the renal plasma flow, with the term total renal plasma flow reserved to designate the total flow, calculated from PAH clearance and the percentage extraction of PAH when the two terms have been measured simultaneously.

Satisfactory measurement of PAH clearance entails the maintenance of uniform plasma concentrations of the substance by constant intravenous infusion, established after the administration of a suitable priming dose. The errors involved in the use of single injection methods are particularly large with any substance which is cleared as rapidly as PAH (Smith, 1951).

In cattle the renal plasma flow has been estimated by clearance procedures using diodrast (Poulsen, 1957), and PAH (Vogel, 1959; Ketz, 1960). The values found for filtration fraction (inulin clearance/diodrast or PAH clearance) were slightly lower than those reported for man (Pitts, 1963). The clearance of PAH has been determined in sheep by Parry and Taylor (1955), who used a single injection technique, and by McDonald and Macfarlane (1958), Vogel (1959), Alexander and Nixon (1962) and Gans and Mercer (1962) using constant infusion procedures. Acceptance of PAH clearance as a measure of renal plasma flow in ruminants assumes a high percentage extraction of PAH in these species as in man. This assumption is supported by the finding that while the ovine kidney filters at a rate comparable with that of man,

the secretory capacity of the ovine kidney for PAH surpasses that of any other large animal so far described (McDonald and Macfarlane, 1958). It has been assumed, therefore, that such secretory ability will achieve an extraction which at least approaches 90%, and that the sheep and the cow are similar in this respect.

Method

Determinations of PAH clearance were carried out simultaneously with inulin clearance using the experimental technique described for inulin (p. 66), with all clearance periods of 15 min duration.

Preparation of PAH sodium

Before each experiment a quantity of the sodium salt of PAH was prepared from the free acid (Light and Co.), using the following procedure.

75 ml. of water were added to 30 g PAH (acid) in a 250 ml. beaker and stirred to make a creamy suspension.

5N NaOH was run in from a burette, stirring continuously, until the solution was just neutral to litmus, and the volume of NaOH added was noted. (The theoretical volume required to neutralise 30g PAH = 30.9 ml. 5N NaOH). The sodium salt formed is more soluble than the free acid and a clear yellow solution resulted.

Water was added to bring the total volume to approximately 145 ml. and the pH checked using narrow range (pH 7.0 - 8.3) indicator paper. More 5N NaOH was added, drop by drop, to bring the pH of the solution to 7.4, and the final volume was made up to 150 ml. with water.

The resulting solution contained 30g PAH as the sodium salt.

PAH infusion

Before infusion was begun, blood and urine samples were taken for PAH blank estimations. Zero values were consistently obtained for urine blanks.

PAH was infused at a concentration of 1g/100 ml. in sterile saline (0.9g/100 ml.) which also contained inulin at a concentration of 2.5 g/100 ml. This solution for infusion was prepared by dissolving 75 g inulin in 2,850 ml. saline to which was then added the solution of PAH sodium, prepared from 30 g of the acid, to make up the total volume to 3 l. A priming dose of 200 ml. of the mixed solution (approximately 20 ml./50 kg body weight) was injected intravenously and constant infusion then begun at 10 ml./min, using an infusion pump (Distillers Co. Ltd., micropump type 'S'). This rate of infusion was chosen since it was known that inulin concentrations of 20 - 25 mg/100 ml. would obtain in plasma, so, assuming inulin clearance to be 1/5 - 1/6 of PAH clearance, PAH concentrations in plasma of 1 - 2 mg/100 ml. would be achieved, as recommended for estimations of renal plasma flow (Smith, 1956).

Measurement of the volume of extracellular fluid

The extracellular fluid comprises the blood plasma, interstitial fluid and lymph, and also a specialised fraction designated transcellular fluid (Pitts, 1963) which includes cerebrospinal fluid, intraocoular, pleural, peritoneal and synovial fluids and the digestive secretions. Transcellular fluid differs from the other divisions of extracellular fluid in that each of its fractions is separated from blood plasma not only by the capillary endothelium but also by a layer of epithelial cells. This layer of epithelial cells modifies the composition of the transcellular fluid in varying degree. However, the volume of transcellular fluid, with the exception of the digestive secretions is a very small fraction of the body weight. In man, moreoever, digestive secretions in the fasting state represent only 1 - 3% of the body weight, although im herbivora they may amount to as much as 6% (Pitts, 1963) because the digestive processes are so prolonged that a comparable fasting state in a normal herbivore is rarely encountered.

The glomerular filtrate, in view of its composition and mode of formation, may be regarded as interstitial fluid, the fraction of the glomerular filtrate volume which is ultimated voided, and the final concentrations of the urinary constituents being determined by renal tubular activity. In the study of kidney function, a measure of the volume of the extracellular fluid is valuable in relating, quantitatively, the effects of observed variations in the rate of excretion of urinary constituents to observed alterations of the concentrations in plasma.

Measurement of the volume of extracellular fluid is possible by dilution techniques, in which a known amount of a substance which distributes throughout the extracellular volume

is injected into the animal. When the substance is uniformly distributed, its concentration in blood plasma is measured and the extracellular volume then calculated from the relationship:

$$V = \frac{M}{C}$$

where V = extracellular volume,

M = weight of substance injected,

and C = plasma concentration of substance.

A number of difficulties are attendant on this method of measuring extracellular fluid volume. The marker substance must be sufficiently diffusible to cross capillary walls readily, to enter the most distant reaches of cell interstices, and yet be absolutely excluded from cells. Allowance for inevitable losses of the substance in urine must be made, and, ideally, the substance should be excreted slowly in comparison with the rate at which it distributes throughout the extracellular volume. Also the substance must be non-toxic and must not itself alter the distribution of fluid in the body. No such ideal substance is known and measures of the extracellular volume vary according to the marker substance used. Estimates of the extracellular fluid volume found in man, for example, using inulin, raffinose, sucrose, mannitol, thiosulphate, radiosulphate, thiocyanate, radiochloride and radiosodium range from 16% of the body weight, for inulin, to over 30% of the body weight for radiosodium (Pitts, 1963). In estimating the extracellular volume, therefore, it is essential that the marker substance employed be specified.

Although isotopic tracers are finding increasing use in the measurement of body compartments (Haxhe, 1964), the volume of distribution of inulin (inulin space) provides a useful approximation of the extracellular fluid volume. Because of its mole-

cular size and the fact that it is not significantly metabolized in the body; inulin most probably does not enter cells, but since its rate of diffusion is low, its distribution throughout the extracellular volume, especially in poorly vascularized structures, can only be achieved after a protracted period of equilibration (Kruhøffer, 1946). In the case of non-nephrectomized animals it appears that continuous infusion must be maintained to allow this equilibration. Schachter, Freinkel and Schwartz (1950) demonstrated that in the dog and in man, after a single injection of inulin, equality of concentrations in plasma and interstitial fluid was evanescent. Before this point in time, plasma concentrations exceed concentrations in interstitial fluid; after it, plasma levels fall below concentrations in the interstitial compartment. These authors concluded that, since, in different subjects, identical concentrations of inulin obtained in plasma and interstitial fluid at such variable intervals after injection, single injection techniques for the determination of the inulin space were unreliable. Levy, Ankeney and Berne (1952), in studying the kinetics of inulin distribution and excretion in the dog, following a constant infusion, assumed that equilibration between plasma and interstitial compartments had been approximated after an infusion of two hours duration, and noted that plasma concentrations of inulin then declined more rapidly than its concentration in the interstitial fluid. The disparity between the rate of excretion of inulin and its rate of diffusion into interstitial fluid seems, therefore, to exclude the use of single injection techniques in determinations of inulin space.

Most determinations of the inulin space in the dog and in man have, therefore, employed constant infusion techniques, to allow equilibration of inulin concentration between plasma and inter-

stitial fluid. The recovery method involves the collection of urine for a period of 5 - 12 hr on stopping the infusion. The inulin content of the collected urine is determined, and the inulin space calculated by dividing the total quantity of inulin excreted after stopping the infusion by the plasma concentration which obtained at that time (Gaudino, Schwartz and Levitt, 1948). In the dog infusion for 2 hr is believed to be adequate for equilibration of inulin throughout its volume of distribution, since increases in inulin space were not found with further prolongation of the infusion (Gaudino and Levitt, 1949), although infusions for 3 hr are reported when no priming dose was given (Raisz, Young and Stanson, 1953).

After stopping the infusion the rate of excretion is asymptotic to complete elimination from the body, necessitating a prolonged period of collection, to ensure complete recovery of the inulin. Levy et al. (1952) point out that the graph relating the logarithm of excretion rate to time is virtually linear during the last 2 hr of a 5 hr collection period, so that the quantity of inulin which could theoretically be recovered until infinity may be calculated from the slope of this line. By adding this amount to that recovered during the collection period, the volume of distribution of inulin may then be calculated by dividing this sum by the plasma concentration at equilibrium.

In other papers (Berne and Levy 1951; Raisz et al., 1953) the measurement of inulin space is described using a procedure in which the amount of inulin distributed in the body at equilibrium is calculated as the difference between the amount infused and the amount excreted up to that time. This procedure (the difference method), although complicated by the need to collect urine during infusion and to know the total amount infused up to

equilibrium, has the advantage over the recovery method that prolonged collection of urine after stopping the infusion is not necessary, and also permits successive measurements of inulin space in the same animal as an experiment proceeds.

There are few reports of the measurement of inulin space in cattle. Ketz (1960), from measurements of the decline in plasma concentration after a single injection, found a mean value for inulin space of 10.3% of the body weight. Using a single injection technique on one cow, Anderson, R.R. and Mixner (1960) found that inulin space was 15.3% of body weight, and these authors claimed that inulin reached equilibrium in the body 45 min after injection. Anderson, R.S. (1964), using an adaptation in cattle of the single injection technique described in man by Robson, Ferguson, Olbrich and Stewart (1949), obtained values for inulin space of 16± 3% of body weight at 27 min after injection, but noted that in all cases the apparent inulin space rose between 27 and 97 min after injection. This author concluded that there was little justification for use of a single injection technique as described by Anderson, R.R. and Mixner (1960) for the measurement of extracellular volume in cattle. In the present work, therefore, a constant infusion technique was used, and inulin space was calculated by the difference method using a similar procedure to that described in the dog by Raisz et al. (1953).

Method

Successive estimates of the inulin space were made during experiments in which the clearances of inulin and PAH were also measured. The animals were prepared using the procedures described for inulin clearance determinations (p. 66)

Inulin infusion

A priming dose of 5 g inulin (approximately 0.5 g/50 kg body weight) was injected intravenously in 200 ml. saline (0.9 g/100 ml.), and urine collection and intravenous infusion was immediately begun. Constant infusion from a measured volume in a reservoir was maintained at a rate of approximately 10 ml/min by an infusion pump (Distillers Co. Ltd., micropump type 'S'). The solution infused was of the same concentration as that used for the priming dose and the infusion tubing was filled before filling the reservoir. The volume remaining in the reservoir was measured at each interval after the onset of infusion when inulin space was determined, to allow calculation of the exact quantity of inulin infused.

Urine collection

Urine was collected continuously by an indwelling urethral catheter as desdribed on p. 58 . Values of inulin space were calculated at intervals of 120, 150, 180, 210, 240 and 270 min after the onset of infusion and the volume of urine collected during each interval was measured and sampled for analysis to allow calculation of the cumulative excretion of inulin.

Plasma samples

Blood samples, for estimation of plasma concentrations of inulin were taken from the jugular vein 7 min before the end of each period of urine collection. Ideally these samples should have been taken at the end of each period of urine collection for measurement of the concentration of inulin which actually obtained at this time. Sampling at the time stated, however, was part of the schedule for concurrent clearance determinations and it was assumed that any differences between the inulin concentrations of these samples and those obtaining in plasma 7 min later was negligible. These samples were taken at intervals from 120 - 270 min after the onset of infusion and during this period variation in plasma concentrations was only slight, as illustrated in Fig. 10.

Inulin estimations

Inulin concentrations in samples of plasma and urine and of the solution infused were measured as described in an earlier section (p. 48). Plasma concentrations were expressed as mg/100 ml. plasma water by multiplying the concentrations found per 100 ml. plasma by the factor:

$$\frac{100}{100 - \text{g of plasma protein/100 ml.}}$$ (Goldring and Chassis, 1944)

Plasma protein concentrations were determined by the biuret method using the biuret reagent described by Weichselbaum (1946).

Values of inulin space were calculated from the equation:

$$\text{Inulin space (l.)} = \frac{C - UV}{10\ P}$$

where, at time t min after the onset of infusion,

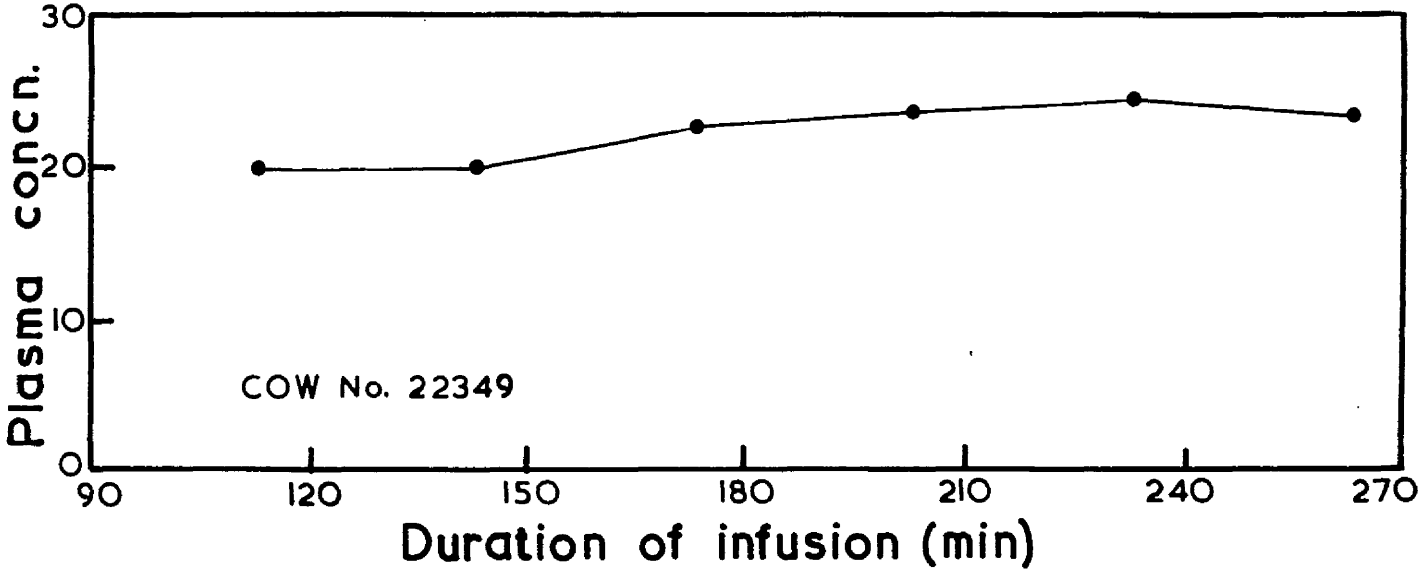

Fig. 10. Variation in plasma concentration of inulin (mg/100 ml.) during constant intravenous infusion at 10 ml./min of a solution containing 2.5 g inulin/100 ml.

C = inulin concentration of solution infused (mg/ml.)

V = total volume of solution infused up to time t, including the priming dose (ml.),

U = urinary inulin concentration (mg/ml.),

V = total volume of urine produced up to time t (ml.),

P = plasma inulin concentration at time t = 7 min (mg/100 ml. plasma water).

RESULTS

AND

DISCUSSION

Variation of rate of urine flow and sodium and potassium excretion in serial collection periods

The effect of the urine collection procedure (p. 58) on urine flow and electrolyte excretion was investigated before attempting clearance procedures. Urine was collected continuously for 3 - 4 hr in five experiments on one cow under the conditions of management described earlier (p. 53). Serial samples were taken at 10 min intervals and the concentrations of sodium and potassium determined by flame photometer (p. 31).

Results

When urine collection was begun within 30 min of administration of the epidural anaesthetic and urethral catheterization, high rates of flow were recorded which declined during subsequent collection periods. The rates of excretion of sodium and potassium ran approximately parallel with the urine flow (Fig. 11). When urine collection was delayed until 40 min after administration of the epidural anaesthetic, this initial diuresis was not seen (Fig. 12).

When the effects of the epidural anaesthetic were wearing off, the animal showed resentment to the presence of the catheter (frequent twitching of the tail and shifting of the hind feet), accompanied by an increase in the rate of urine flow. These signs were abolished on reinforcing the anaesthetic and urine flow returned to lower levels (Figs. 11, 12).

Peaceful conditions were not maintained in one experiment

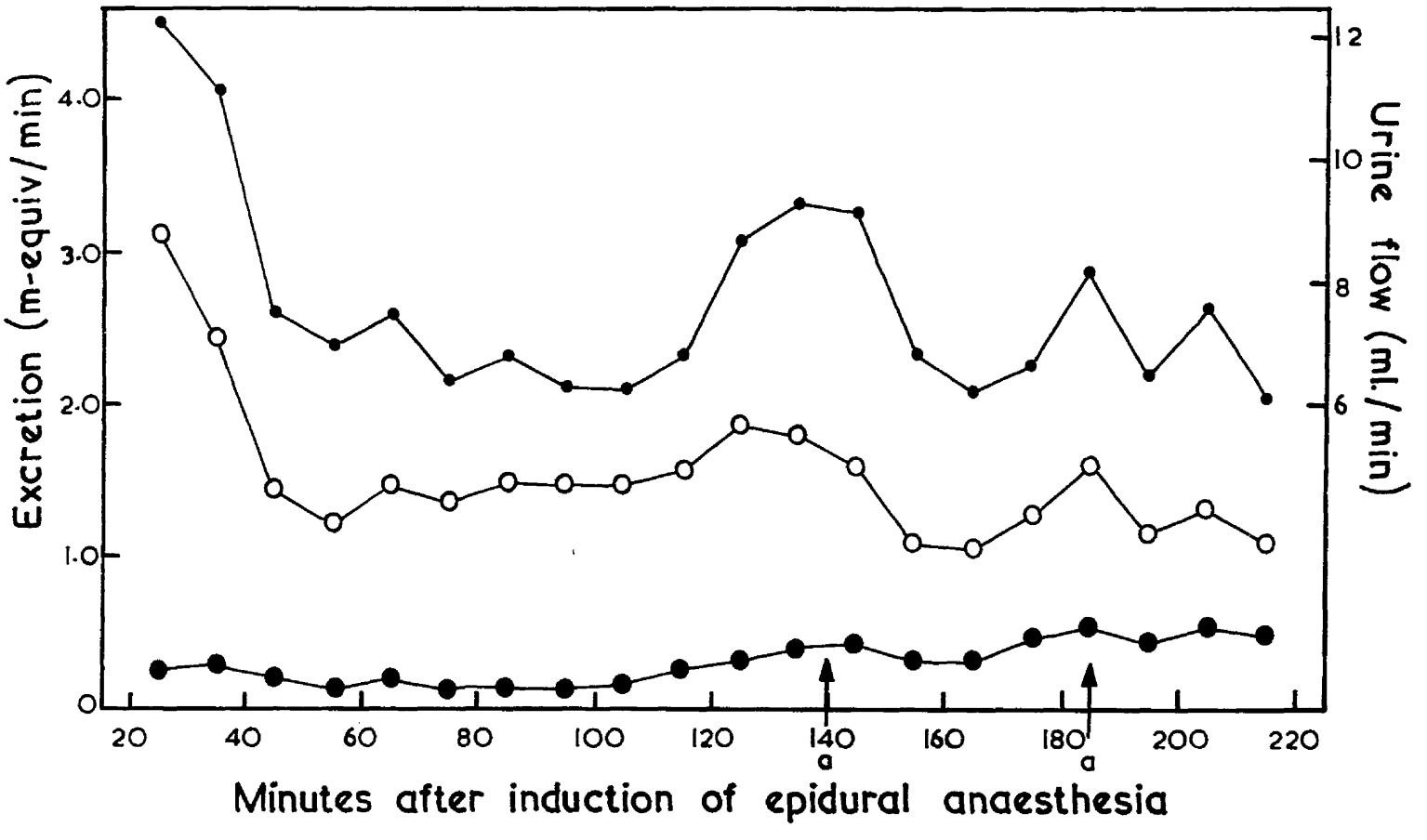

Fig. 11. Urine flow (•——•), sodium (●——●) and potassium (O——O) excretion in serial 10 min collection periods. Epidural anaesthesia was reinforced at 'a'.

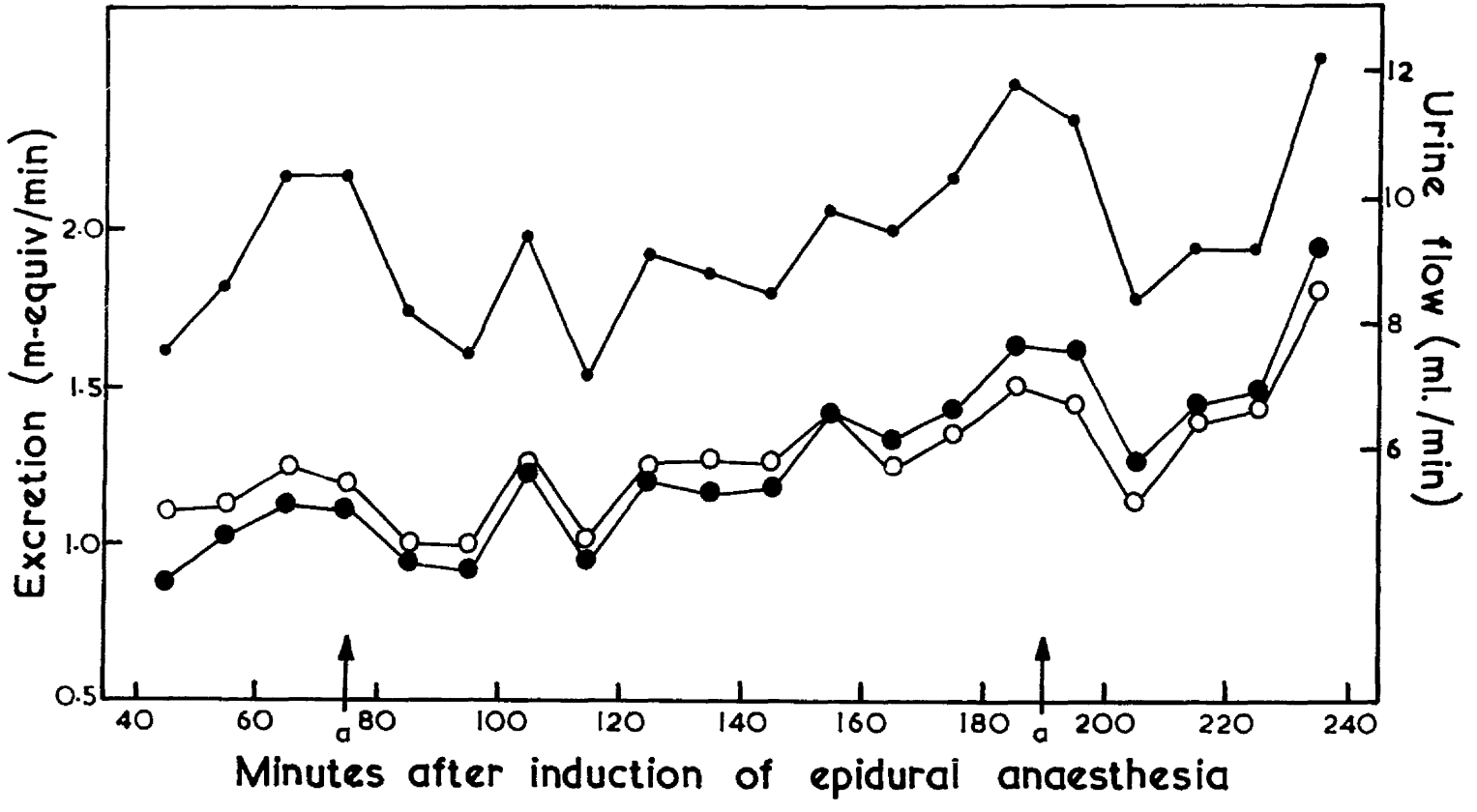

Fig. 12. Urine flow (●——●), sodium (●——●) and potassium (○——○) excretion in serial 10 min collection periods. Epidural anaesthesia was reinforced at 'a'.

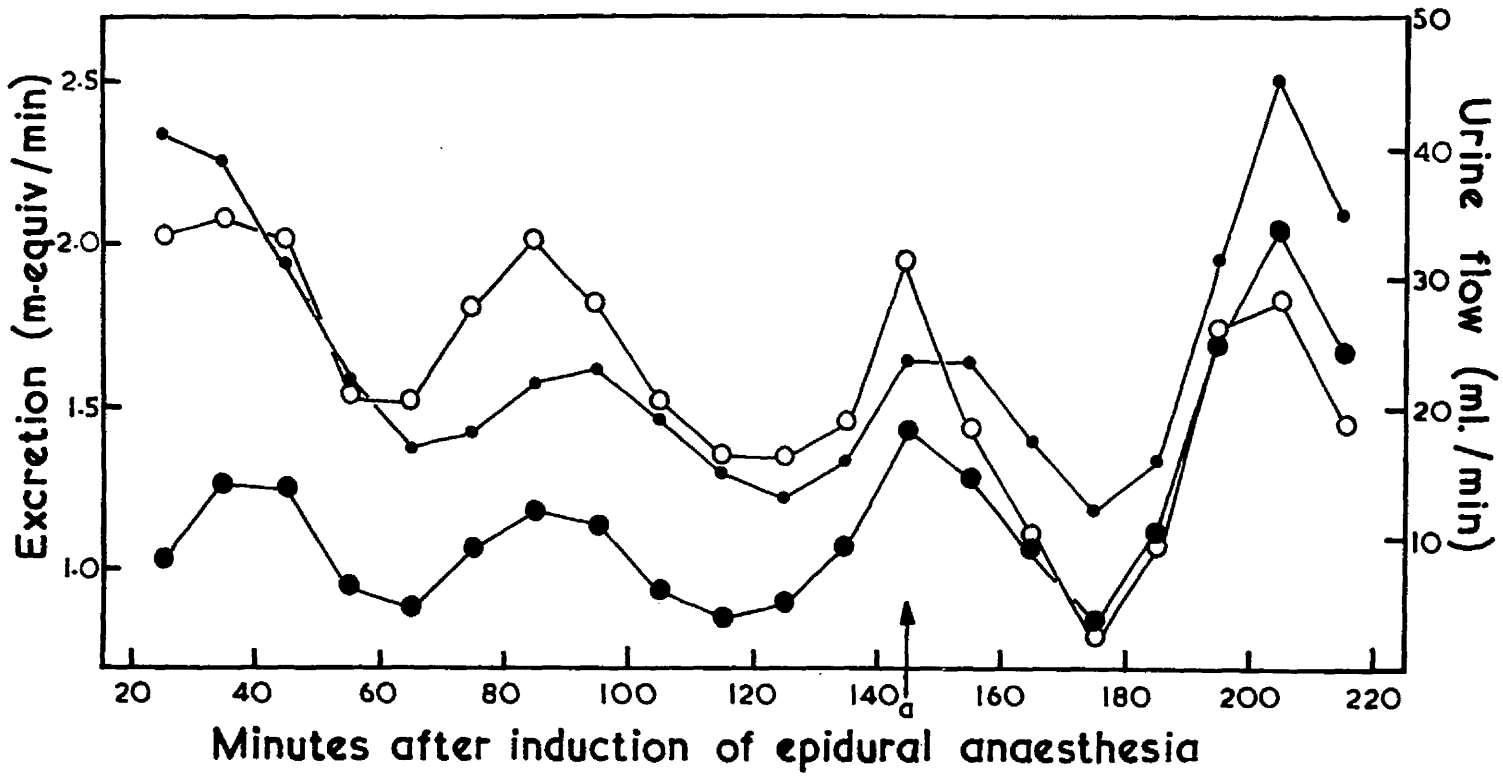

Fig. 13. Urine flow (•—•), sodium (●—●) and potassium (O—O) excretion in serial 10 min collection periods. Epidural anaesthesia was reinforced at 'a'. The experimental animal was intermittently disturbed by extraneous noise and personnel (see text).

which was conducted in the large animal demonstration room. There were frequent intrusions from hospital staff who were preparing to receive a batch of calves from the market. This experiment is illustrated in Fig. 13. About forty calves were admitted to the room during the last 40 min of this experiment and the rate of urine flow rose markedly as noise and general disturbance increased.

Discussion

These findings were obtained at the outset of the investigations described in this thesis. Whilst recognising that different animals may react in different degree, the results confirmed the effect of stress on the rate of urine flow and electrolyte excretion in cattle described by Knudsen (1960) and Anderson (1961), and emphasised the need for careful control of the experimental conditions in studies involving urine collection over short periods. For subsequent clearance experiments, therefore, the present findings suggested that at least 30 min should be allowed to elapse after catheterization before commencing an experiment; that when necessary, the initial epidural anaesthesia should be promptly reinforced; and that possible distraction or disturbance of the experimental animal from extraneous noise or the presence of unfamiliar personnel must be prevented.

Determinations of glomerular filtration rate

Using the methods described on p. 66 , clearance experiments were carried out on seven non-pregnant, non-lactating Ayrshire cows whose weights ranged from 410 to 547 kg. Values found in a typical experiment are shown in Table 10, in which four successive determinations of inulin clearance showed agreement within ± 10% of the mean value.

Experiments were repeated at least once on each animal and, in most cases, inulin clearance values were determined at intervals over a period of several months. Table 11 shows the collected results of six experiments (26 observations) on one cow which extended over a period of 18 months. Most values were within ± 10% of the overall mean and there was no constant trend for the values to rise or fall over this period. However, significant differences were found between mean clearance values determined on different days. For example, although the mean clearance value found on 1/8/60 (1031 ± 59 ml./min) was almost identical with that found on 10/11/60 (1028 ± 141 ml./min), it was significantly higher ($P<0.01$) than that found on 7/9/60 (893 ± 39 ml./min). Similar findings were recorded in other cows, but in no case was a general trend detected. Tables 12 - 17 show the results of inulin clearance measurements on the other six animals.

Table 18 shows the mean values found for the seven cows in a total of 30 experiments involving 103 measurements of the renal clearance of inulin. The mean rate of inulin clearance varied between animals from 838 ± 96 ml./min to 1234 ± 133 ml./min, giving a value for the overall mean of 1005 ± 147 ml./min. When the mean value for each animal was calculated on a unit weight basis, the overall mean for the seven animals was 1,100 ± 236

T A B L E 10

Cow No. 12916 (450 kg): Inulin clearance determinations

Period	Time	Duration (min)	Urine flow (ml./min)	Urine concn. (mg/100 ml.)	Plasma concn.* (mg/100 ml.)	Clearance (ml./min)	Difference from mean (%)
1	2.20 - 2.53	33	20.8	542.0	10.0 9.4 (9.8) 10.0	1150	5
2	3.09 - 3.31	31	17.7	636.6	9.8 10.5 (10.0) 9.8	1128	3
3	3.40 - 4.10	30	18.4	636.2	10.5 10.0 (10.6 11.2	1110	1
4	4.20 - 4.50	30	18.6	580.8	11.2 10.8 (10.9) 10.8	987	10

Mean 1094

*In this experiment, blood samples were taken at the beginning, middle and end of each period. The figures in brackets are mean values of the three concentrations measured in each period.

TABLE 11

Inulin clearance determinations

Cow No. 14711 (457 kg)

Date	G.F.R. (ml./min)	Mean daily G.F.R. ml./min)
8.2.60	868	
	1018	943
23.2.60	1000	
	920	960
4.8.60	996	
	946	
	1068	
	1058	
	1087	1031 ± 59
7. 9.60	918	
	843	
	941	
	929	
	894	
	864	898 ± 39
10.11.60	1111	
	1256	
	846	
	956	
	998	
	1000	1028 ± 141
20.7.61	892	
	893	
	1026	
	907	
	920	927 ± 56
Mean and S.D.	968 ± 95	965 ± 54

TABLE 12

Inulin clearance determinations

Cow No. 12916 (449 kg)

Date	G.F.R. (ml./min)	Mean daily G.F.R. (ml./min)
8. 6.59	1207	
	1317	
	1209	
	1230	1240 ± 52
7. 7.59	1151	
	1128	
	1110	
	986	1094 ± 74
9. 7.59	1359	
	1207	
	1219	
	961	1187 ± 165
11.8.59	1205	
	1117	1161
25. 7.60	1396	
	1224	
	1502	
	1437	
	1292	1370 ± 112
3. 8.60	1265	
	1199	
	1274	
	1142	
	1162	
	1331	1229 ± 73
17.11.60	1537	
	1232	
	1285	
	1098	1288 ± 193
Mean and S.D.	1234 ± 133	1223 ± 89

T A B L E 13

Inulin clearance determinations

Cow No. 14948 (477 kg)

Date	G.F.R. (ml./min)	Mean daily G.F.R. (ml./min)
8. 8.60	933	
	973	
	1160	
	884	
	1055	
	936	990 ± 102
20.10.60	1036	
	894	
	959	
	982	
	1023	
	989	981 ± 51
24.11.60	897	
	972	
	977	
	800	912 ± 83
Mean and S.D.	967 ± 83	961 ± 79

T A B L E 14

Inulin clearance determinations

Cow No. 1122/237 (484 kg)

Date	G.F.R. (ml./min)	Mean daily G.F.R. (ml./min)
4. 3.59	908	908
28. 4.59	784	
	853	819
12. 5.59	1096	
	1095	
	933	1041
25. 5.59	777	
	702	739
27. 5.59	869	
	850	860
Mean and S.D.	887 ± 133	873 ± 112

T A B L E 15

Inulin clearance determinations

Cow No. 13107 (423 kg)

Date	G.F.R. (ml./min)	Mean daily G.F.R. (ml./min)
5.5.59	921	
	964	
	951	945
18.5.59	890	
	817	854
20.5.59	780	
	800	790
1.6.59	694	
	716	705
3.6.59	925	
	762	844
Mean and S.D.	838 ± 96	828 ± 88

TABLE 16

Inulin clearance determinations

Cow No. 1122/315 (401 kg)

Date	G.F.R. (ml./min)	Mean daily G.F.R. (ml./min)
13. 7.59	1438	
	1321	
	1295	
	1119	1293 ± 132
15. 7.59	1103	
	1090	
	1103	
	989	1071 ± 55
Mean and S.D.	1182 ± 151	1182 ± 94

TABLE 17

Inulin clearance determinations

Cow No. 12401 (418 kg)

Date	G.F.R. (ml./min)	Mean daily G.F.R. (ml./min)
21. 4.59	894 1048	1021
8. 4.59	940	940
Mean and S.D.	961 ± 79	981 ± 57

TABLE 18

Collected values of inulin clearance determinations

Cow No.	Body Weight* kg	No. of Experiments	No. of Observations	Mean Clearance (ml./min)
12916	449	7	29	1234 ± 133
14711	547	6	26	968 ± 95
14948	477	3	16	967 ± 83
13107	423	5	11	838 ± 96
1122/237	484	5	10	887 ± 133
1122/315	401	2	8	1182 ± 151
12401	418	2	3	961 ± 79
	457 ± 50	30	103	1005 ± 147
	Mean clearance ml./min/500 kg 1100 ± 236			

* Average weight over the period of experimentation

ml./min/500 kg body weight. The increase in the standard deviation when the overall mean was expressed in this way indicated that no relationship was apparent in these animals between inulin clearance and body weight.

No untoward reactions to the experimental techniques were noted except on one occasion, when, after infusion with 2.5% inulin solution at 5 ml./min for 60 min, an animal showed tachypnoea, dyspnoea and coughing. A venous blood sample taken at this time was noticeably darker in colour. The infusion was stopped and the animal recovered within two hours of the onset of respiratory distress. On several occasions transient coughing occurred in other animals during, or immediately after, injection of the priming dose of inulin solution into the jugular vein, but no subsequent reactions were seen.

Discussion

The overall mean and the range of inulin clearance values found in the present study and by earlier workers are shown in Table 19. For comparison, all figures are expressed as ml./min/500 kg body weight. The results of Sellers et al. (1958) and Ketz (1960) were expressed in the original text as ml./min/sq m body surface and have been converted to ml./min/500 kg by using the formula:

$$A = 0.10 \times W^{\frac{2}{3}} \quad \text{(Dukes, 1955)}$$

where A = body surface area (sq m)

W = body wt (kg).

These authors did not specify how they estimated the body surface area of the animals used. Since the value of the mean

T A B L E 19

Inulin clearance values found in adult cows by various workers

Author	No. of cows	No. of experiments	No. of observations	Inulin clearance (ml./min/500 kg)
Poulsen (1957)	10	16	72	919 ± 161(547-1262)
Sellers et al. (1958)	4			951* (894-1360)
Ketz (1960)	19		76	454 ± 54*
Pickering (1966)	7	30	103	1100 ± 236(878-1474)

* These values were calculated from those expressed in the original papers as ml./min/sq m of body surface, by substituting in the formula $A = 0.10\ W^{\frac{2}{3}}$ (see text).

inulin clearance per 500 kg body weight calculated from the figure given by Ketz (1960) is less than half of the others quoted, it is probable that he used some formula different from that given above. This discrepancy is discussed at greater length in the following section.

The clearance technique used in the present study was developed jointly with R. S. Anderson whose earlier investigation of the repeatability of the chemical methods has been summarized in an earlier section (p. 48). In the absence of significant errors arising from this source, the remaining variability in the results obtained may be attributed to two main factors: (a) failure to eliminate completely errors in urine collection, and (b) actual variations in glomerular filtration rate.

Errors in urine collection must be considered because of the possibility of small volumes of urine remaining uncollected in the bladder at the end of the clearance periods. The repeatability achieved in clearance determinations in any one experiment, however, was as consistent as that commonly obtained in experiments using similar techniques in dogs and man, in which uncertainties of ± 10% (Winton, 1956) have been ascribed to the difficulty of obtaining complete bladder drainage (Smith, 1951).

On the other hand, there is some evidence in the present work to indicate that observed variations in mean clearance values measured on different days may be attributed to actual variations in glomerular filtration rate. Variability in actual rates of glomerular filtration are well recognised, particularly under experimental conditions (Deyrup, 1947; Davies and Schock, 1950; Wolf, 1950; Smith, 1951; Winton, 1956), and statistically significant variations in day to day clearance values, as

shown in Table 11, were also evident in the experimental records of Poulsen (1956).

McDonald and Macfarlane (1958) described, in sheep, long term responses of glomerular filtration rate to climatic changes. In summer, glomerular filtration rate reached twice the value found in winter; it rose during 2-9 weeks exposure to heat, but there was no change of glomerular filtration rate during 4 hr acute heating. In the present study, however, day to day variations in inulin clearance values were not associated with seasonal or climatic changes, nor was there any trend for values to diminish towards the end of a series of determinations on each day, such as was described by Wolf (1950) and by Smith (1951).

The present study was undertaken to establish a reliable technique for measuring the glomerular filtration rate in cows to permit quantitative study of the excretion and reabsorption of electrolytes by the bovine kidney. The results indicate that the method was comparable in reliability to clearance techniques which have been widely used in man and the dog under controlled laboratory conditions. Serial clearance measurements on individual animals suggest that true variations in filtration rate are measured by this technique if differences greater than $\pm$ 10% of control values are recorded.

Determinations of renal plasma flow and filtration fraction

Using the methods described on p. 74 , clearance experiments were carried out on two non-pregnant, non-lactating Ayrshire cows weighing 502 and 409 kg. The values found for PAH and inulin clearances are given in Tables 20 and 21. These figures are control values measured before the administration of acetazolamide in experiments which are described fully in a later section. Filtration fraction was calculated for each clearance period as the ratio of inulin clearance (glomerular filtration rate) to PAH clearance (renal plasma flow).

The range and overall mean values found for PAH and inulin clearances, expressed per 500 kg body weight, and for filtration fraction, are compared in Table 22 with values given by earlier workers. Both Vogel (1959) and Ketz (1960) expressed their findings as ml./min/sq m body surface and these values have been converted to ml./min/500 kg body weight using the formula $A = 0.10 \times W^{\frac{2}{3}}$ as described in the preceding section. Vogel (1959) did not state figures for inulin and PAH clearances: the values quoted in Table 22 were calculated from figures taken from a graph in the original text. Poulsen's (1957) figures for diodrast clearance are of interest since diodrast and PAH clearances are both acceptable as measures of the effective renal plasma flow (Smith, 1951).

Discussion

The values found for filtration fraction in the present study agree closely with those found by Poulsen (1957), Vogel (1959) and Ketz (1960): apparently one sixth to one seventh of the volume

TABLE 20

Inulin and PAH clearances

Cow No. 22349

Body wt. (kg)	C_{PAH} (ml./min)	C_{IN} (ml./min)	C_{IN}/C_{PAH}
511	6,725	1,087	0.16
	7,059	1,138	0.16
	7,142	1,111	0.16
	6,949	1,153	0.17
500	7,580	1,268	0.17
	7,987	1,280	0.16
485	6,940	1,020	0.15
	7,581	1,093	0.14
Mean ± S.D	7,245 ± 426	1,144 ± 90	0.16 ± 0.01

TABLE 21

Inulin and PAH clearances

Cow No. 21271

Body wt. (kg)	C_{PAH} (ml./min)	C_{IN} (ml./min)	C_{IN}/C_{PAH}
427	6,913	1,155	0.17
	6,387	1,006	0.16
422	8,645	1,167	0.14
	8,548	1,158	0.14
405	7,136	1,111	0.16
	7,498	1,084	0.15
Mean ± S.D	7,521 ± 908	1,114 ± 62	0.15 ± 0.01

TABLE 22

Values for glomerular filtration rate, renal plasma flow and filtration fraction in cows found by various workers.

Author	No. of cows	No. of expts.	No. of observations	G.F.R. (ml./min/ 500 kg)	R.P.F. (ml./min/ 500 kg)	F.F. (G.F.R./R.P.F.)
Poulsen (1957)	6	8	25	-	4556±1223 (2728-6592)	-
Poulsen (1957)	2	2	6	-	-	0.16 (0.14-0.17)
Vogel (1959)	11	11	-	378*	2,520*	0.15
Ketz (1960)	19	-	76	454*-54*	2979±183*	0.15
Pickering (1966)	2	7	14	1222±127 (1060-1383)	7984±1210 (6582-10242)	0.16 (0.14-0.17)

* Using the formula $A = 0.10\ W^{\frac{2}{3}}$ (Dukes, 1955), these values were calculated from those expressed in the original papers as ml./min/sq m of body surface

Poulsen's figures for renal plasma flow are values of diodrast clearance. This author did not give the values of inulin and diodrast clearances from which the cited values of filtration fraction were calculated.

of plasma perfusing renal corpuscles on the bovine kidney normally is separated as glomerular filtrate. However, considerable differences are apparent in the absolute values found for renal plasma flow and glomerular filtration rate. Poulsen (1957) does not give the figures for diodrast and inulin clearances from which he calculated the filtration fraction, but the mean value found for maximal diodrast clearance in his other experiments was only 60% approximately, of the mean PAH clearance found in the present work. If clearance values of diodrast and PAH are comparable in the cow as in man, Poulsen's findings may reflect true differences in renal plasma flow, expressed on a unit weight basis, of his animals (Red Danish) compared with those of the Ayrshire breed used in the present study. Similarly, the mean value of inulin clearance measured in Red Danish cows by Poulsen was also significantly lower ($P < 0,01$) than the mean value of inulin clearance found using Ayrshire cows in the present work (Table 19). In consequence, no difference was reflected in the respective values found for filtration fraction (Table 22).

The values for inulin and PAH clearance calculated from the findings of Ketz (1960) and Vogel (1959) are only 30 - 40% of the present mean findings. Ketz used lowland black cattle, while the breed used by Vogel was not specified, but it is unlikely that discrepancies of this order may be explained on the basis of breed differences. Neither author describes how values of inulin and PAH clearance, expressed per unit of body surface, were derived, and it is possible that the differences under discussion have arisen in part through the use of different formulae relating body weight to surface area. Sellers et al. (1958) expressed the inulin clearance of cows per unit of body surface

and their mean values when recalculated on a body weight basis are more than twice those stated by Ketz (1960) and plotted by Vogel (1959). Similarly the mean values of inulin and PAH clearances in sheep plotted by Vogel (1959) are only 50%, or less, of values found in this species by McDonald and Macfarlane (1958).

In view of these findings the figures for PAH and inulin clearance given by Vogel (1959) and Ketz (1960) cannot be accepted without further details of their derivation; but there is close agreement in the values of filtration fraction found in cattle in the present study with those found by Poulsen (1957), and with those found by the German authors. This is consistent with the suggestion that the differences in clearance values seen when comparing the present findings with those of the German workers are in part attributable to the method used to express the values per unit of body surface. Unfortunately, repeated attempts to contact these authors have failed, so it has not been possible to establish what method was used.

Determinations of inulin space

Using the methods described on p. 81 , forty eight determinations of the volume of distribution of inulin were made in eight experiments on two non-pregnant, non-lactating Ayrshire cows. Between experiments the animals were kept at grass with access to drinking water ad libitum. The experiments were conducted to a strict time schedule so that the successive estimations were made in each experiment at the same time of day.

Detailed results of one experiment are given in Table 23, to illustrate the method of calculation. The values found in all experiments are given in Tables 24 and 25 and the successive values obtained in each experiment are plotted separately against duration of infusion in Fig. 14. Overall mean values of inulin space for each animal were 13.5 ± 3.5% and 15.7 ± 4.0% respectively (l./100 kg body weight).

Discussion

The mean values found in the present study are similar to those reported by other workers using single injection techniques. Retz (1960) in 16 determinations on 10 animals, found a mean value of 10.3 ± 1.3% of the body weight, and Anderson, R.R. and Mixner (1960) in one cow reported inulin space to be 15.3% of the body weight when calculated 45 min after the injection. Anderson, R.S. (1964), using a single injection technique on five animals, reported a mean value of 16 ± 3% at 27 min after injection but in all experiments noted a marked increase in the

TABLE 23

Successive estimates of inulin space

Cow No. 22349 (485 kg)

Duration of infusion (min)	Total vol.* infused: v (ml.)	Total amt. inulin infused: Cv (mg)	Total amt.+ inulin excreted: UV (mg)	Amt. inulin retained: Cv - UV (mg)	Plasma inulin concn.: $\frac{P}{100-pr}$ (mg/ml. plasma H_2O)	Inulin space: $(Cv - UV)\frac{(100-pr)}{1000P}$ (l.)
120	1355	34,688	21,627	13,061	23.8	55
150	1643	42,061	27,923	14,138	22.6	63
180	1926	49,306	34,561	14,745	23.3	63
210	2204	56,422	40,588	15,834	26.9	59
240	2488	63,693	46,908	16,785	28.8	58
270	2773	70,989	54,035	16,954	26.2	65

C = concentration of inulin (mg/ml.) in solution used for infusion and for priming dose
P = concentration of inulin found in plasma samples (mg/100 ml. plasma)
pr = plasma total protein (g/100 ml. plasma)
* Figures include volume of priming dose
+ Figures are of cumulative excretion calculated from concentrations $U_1 \ldots\ldots U_6$ (mg/ml.) found in samples of of urine volumes $V_1 \ldots\ldots V_6$ (ml.) collected successively from onset of infusion.

TABLE 24

Inulin space determinations

Cow No. 22349

Date and wt. (kg)	Inulin space (l.)	Mean daily inulin space (l.)	Mean as l./100 kg (%)
8.7.63	55		
	63		
(485)	63		
	59	60.5 ± 3.8	12.5
	58		
	65		
15.7.63	85		
	86		
(500)	85		
	86	90.3 ± 7.5	18.1
	99		
	101		
25.7.63	48		
	52		
(513)	51		
	46		
	49	49.0 ± 7.5	9.6
	48		
1.8.63	65		
	71		
(511)	68		
	70		
	73	71.3 ± 5.5	14.0
	81		
Overall mean ± S.D.		67.8 ± 16.3	13.5 ± 3.5

T A B L E 25

Inulin space determinations

Cow No. 21271

Date and wt. (kg)	Inulin space (l.)	Mean daily inulin space (l.)	Mean as l./100 kg (%)
1.7.63 (382)	74		
	78		
	70		
	72	73.5 ± 3.9	19.2
	69		
	78		
11.7.63 (405)	46		
	45		
	44		
	41	47.0 ± 5.1	11.6
	51		
	55		
23.7.63 (422)	46		
	59		
	62		
	58	58.2 ± 6.2	13.8
	63		
	61		
30.7.63 (427)	66		
	79		
	78		
	70		
	82	77.5 ± 8.6	18.2
	90		
Overall mean ± S.D.		64.1 ± 13.7	15.7 ± 4.0

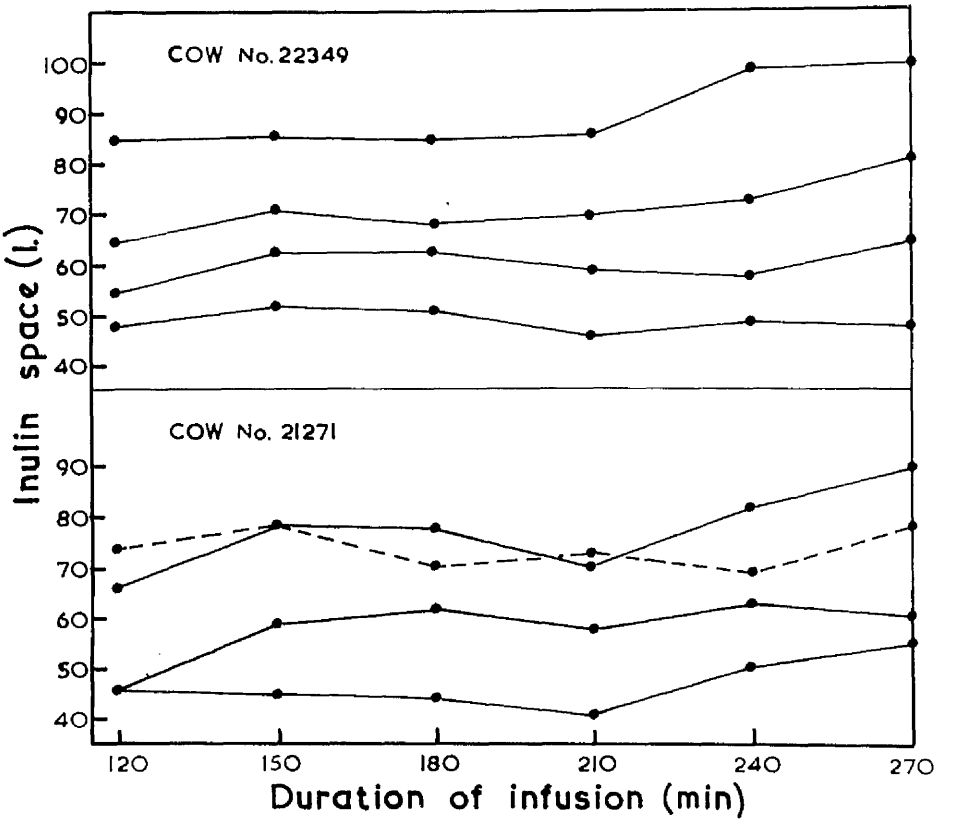

Fig. 14. Successive values of inulin space plotted against duration of infusion for individual experiments.

apparent inulin space when calculated 97 min after injection.

Chien and Gregersen (1960) state that infusion is a necessary procedure for determinations of inulin space in the dog and in man, and give normal values for inulin space in these species of 21% and 16% of body weight respectively. They state that when repeated determinations of inulin space are made in the same subject on different days over a period of 1-2 months, the average deviation from the mean value is less than 5% of the mean. This figure included not only the error of the method but also the physiological variation of the volume with time. In the present study, however, the average deviation of values obtained on different days from the overall mean value for each animal were 19% and 18% respectively, although the mean values of average deviations within days were only 5.3% and 7.0% respectively.

The large variation between values found on different days is difficult to explain. The body weights of the animals showed little change over the month during which the experiments were carried out (Tables 24 and 25). Efforts to maintain identical conditions during the experiments were outwardly successful, and the determinations in each experiment were made at precisely the same time of day. Simultaneous estimations of inulin clearance showed an average deviation from the overall mean value for each animal of only 5.1% and 5.2% respectively, so that errors in urine collection or in the analysis of plasma and urine samples are presumably not involved. On each day, sufficient inulin solution for infusion was made up in bulk. No precipitation was was observed in the solution during the experiments and in each experiment the concentration of inulin in the solution was determined, in duplicate, by including a diluted aliquot with the

urine and plasma samples. The possibility that variation arose from mistakes made in measuring the volumes added to, or remaining in, the infusion reservoir is in itself unlikely and may be excluded since large variations did not occur between successive estimations in individual experiments.

The large variations between values of inulin space found on different days, therefore, appear to represent true day-to-day differences in the volume of distribution of inulin in the animals studied. This does not necessarily imply that the volume of extracellular fluid fluctuated to the same extent, but only that the distribution of inulin through the extracellular volume varied on different days. Inulin has a low coefficient of diffusion (Bunim, Smith and Smith, 1937) largely because of its molecular size. Bassir (1956) showed that solutions of commercial samples of inulin from various parts of the world had different physico-chemical properties and that, in man, heterogeneity of particle size appeared to be related to the pattern of excretion after a single intravenous injection. Variation between the values of inulin space found in repeated estimations might therefore arise because of molecular inhomogeneity of the inulin used for infusion in different experiments. In the present study, however, the inulin solutions used were made up from the same batch (2 x 500 g) of commercial inulin.

Chien and Gregersen (1960) point out that inulin does not reach all parts of the extracellular fluid because of its extremely slow rate of entry into poorly vascularized structures such as dense connective tissue, cartilage and bone. The entry of inulin into tissue spaces of other structures, therefore, may be variably limited by local reductions in blood flow. Thus the possibility remains that the day-to-day variations in inulin

space observed in the present work occurred because of day-to-day differences in the patency of some capillary beds in the animals studied. Further investigation of this hypothesis is required. It should be noted that reasonable equilibration of inulin within its volume of distribution was apparently achieved in each experiment after 2 hr of infusion since no clear upward or downward trend was apparent in values calculated during infusion for a further 2½ hr (Fig. 14).

Effects of intravenous infusion of potassium chloride on potassium and sodium excretion and on the rate of urine formation

Comparison of bovine and human urine (p. 43) showed striking differences between species in urinary concentrations of potassium and sodium, which are in agreement with figures quoted for daily urinary outputs of these elements (Spector, 1956). These differences presumably arise from differences in dietary intake since it is well known that the normal diet of cattle compared with that of man (and the dog) is high in potassium and low in sodium content (Morrison, 1950). Renal mechanisms promoting the excretion of potassium and the conservation of sodium are presumably more active in the cow than in man and the dog. The work to be described was therefore carried out in order to determine whether the renal response of the cow to intravenous infusion of a potassium salt resembles that reported for man and the dog in view of the differences in the electrolyte content of the diet.

Eleven experiments were carried out on three non-pregnant, non-lactating Ayrshire cows. The animals were at grass for approximately half the period during which the work was carried out but were kept indoors for the remainder. They were prepared for clearance estimations during intravenous infusion of solutions of inulin and potassium chloride as described on p. 66, with two infusion reservoirs attached to one jugular catheter via a Y tube. Urine was collected continuously by urethral catheter as described on p. 58.

After one or two control clearance periods when inulin was infused alone, intravenous infusion of potassium chloride was

started and continued for up to 4 hr. Normal KCl, in solution in sterile water, was infused at a rate of 7 - 9 ml./min from an aspirator bottle as described for inulin infusion, and the rate was checked by measuring the residual volume at the end of the experiment. Clearance periods were of 15 min duration, blood samples being taken for estimation of plasma concentrations of inulin, sodium, and potassium at the mid-point of each period. Details of the analytical methods used for urine and plasma samples are given in earlier sections (pp. 31, 48).

Results

Results obtained in a typical experiment are shown in Table 26.

Plasma clearance of potassium

In all experiments, plasma concentrations of potassium rose 1 - 2 m-equiv/l. as a result of the infusion, but progressive increase in plasma potassium did not occur. The individual animals showed some variation but, in general, the increase in plasma concentration occurred within 2 hr of the onset of infusion and was sustained around this level for the duration of the experiment, as shown in Fig. 15. At the same time the rate of excretion of potassium increased, and, within 2 hr. of the onset of infusion, the rate of excretion had risen to equal or exceed the rate of administration (Fig. 16).

In all experiments potassium clearances rose to exceed inulin clearance values, giving ratios of potassium clearance to inulin clearance greater than unity and ranging as high as 2.0 towards the end of the infusion period (Fig. 17). Inulin

TABLE 26

Renal responses of intravenous infusion of N-KCl.

Cow No. 14711 (565 kg).

Time	Urine flow (ml./min)	Plasma Na (m-equiv/l.)	Plasma K (m-equiv/l.)	Inulin clearance (ml./min)	Na excretion (m-equiv/min)	K excretion (m-equiv/min).	K filtered* (m-equiv/min)	K clearance (ml./min)	$C_K:C_{IN}$
-70 to -55	15.4	138	3.8	918	0.32	3.05	3.32	791	0.86
-35 to -20	7.9	140	3.7	843	0.18	1.82	2.96	498	0.59
0	-	start intravenous infusion of N-KCl at 8.5 ml./min							
60-75	29.9	140	6.1	941	2.57	6.43	5.45	1057	1.12
115-130	32.2	138	5.4	931	2.24	8.47	4.78	1573	1.69
170-185	27.7	142	5.3	894	1.46	8.60	4.50	1614	1.81
225-240	26.3	140	5.1	864	1.22	8.62	4.19	1706	1.97

* Product of inulin clearance, plasma potassium concentration and a Donnan factor for univalent cations of 0.95 (Berliner et al, 1950)

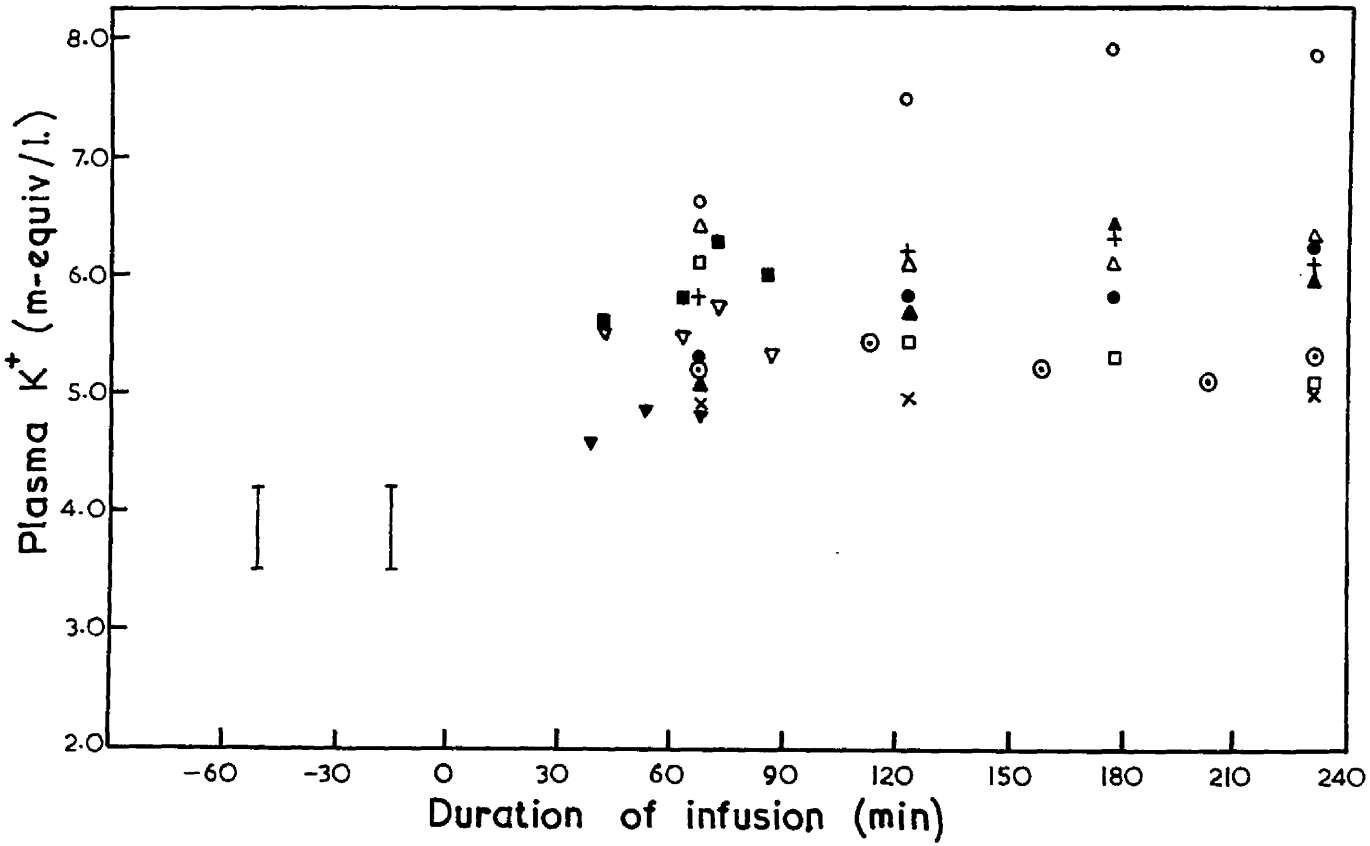

Fig. 15. The effect of intravenous infusion of N - KCl at rates of 7.0 - 9.0 ml./min on plasma potassium concentration. Different symbols are plotted to distinguish individual experiments. Pre-infusion values in all experiments lie within the indicated range.

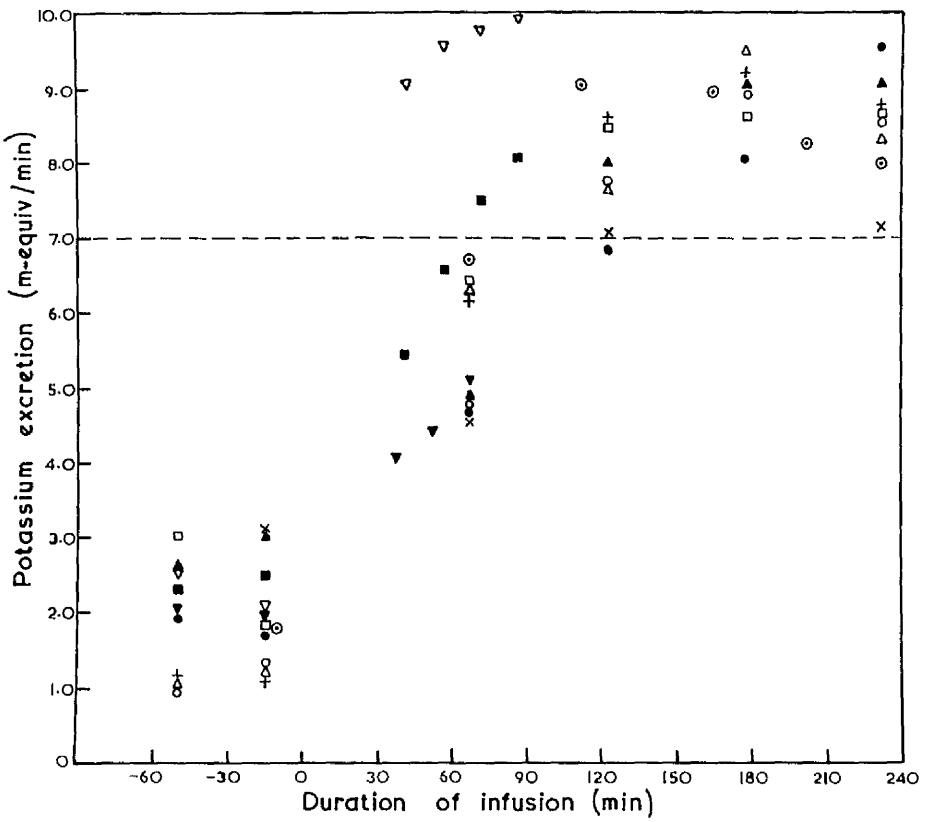

Fig. 16. The effect of intravenous infusion of N - KCl at rates of 7.0 - 9.0 ml./min on the rate of excretion of potassium. Different symbols are plotted to distinguish individual experiments. The horizontal broken line indicates a rate of excretion equal to the lowest rate of infusion.

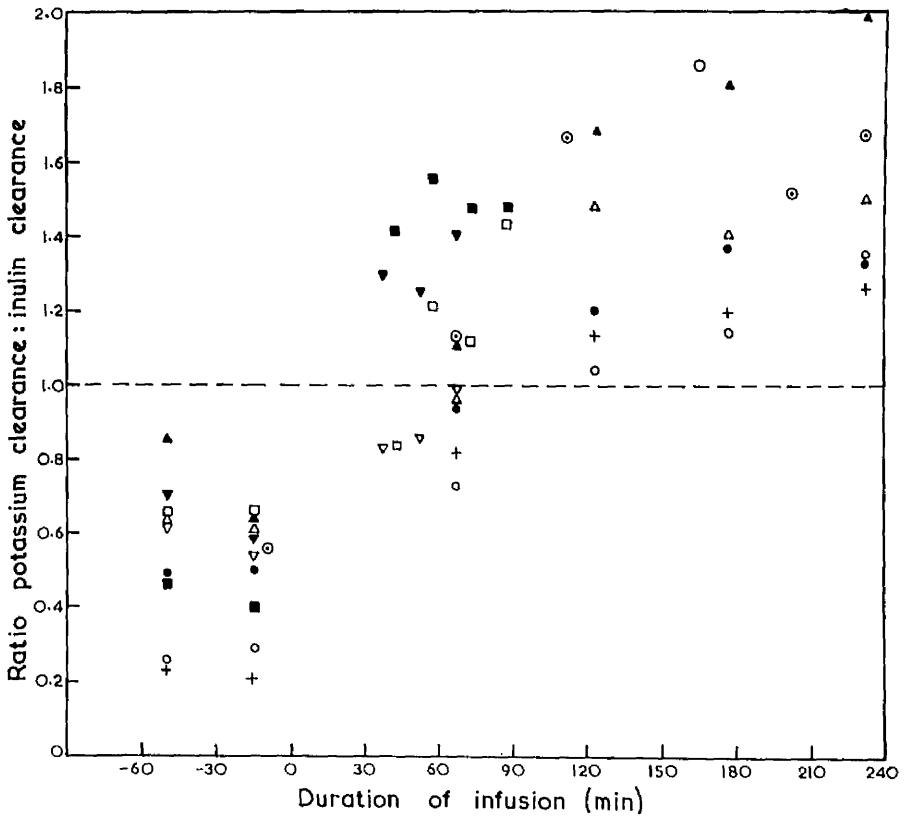

Fig. 17. The effect of intravenous infusion of N-KCl at rates of 7.0 - 9.0 ml./min on the ratio of potassium clearance to inulin clearance. Different symbols are plotted to distinguish individual experiments.

clearance values were not affected by the infusion (Fig. 18).

Rate of urine flow and sodium excretion

The rate of urine flow before infusion ranged from 3.9 to 25.1 ml./min, with a mean rate for all experiments of 13.26 ± 5.64 (S.D.) ml./min. In all experiments the onset of infusion was followed by a marked increase in urine flow, but this diuresis was not sustained. In most experiments urine flow followed a distinct pattern, with a three - to sevenfold increase over the immediate pre-infusion rate occurring 1-2 hr after the onset of infusion (Fig. 19). The diuresis then declined gradually but had not returned to pre-infusion values 4 hr after starting the infusion. Peak diuresis ranged from 3 to 7.7 times the pre-infusion rate, with a mean peak diuresis for all experiments of 4.6 times the pre-infusion rate.

The rate of excretion of sodium varied widely between experiments. In the twenty clearance periods conducted before potassium infusion was begun, sodium excretion ranged from 0.03 to 4.20 m-equiv/min, with a mean rate of 1.16 ± 1.41 (S.D.) m-equiv/min. During infusion sodium excretion in every experiment increased, and reached peak values 1-2 hr after the start of infusion. Like the concomitant diuresis, these values then fell progressively but persisted above pre-infusion values during 4 hr of infusion (Fig. 20). During this time no changes were noted in the plasma sodium concentration. Maximum increases in the rate of sodium excretion ranged from two to fifty times the pre-infusion figure, the larger percentage increases being noted for the lower pre-infusion rates of excretion.

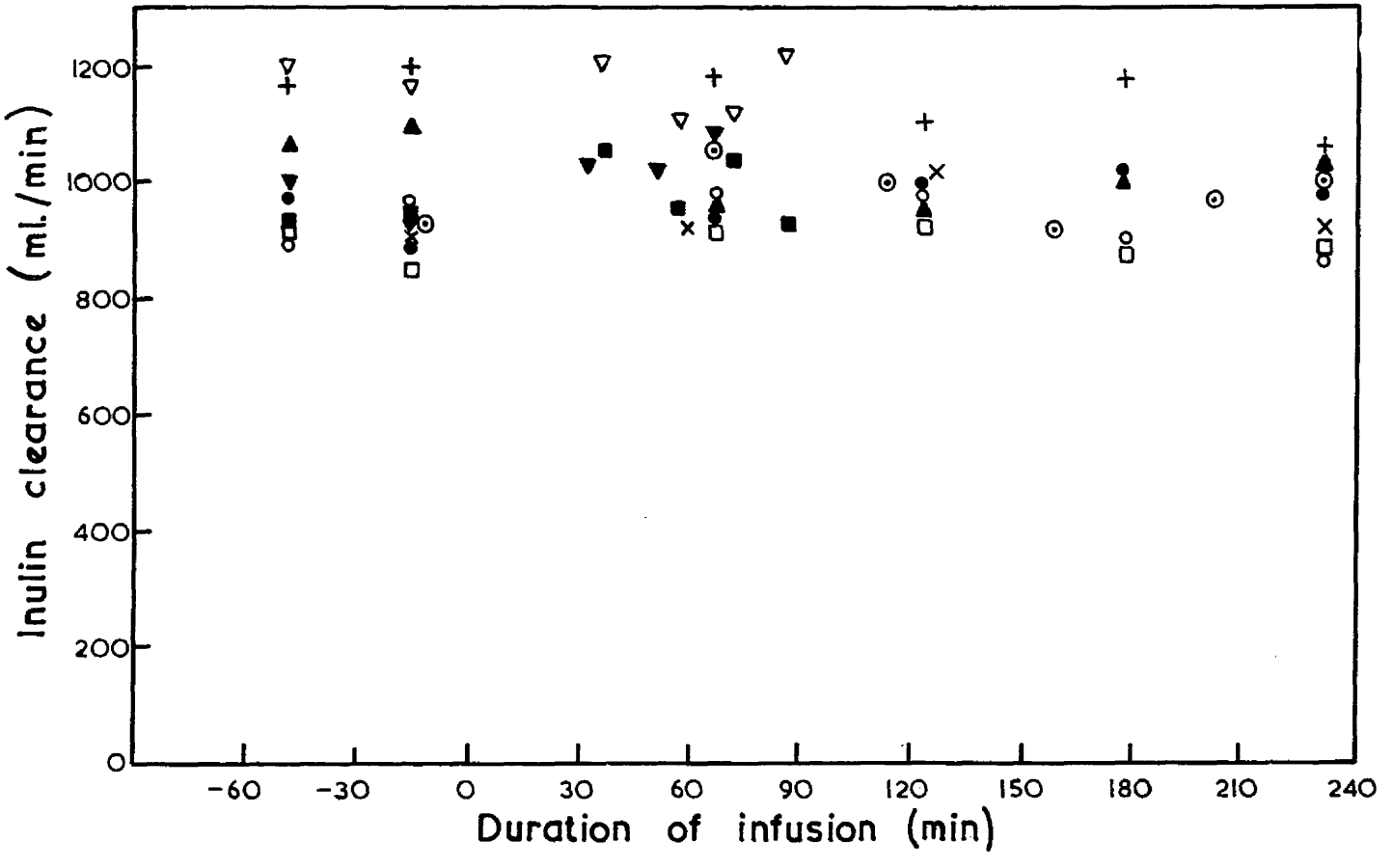

Fig. 18. Collected results showing inulin clearance values measured during intravenous infusion of M-KCl at rates of 7.0 - 9.0 ml./min. Different symbols are plotted to distinguish individual experiments.

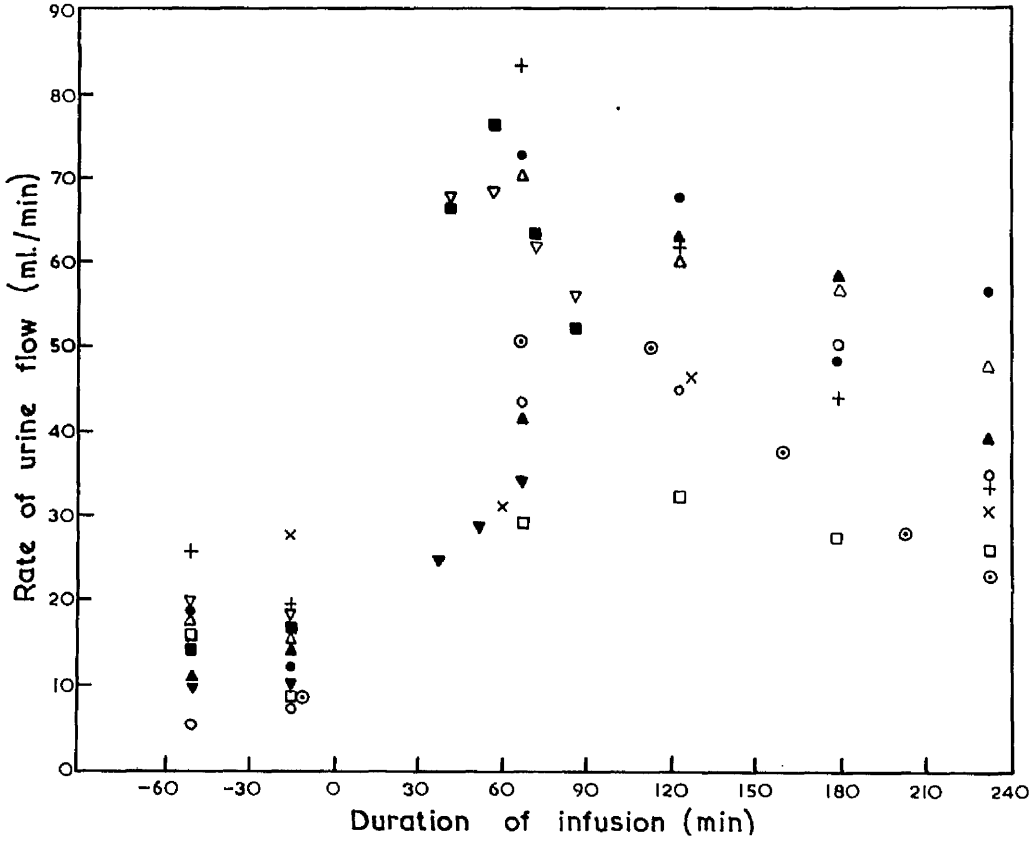

Fig. 19. The effects of intravenous infusion of N-KCl at rates of 7.0 - 9.0 ml./min on the rate of urine flow. Different symbols are plotted to distinguish individual experiments.

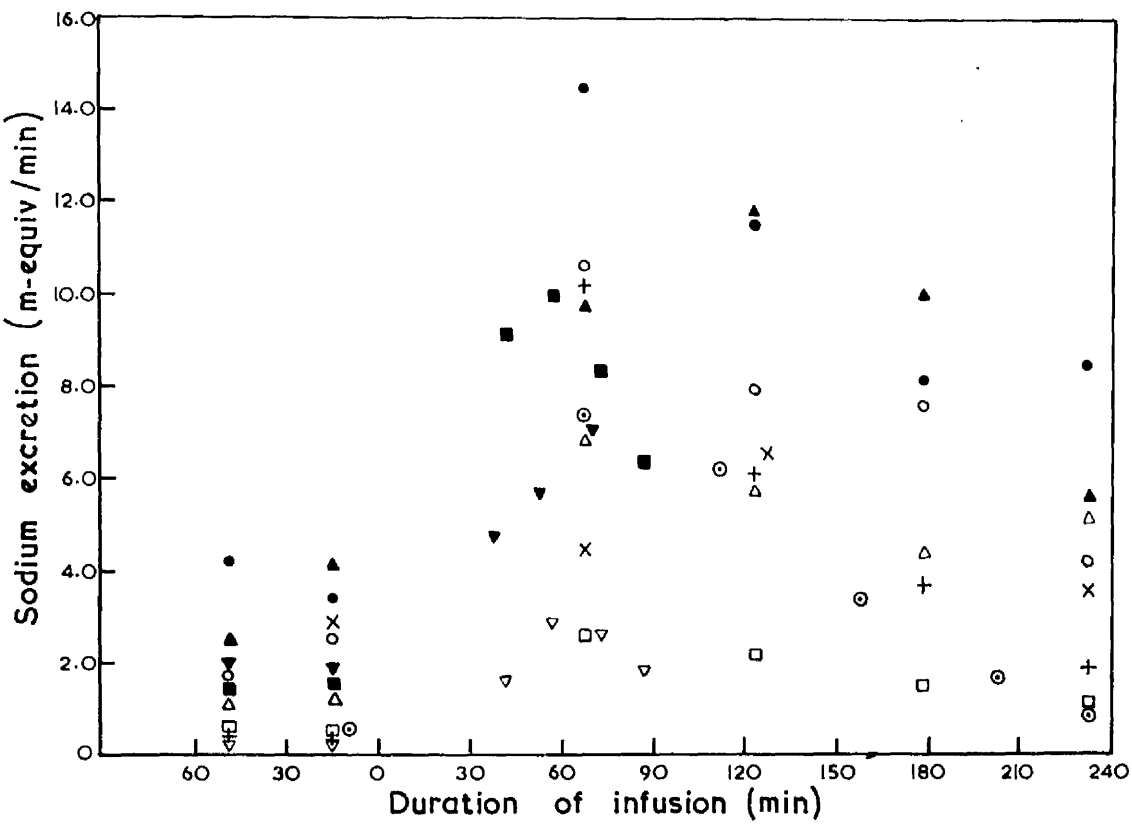

Fig. 20. The effects of intravenous infusion of N-KCl at rates of 7.0 - 9.0 ml./min on the rate of excretion of sodium. Different symbols are plotted to distinguish individual experiments.

Discussion

These experiments showed that slow intravenous infusion of potassium chloride in the cow caused some rise in plasma concentration of potassium, but no progressive rise with continuing infusion. In addition, the rate of potassium excretion rose rapidly to equal the rate of administration and clearance determinations indicated an excess of potassium excreted over that filtered. Similar findings have been reported in the cow and in the sheep by Vogel (1959). In cattle this author obtained values approaching 2.0 for the ratio of potassium to inulin clearance during potassium infusion, although the rate of infusion used was only one third, approximately, of that used in the present work. However, Vogel (1959) recorded potassium clearances before infusion which were already slightly larger than inulin clearance values, which suggests that the normal dietary turnover of potassium in his animals was greater than in those used in the present study.

The dog has been shown to tolerate potassium loading less readily than the cow. The increases in urinary excretion of potassium in the normal dog during slow intravenous infusion of potassium salts failed to equal the rate of infusion, so that plasma potassium concentrations increased progressively, and potassium clearance values rarely rose high enough to exceed the glomerular filtration rate (Berliner, Kennedy and Hilton, 1950). However, a response similar to that described in the cow occurred readily in the dog if a supplement of a potassium salt had been fed over a period preceding the intravenous infusion (Berliner *et al.*, 1950; Mudge, Ames, Foulks and Gilman, 1950). Since the

normal diet of herbivora is high in potassium content it appears that the ability to tolerate intravenous loading with potassium salts is the result of adaptation to a large dietary intake of this element and involves a large excretory reserve capacity rather than tolerance of high potassium concentrations in extracellular fluid. In this, the secretory capacity of the renal tubules plays a greater part than is at first apparent. Comparison of clearance values of potassium and inulin probably gives an erroneously low estimate of the quantity of potassium secreted since there is good evidence that essentially all the potassium filtered in the mammalian kidney is reabsorbed in proximal segments of the renal tubules (Black and Emery, 1957; Davidson, Levinsky and Berliner, 1958). The technique of stop-flow analysis indicates that secretion of potassium occurs in a distal tubular site (Pitts, Gurd, Kessler and Hierholzer, 1958), where potassium is believed to compete with hydrogen ions for secretion by an ion-exchange process involving concurrent reabsorption of sodium (Berliner, Kennedy and Orloff, 1951). However, the physiological mechanism promoting this large excretory reserve capacity apparently associated with a large dietary intake of potassium is not clear. So far no evidence has been reported which indicates that adrenocortical activity is increased, although there is good evidence that aldosterone and other mineralocorticoids stimulate tubular exchange of potassium and sodium (Giebisch, 1962; Denton, 1965), and Vander, Wilde and Malvin (1961) argue from analysis of stop-flow data that aldosterone may act by increasing the number of distal tubular carrier sites.

In the present work, however, the increases in potassium excretion did not occur with concurrent reductions in sodium

excretion. In all experiments a well-marked natriuresis was observed which declined as infusion continued (Fig. 20). Vogel (1959) stated that sodium excretion was unchanged in his experiments, although his graphs show a fourfold increase in sodium excretion during KCl infusion in the sheep. In the present work sodium excretion reached peak values 1 - 2 hr after the start of infusion, at the same time as the maximum diuretic response was observed. This diuresis may be due in part to an increased solute load in the glomerular filtrate as a result of the infusion of the hypertonic solution of potassium chloride, with the subsequent decline in urine flow the result of increased secretion of antidiuretic hormone because of the hypertonicity of the infusion, and the water lost during the initial marked diuresis. This initial diuresis may give rise to natriuresis by sweeping sodium out of the proximal tubules more rapidly than it can be reabsorbed distally (Smith, 1951).

The decline in natriuresis was observed as potassium excretion became established at a high level, when the increased tubular secretion of potassium, by ion-exchange with sodium, presumably resulted in enhanced reabsorption of sodium. The persistence of sodium excretion above pre-infusion values for the duration of the infusion is explicable by considering competition between hydrogen ions and potassium for secretion by the distal tubules. Competition of this type has been shown in dogs by the infusion of acetazolamide, but the competition was not, apparently, on a one-to-one basis, since acetazolamide caused increases in potassium excretion considerably less than the concurrent decrease in hydrogen ion excretion (Berliner, Kennedy and Orloff, 1951). It is therefore suggested that during potassium loading in the present work, a greater suppression of

hydrogen ion excretion occurred than increase in potassium, which gave rise to a net reduction in sodium reabsorption, so that sodium excretion did not decline to pre-infusion values. This increased excretion of urinary solutes presumably was associated with increased restriction on the tubular reabsorption of water, since the rate of urine flow similarly persisted above pre-infusion values for the duration of each experiment.

Effects of sulphonamyl diuretics in normal cattle

Investigation of the effects of the carbonic anhydrase inhibitor acetazolamide was undertaken in view of its reported effects on potassium and sodium excretion in the dog (Berliner, Kennedy and Orloff, 1951) and in man (Counihan, Evans and Milne, 1954). No report was available of the effects of this drug on bovine renal function, although its clinical use as a diuretic in cattle has been described (Vigue, 1961a). Responses to the heterocyclic sulphonamide hydrochlorothiazide, which has little carbonic anhydrase-inhibiting activity, were also studied since this compound has been shown in man to have a potent natriuretic effect associated with little increase in potassium excretion (Bartorelli, Gargano and Zanchetti, 1959; Richterich, 1959). Although hydrochlorothiazide has been widely used in the treatment of various oedematous conditions in cattle (Cowie, 1960; Fluckiger and Hofer, 1960; Johnston, 1961; Vigue, 1961b), little detailed information was available on its effects on urine flow and electrolyte excretion in the cow.

Comparison of responses to acetazolamide and hydrochlorothiazide

This work was undertaken in collaboration with R.S. Anderson whose interest in the excretion of bicarbonate, by converging with the present interest in cationic excretion, permitted a more complete description of the renal responses of the cow to these compounds.

Twelve experiments were performed on three non-pregnant

non-lactating Ayrshire cows of 355-412 kg body weight. In eight experiments acetazolamide ('Diamox sodium (parenteral)', Lederle) was injected intravenously at a dosage of 5 mg/kg (approximately 2.0 g per animal), followed, in four of these experiments, by an intravenous infusion of the drug at a rate equal to twice the initial dose per hour. Hydrochlorothiazide ('Vetidrex', CIBA) was administered in four experiments, by a single, deep intramuscular injection, according to the manufacturers' recommendation, at a dosage of 0.6 - 0.7 mg/kg (250 mg per animal).

Urine was collected continuously by urethral catheter as described on p. 58. Both jugular veins were cannulated to permit blood sampling, drug administration and infusion of inulin solution, as described on p. 66 . Inulin infusion was established and urine flow was measured continuously for at least 2 hr before administration of the diuretic drug. During this time blood and urine samples were taken for analysis during two 15 min clearance periods, to establish control values for glomerular filtration rate, for plasma concentrations of sodium, potassium and chloride, for urinary pH and for excretion rates of sodium, potassium, chloride and bicarbonate. Urinary sodium, potassium, chloride and inulin estimations were carried out on samples of the collected volumes; urinary pH, total CO_2 and bicarbonate concentrations were determined on additional samples taken anaerobically at the mid-point of each clearance period. After administration of the drug, blood and urine samples were taken for analysis during four 15 min clearance periods, the first of these starting 5 - 15 min after dosing, with 15 min between successive clearance periods. Thus observations were continued for 2 hr following administration of the drugs. Details of the analytical methods used for urine and plasma

samples are given in earlier sections (pp. 31-49).

Results

Detailed results of all experiments are given in Tables 27 to 38.

Urine flow

Large differences were noted between pre-dosing rates of urine flow in experiments performed on the same animals on different days, but, in general, the pre-dosing rate of urine formation was maintained at a steady level in each experiment. Both drugs increased the rate of urine formation, a diuresis being seen within 10 min of their administration, with no delay apparent in the response to intramuscular hydrochlorothiazide when compared with intravenous acetazolamide. Peak diuresis varied from a half to a tenfold increase of the pre-dosing rate of urine flow, the lower percentage increases being noted when the pre-dosing rate of flow was high ($>$ 20 ml./min). Representative experiments are illustrated in Figs. 21 and 22.

Maximum diuresis occurred usually within 30 min of drug administration and, although remaining above pre-dosing values, in most experiments the urine flow then declined to much lower levels. This decline did not occur in one experiment with hydrochlorothiazide and, in one experiment with acetazolamide, in which the pre-dosing rate of urine flow was very high, the urine flow fell below the pre-dosing rate within 2 hr of administration of the drug. In the four experiments in which acetazolamide was

TABLE 27

Renal responses to acetazolamide

Cow No. 21271 (355 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3		Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	pH	(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-55 to -40	138.8	3.3	100.5	19.5	145.0	39.5	68.4	7.57	4.87	0.095	0.706	0.192	0.333	902	0.24
-25 to -10	138.8	3.3	103.5	36.5	141.3	46.5	87.8	7.65	4.40	0.161	0.623	0.205	0.386	728	0.26
0				2.0 g acetazolamide i/v											
5-20	140.0	3.0	102.5	74.5	65.5	18.0	130.9	7.72	37.73	2.792	2.471	0.679	4.939	566	1.46
35-50	140.0	2.8	103.5	137.5	77.0	21.5	191.0	7.84	23.73	3.263	1.827	0.510	4.532	515	1.27
65-80	138.8	2.9	103.0	147.5	89.0	19.5	209.2	7.85	18.80	2.478	1.495	0.238	3.515	492	1.05
95-110	138.8	2.8	107.5	148.8	105.0	17.0	227.9	7.88	12.73	1.894	1.337	0.216	2.901	610	0.78

TABLE 28

Renal responses to acetazolamide

Cow No. 21271 (355 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml/min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-55 to -40	142.5	2.4	103.0	102.0	75.0	113.0	62.2	7.58	29.5	3.012	2.215	3.337	1.837	1030	0.88
-25 to - 10	142.5	2.7	109.0	112.5	92.0	123.5	83.9	7.63	19.3	2.171	1.776	2.384	1.619	897	0.75
0	2 g acetazolamide i/v														
5-20	143.8	2.4	107.5	125.0	39.0	49.5	120.1	7.67	78.7	9.841	3.070	3.897	9.455	657	1.94
35-50	141.3	2.6	109.5	146.0	48.0	55.5	142.6	7.74	56.1	8.186	2.691	3.112	7.996	788	1.32
65-80	138.8	3.0	109.5	157.5	62.5	57.5	157.1	7.74	40.1	6.311	2.504	2.304	6.295	777	1.09
95-110	138.8	2.5	108.0	161.3	86.0	49.0	200.1	7.80	24.4	3.936	2.098	1.196	4.882	678	1.22

TABLE 29

Renal responses to acetazolamide

Cow No. 21270 (376 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-80 to -65	145.0	3.1		197.5	207.5	233.0			4.40	0.869	0.913	1.025		670	0.44
-50 to -35	145.0	3.1		161.3	187.5	189.0			4.87	0.786	0.913	0.926		599	0.49
-20 to -5	143.8	3.1		162.5	235.0	219.0			2.73	0.444	0.642	0.591		507	0.41
0				2 g acetazolamide $^1/_V$											
10-25	145.0	3.1		170.0	87.5	34.0			21.13	3.592	1.849	0.717		527	1.13
40-55	143.8	2.9		210.3	105.0	33.5			21.67	4.553	2.275	0.724		711	1.10
70-85	143.8	3.0		230.0	117.5	26.0			7.67	1.764	0.901	0.200		310	0.96

TABLE 30

Renal responses to acetazolamide

Cow No. 21270 (376 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-55 to -40	143.8	3.0	101.5	153.8	155.0	142.5	131.8	7.72	6.60	1.015	1.023	0.941	0.870	670	0.51
-25 to -10	143.8	3.3	104.5	165.0	141.3	152.5	129.7	7.67	7.67	1.266	1.084	1.170	0.995	689	0.48
0				2.25 g acetazolamide i/v											
5-20	145.0	3.0	105.0	143.8	42.8	56.5	130.0	7.72	43.80	6.298	1.875	2.475	5.694	697	0.90
35-50	145.0	3.2	106.5	163.8	46.0	58.5	155.5	7.79	33.20	5.438	1.527	1.942	5.163	399	1.20
65-80	143.8	2.8	105.5	155.0	43.0	52.5	144.1	7.81	30.87	4.785	1.327	1.621	4.448	401	1.18
95-110	143.8	2.8	103.5	173.8	59.5	47.5	182.5	7.84	19.87	3.453	1.182	0.944	3.626	414	1.02

TABLE 31

Renal responses to acetazolamide

Cow No. 20636 (412 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-49 to -33	143.7	2.5	98.0	59.5	139.0	58.5	92.3	7.57	9.88	0.588	1.373	0.578	0.912	909	0.62
0	2.0 g acetazolamide i/v			Infuse acetazolamide at 27 mg/min											
25-45	143.7	2.4	99.0	173.5	87.0	11.0	117.1	7.78	28.35	4.919	2.466	0.312	3.320	578	1.82
95-115	143.7	2.2	100.0	187.5	117.5	8.5	275.1	7.79	17.80	3.338	2.092	0.151	4.897	643	1.48
160-175	143.7	2.3	101.1	200.0	152.5	7.5	307.2	7.83	13.13	2.626	2.002	0.098	4.034	840	1.04

TABLE 32

Renal responses to acetazolamide

Cow No. 21270 (382 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-65 to -50	146.3	3.9		15.5	313.8	221.0	41.0	7.19	2.67	0.041	0.838	0.590	0.109	532	0.41
-35 to -20	146.3	3.9	112.0	17.0	237.5	213.5	36.8	7.26	5.60	0.095	1.330	1.196	0.206	697	0.50
0	2 g acetazolamide $^{i}/_{v}$ (loading dose) Infuse acetazolamide at 100 mg/min														
15 - 30	146.3	3.2	110.5	58.0	207.5	34.5	197.7	7.80	10.93	0.634	2.268	0.377	2.160	370	1.95
45 - 60	146.3	3.1	111.0	107.0	190.0	25.5	243.2	7.86	21.07	2.265	4.003	0.537	5.124	563	2.29
60	Stop infusion														
75 - 90	146.3	3.1	111.5	133.8	180.0	23.5	272.0	7.88	12.67	1.695	2.281	0.298	3.446	419	1.76
105 - 120	146.3	3.1	110.0	158.8	181.3	23.5	302.9	7.91	11.20	1.778	2.030	0.263	3.392	428	1.53

TABLE 32

Renal responses to acetazolamide

Cow No. 21271 (355 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-45 to -3)	141.3	2.7	96.0	167.5	85.0	96.5	124.8	7.85	13.73	2.300	1.167	1.325	1.714	818	0.53
-15 to 0	141.3	2.7	97.0	180.0	94.0	111.0	136.1	7.92	9.20	1.656	0.865	1.021	1.252	726	0.44
0	2 g acetazolamide $^{i}/_{v}$ (loading dose) Infuse acetazolamide at 66 mg/min														
15 - 30	141.3	2.5	97.5	151.3	30.0	44.5	132.7	7.80	59.60	9.018	1.788	2.652	7.909	582	1.23
45 - 60	141.3	2.4	98.0	170.0	36.5	39.5	167.8	7.84	41.27	7.016	1.506	1.630	6.925	546	1.15
60	Stop infusion														
75 - 90	141.3	2.4	100.5	175.0	37.0	37.0	173.2	7.89	28.53	4.993	1.056	1.053	4.941	457	0.96
105 - 120	141.3	2.5	101.5	186.3	43.0	35.0	193.9	7.90	25.47	4.745	1.095	0.891	4.939	553	0.79

TABLE 34

Renal responses to acetazolamide

Cow No. 21271 (355 kg)

Time (min)	Plasma Na (mM/l.)	Plasma K (mM/l.)	Plasma Cl (mM/l.)	Urine Na (mM/l.)	Urine K (mM/l.)	Urine Cl (mM/l.)	Urine HCO_3 (mM/l.)	pH	Vol (ml./min)	Excretions Na (mM/min)	Excretions K (mM/min)	Excretions Cl (mM/min)	Excretions HCO_3 (mM/min)	C_K (ml./min)	$\frac{C_K}{C_{IN}}$
-50 to -35	135.0	2.4	96.5	92.5	56.5	86.5	56.0	7.54	40.13	3.712	2.267	3.471	2.246	495	1.90
-20 to -5	138.8	2.3	97.5	108.8	56.8	107.5	52.2	7.50	43.93	4.780	2.495	4.723	2.292	333	3.26
0	2 g acetazolamide $^{i}/_{v}$ (loading dose) Infuse acetazolamide at 66 mg/min														
20 - 25	135.0	2.1	96.0	132.5	33.0	55.5	107.6	7.66	72.53	9.610	2.393	4.025	7.793	439	2.60
50 - 65	132.5	2.0	98.5	135.0	33.8	53.5	126.0	7.68	58.00	7.830	2.204	3.103	7.308	286	3.85
65	Stop infusion														
80 - 95	130.0	1.9	98.0	146.3	43.5	52.5	147.6	7.75	44.47	6.506	1.934	2.335	6.568	534	1.91
110 - 125	130.0	1.9	98.0	155.0	65.6	45.5	176.9	7.82	28.00	4.340	1.834	1.274	4.953	334	2.89

TABLE 35

Renal responses to hydrochlorothiazide

Cow No. 21270 (376 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
- 45 to - 30	143.8	2.9	109.0	177.5	72.5	146.0	82.1	7.68	13.00	2.308	0.943	1.898	1.067	689	0.47
- 20 to - 5	143.8	2.9	105.5	195.0	90.0	155.5	106.8	7.72	9.47	1.847	0.852	1.470	1.011	717	0.41
0	250 mg hydrochlorothiazide i/m														
10-25	143.8	2.8	104.0	180.0	32.5	169.5	41.9	7.42	37.53	6.755	1.220	6.361	1.573	690	0.63
40-55	143.8	2.7	105.5	208.8	36.5	193.5	47.2	7.46	29.47	6.153	1.076	5.702	1.391	663	0.60
70-85	143.8	2.9	101.5	201.3	41.5	184.5	60.3	7.54	25.33	5.089	1.051	4.673	1.510	643	0.57
100-115	143.8	2.8	104.0	206.3	41.5	201.5	45.1	7.45	22.07	4.553	0.916	4.447	0.995	673	0.49

TABLE 36

Renal responses to hydrochlorothiazide

Cow No. 21270 (376 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
- 50 to - 35	141.3	2.7	103.5	132.3	45.5	115.5	52.9	7.60	32.40	4.287	1.474	3.742	1.714	347	1.57
-20 to - 55	141.3	2.7	101.5	143.0	67.0	133.5	66.3	7.70	15.60	2.231	1.045	2.083	1.034	245	1.58
0			250 mg hydrochlorothiazide i/m												
10-25	141.3	2.5	101.0	144.5	32.0	133.0	43.4	7.49	53.33	7.706	1.707	7.093	2.315	301	2.27
40-55	141.3	2.8	103.0	147.0	36.8	139.5	40.1	7.43	40.07	5.890	1.475	5.590	1.607	302	1.75
70-85	141.3	2.5	98.5	143.8	36.3	139.0	37.6	7.40	36.80	5.290	1.336	5.115	1.384	283	1.89
100-115	141.3	2.7	99.0	128.5	30.0	124.5	32.0	7.40	48.27	6.203	1.448	6.010	1.545	498	1.08

TABLE 37

Renal responses to hydrochlorothiazide

Cow No. 21271 (355 kg)

Time	Plasma			Urine						Excretions				G.F.R.	$\frac{C_K}{C_{IN}}$
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
- 50 to -35	140.0	2.4	98.5	111.0	90.5	108.5	81.9	7.62	27.13	3.011	2.455	2.944	2.222	770	1.33
- 20 to - 5	138.8	2.4	97.5	103.0	111.5	103.0	95.2	7.65	18.73	1.929	2.088	1.929	1.783	775	1.15
0	250 mg hydrochlorothiazide i/m														
10-25	138.8	2.4	95.5	145.0	60.0	136.0	63.8	7.52	43.00	6.235	2.580	5.848	2.743	677	1.59
40-55	138.8	2.4	98.5	156.0	61.0	153.5	59.2	7.50	34.27	5.346	2.091	5.260	2.029	788	1.11
70-85	140.0	2.4	96.5	165.0	69.5	166.5	55.4	7.41	27.20	4.488	1.890	4.529	1.507	787	1.00
100-115	138.8	2.5	89.5	160.0	70.5	175.5	47.7	7.36	24.13	3.861	1.701	4.235	1.151	732	0.93

TABLE 38

Renal responses to hydrochlorthiazide

Cow No. 21271 (355 kg)

Time	Plasma			Urine						Excretions				G.F.R.	CK
	Na	K	Cl	Na	K	Cl	HCO_3	pH	Vol	Na	K	Cl	HCO_3		C_{IN}
(min)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)	(mM/l.)		(ml./min)	(mM/min)	(mM/min)	(mM/min)	(mM/min)	(ml./min)	
-47 to - 32	139.0	2.4	89.5	120.0	43.0	80.5	79.3	7.73	38.00	4.560	1.634	3.059	3.013	600	1.134
- 20 to - 5	139.0	2.3	93.5	126.0	54.5	76.5	96.5	7.75	30.47	3.839	1.661	2.331	2.940	593	1.218
0	250 mg hydrochlorothiazide i/m														
12-27	139.0	2.3	89.5	157.0	36.5	120.5	64.9	7.59	61.53	9.660	2.246	7.414	3.993	613	1.592
40-58	139.0	2.3	88.0	165.0	37.0	135.0	67.3	7.58	42.33	6.985	1.566	5.715	2.849	534	1.276
70-85	139.0	2.4	90.5	157.0	35.0	125.5	64.1	7.58	51.80	7.056	1.568	5.622	2.872	696	0.938
100-115	139.0	2.4	90.5	142.0	30.0	106.5	60.5	7.56	44.40	6.327	1.332	4.729	2.686	686	0.809

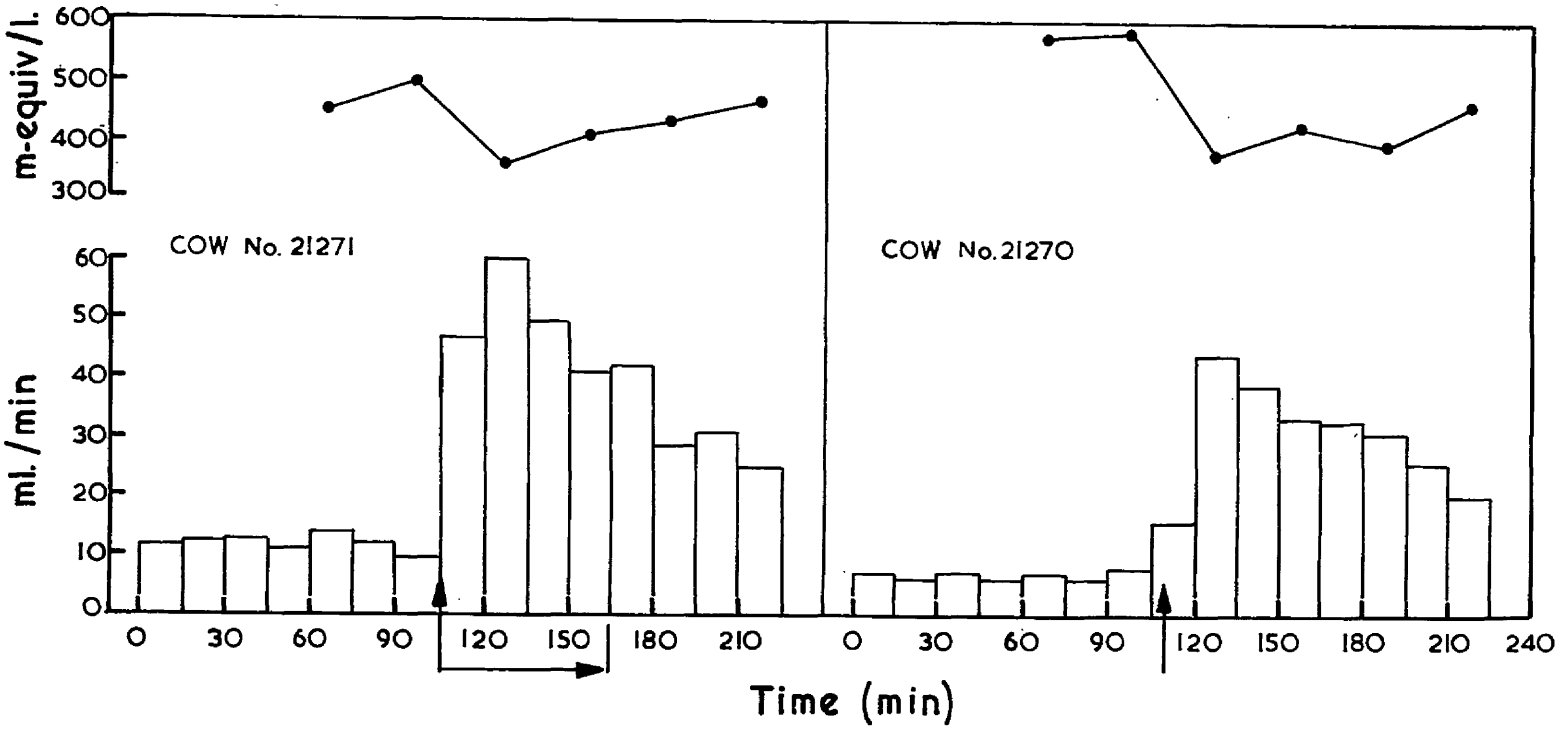

Fig. 21. Diuretic response to intravenous acetazolamide. Changes in the total concentration of the main urinary electrolytes (Na, K, Cl, HCO_3) are shown above the urine flow histograms. 2.0g acetazolamide were injected at the arrows followed in one experiment (Cow No. 21271) by intravenous infusion at 66 mg/min for 1 hr.

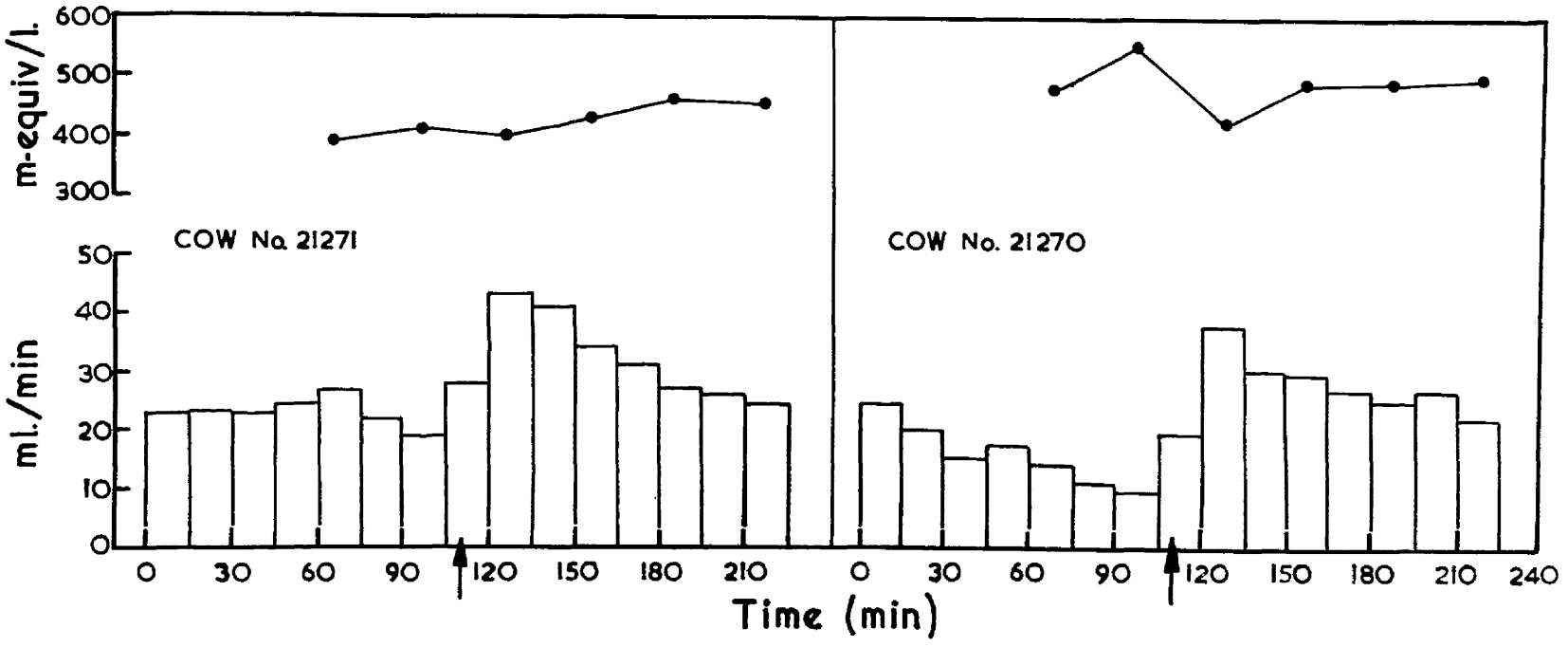

Fig. 22. Diuretic response to intramuscular hydrochlorothiazide. Changes in the total concentration of the main urinary electrolytes (Na, K, Cl, HCO_3) are shown above the urine flow histograms. 250 mg hydrochlorothiazide were injected at the arrows.

infused after its initial dose, the infusion did not sustain the initial peak diuresis (Fig. 21).

Excretion of electrolytes

The total concentration of the main urinary electrolytes (sodium, potassium, chloride and bicarbonate) in urine samples taken before drug administration varied inversely with the rate of urine flow and lay within the range 300 - 650 m-equiv/l. During the diuresis produced by either drug the reduction in the sum of the urinary concentrations of sodium, potassium, chloride and bicarbonate was slight when compared with the increase in urine flow (Figs. 21, 22) and big increases were seen in rates of excretion (urinary concentration x minute volume), which showed striking differences between drugs. Results from representative experiments are plotted in Figs. 23 and 24. Hydrochlorothiazide increased the rate of excretion of sodium to 200-300% of the pre-dosing figure, and this increase was paralleled closely by increased urinary losses of chloride, while the rates of excretion of potassium and bicarbonate were little affected (Fig. 23). Acetazolamide caused increases in sodium excretion quantitatively similar to those seen after hydrochlorothiazide but, with acetazolamide, the increase in sodium excretion was accompanied by an increase in bicarbonate excretion while the excretion of chloride was little affected by this drug (Fig. 24). In general the increases in potassium excretion after acetazolamide were only slight, although more marked increases were recorded in two experiments in which kaliuresis approached and exceeded, respectively, the natriuresis (Fig. 25). Infusion of acetazolamide did not sustain the effects of the initial dose of the drug (Figs. 24, 25). Although large increases in potassium

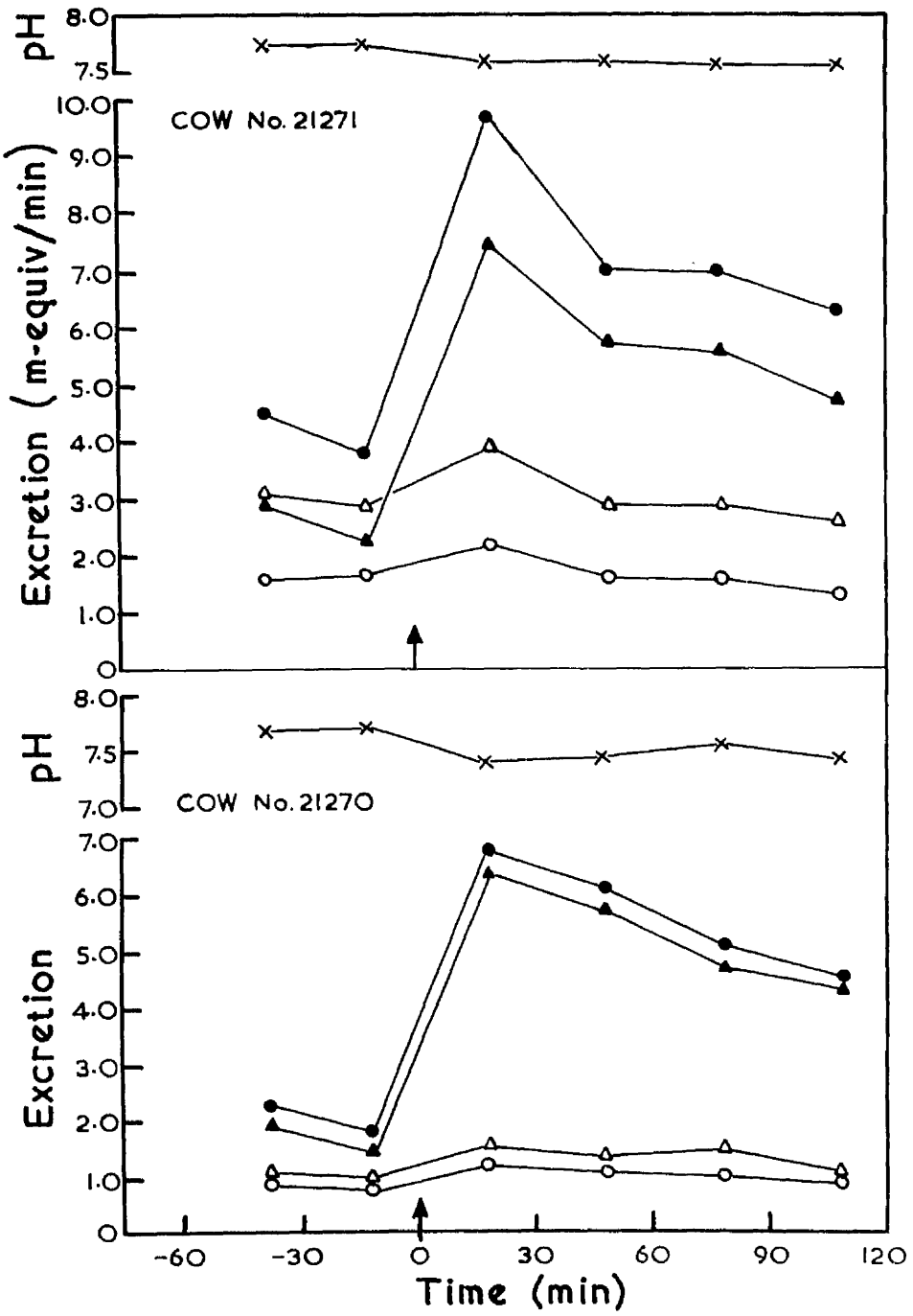

Fig. 23. Effect of hydrochlorothiazide on urinary pH (×——×) and on the rates of excretion (m-equiv/min) of sodium (●——●), potassium (o——o), chloride (▲——▲) and bicarbonate (△——△). 250 mg hydrochlorothiazide were injected intramuscularly at time 0.

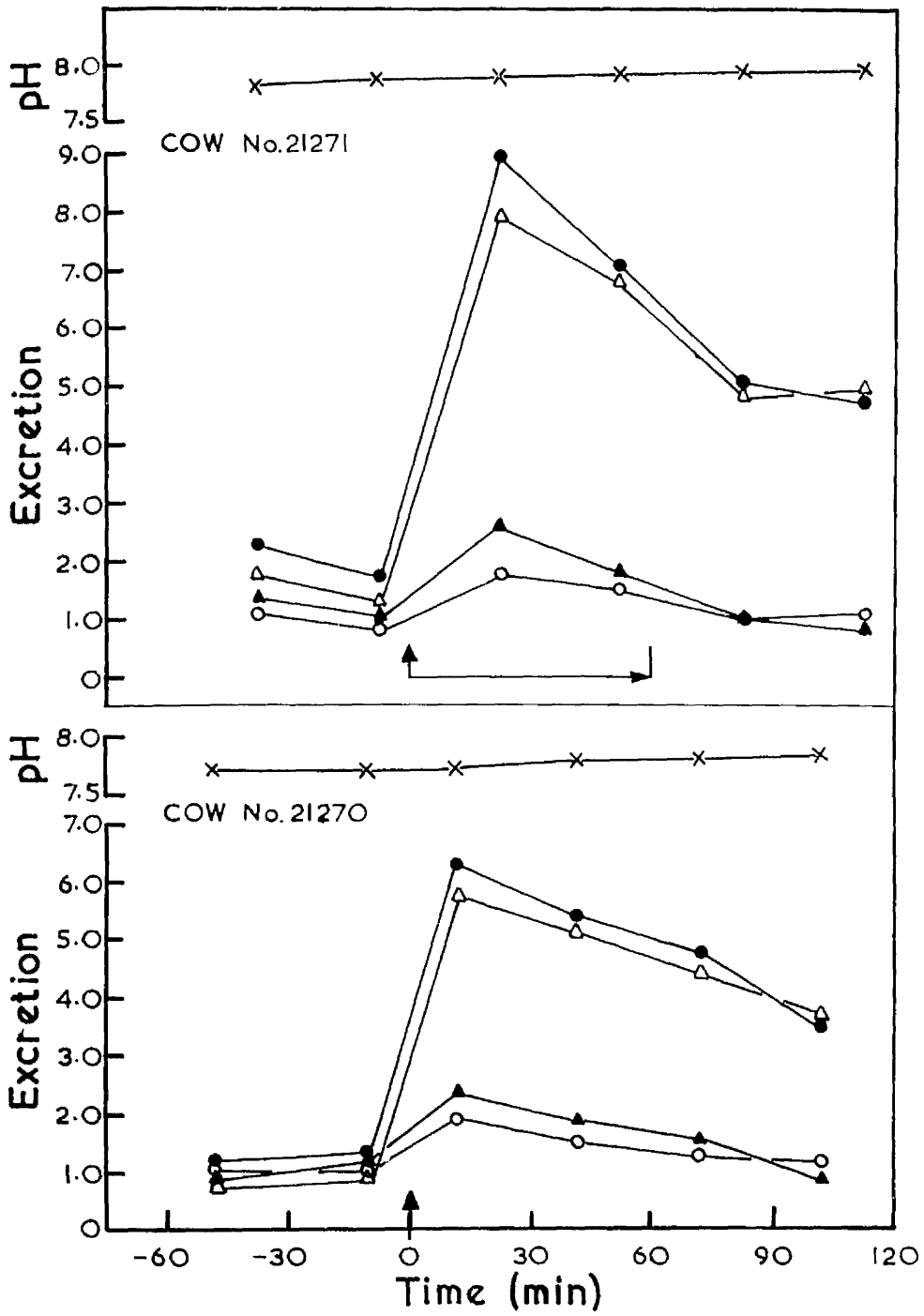

Fig. 24. Effect of acetazolamide on urinary pH (×——×) and on the rates of excretion (m-equiv/min) of sodium (●——●), potassium (○——○), chloride (▲——▲) and bicarbonate (△——△). 2.0g acetazolamide were injected intravenously at time 0, followed, in the experiment on cow No. 21271 by intravenous infusion at 66 mg/min for 1 hr.

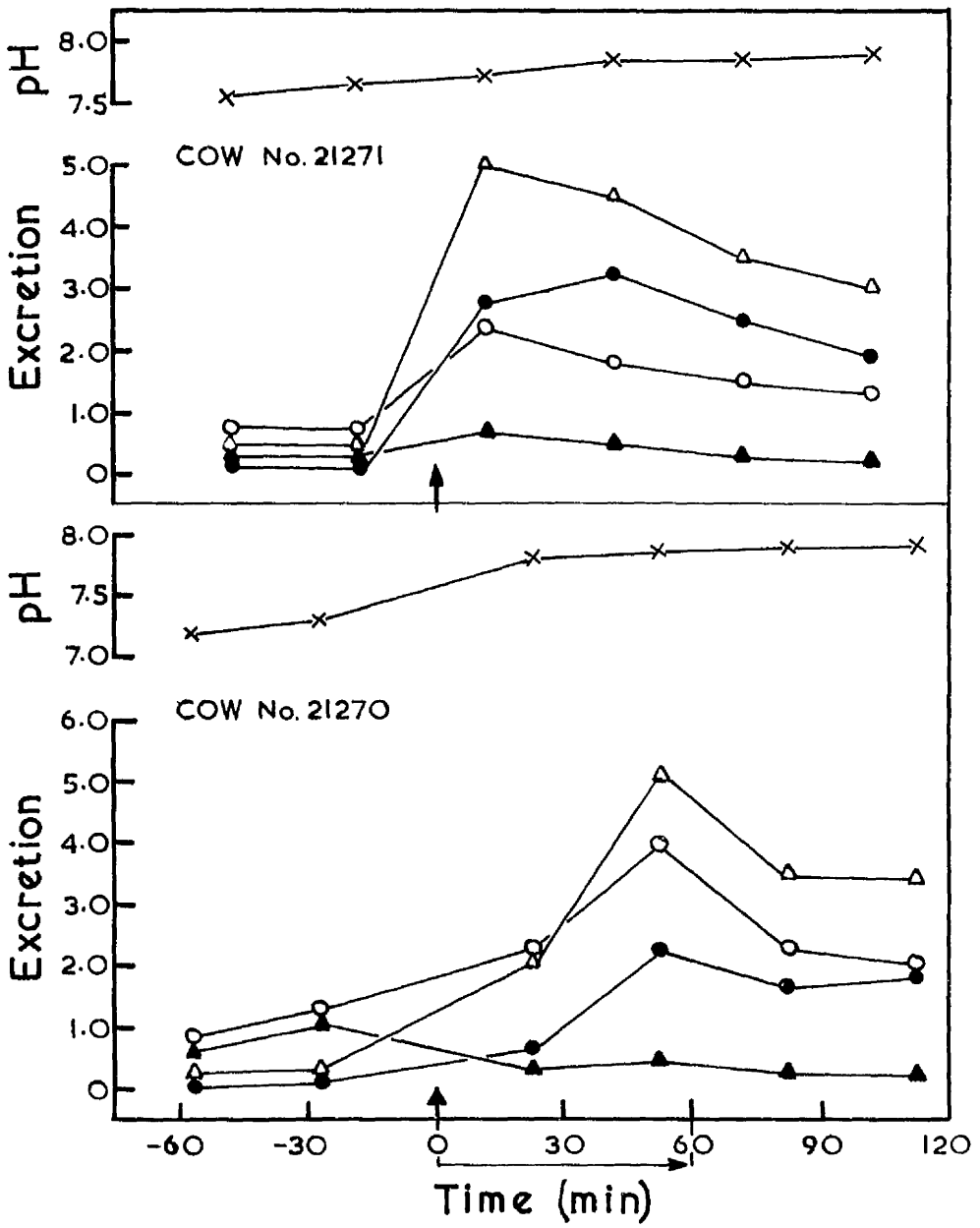

Fig. 25. Effect of acetazolamide on urinary pH (×——×), and on the rates of excretion (m-equiv/min) of sodium (●——●), potassium (o——o), chloride (▲——▲) and bicarbonate (△——△). 2.0g acetazolamide were injected intravenously at time 0 followed, in the experiment on cow no. 21270 by intravenous infusion at 66 mg/min for 1 hr.

excretion were rarely seen, in every experiment with acetazolamide the ratio of potassium clearance to inulin clearance rose to exceed unity, largely because of the concurrent reduction in inulin clearance which followed administration of this drug (q.v.)

Urinary pH showed alteration in opposite directions as a result of administration of the different drugs (Figs. 23 - 25): after acetazolamide the urine became slightly more alkaline and after hydrochlorothiazide slightly less alkaline.

Plasma composition

No alterations in plasma concentrations of sodium and chloride were seen after either drug, nor was there any fall in plasma potassium levels after hydrochlorothiazide. Some reduction in plasma potassium concentrations, however, was seen after acetazolamide (Fig. 26), but in no experiment was the reduction greater than 1.0 m-equiv/l. The two experiments with acetazolamide illustrated in Fig. 26 are those in which the fall in plasma potassium was most marked, and these two experiments also showed the greatest increases in urinary excretion of potassium after the drug. In six other experiments with acetazolamide, increases in the rate of excretion and reduction in the plasma concentration of potassium were not so pronounced, although all showed the trend towards hypokalaemia.

Glomerular filtration rate

Hydrochlorothiazide had no effect on inulin clearance, but after administration of acetazolamide inulin clearance values fell to 50 - 70% of the pre-dosing figures. Typical responses are illustrated in Fig. 27.

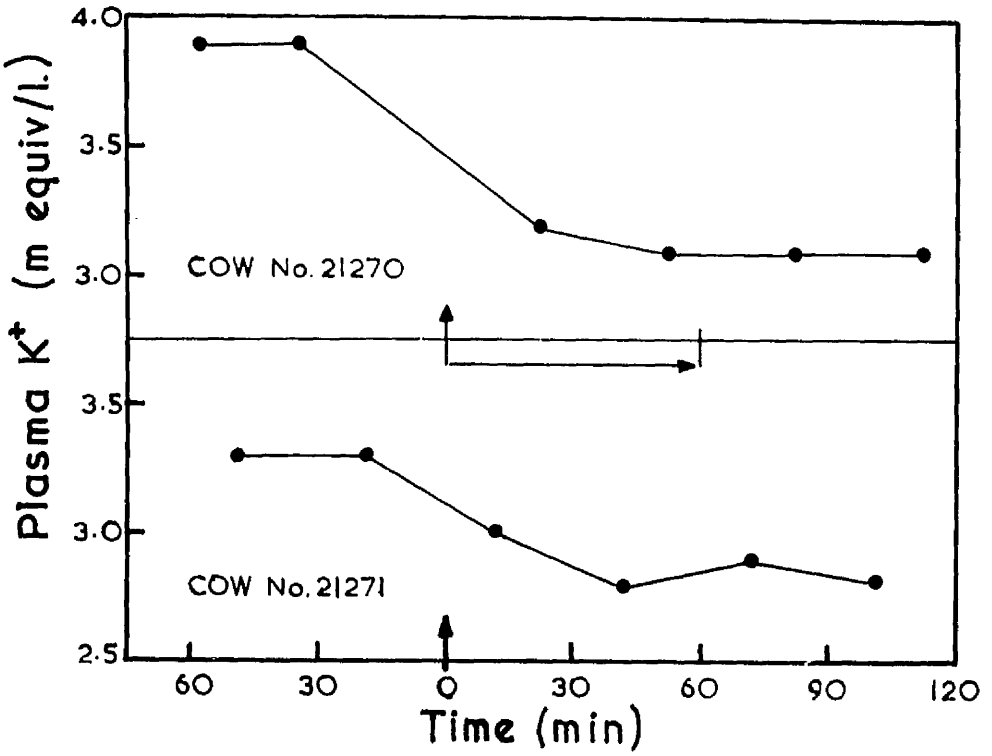

Fig. 26. Effect of acetazolamide on plasma potassium concentration. 2.0 g acetazolamide were injected intravenously at time 0 followed, in the experiment on cow no. 21270 by intravenous infusion at 66 mg/min for 1 hr.

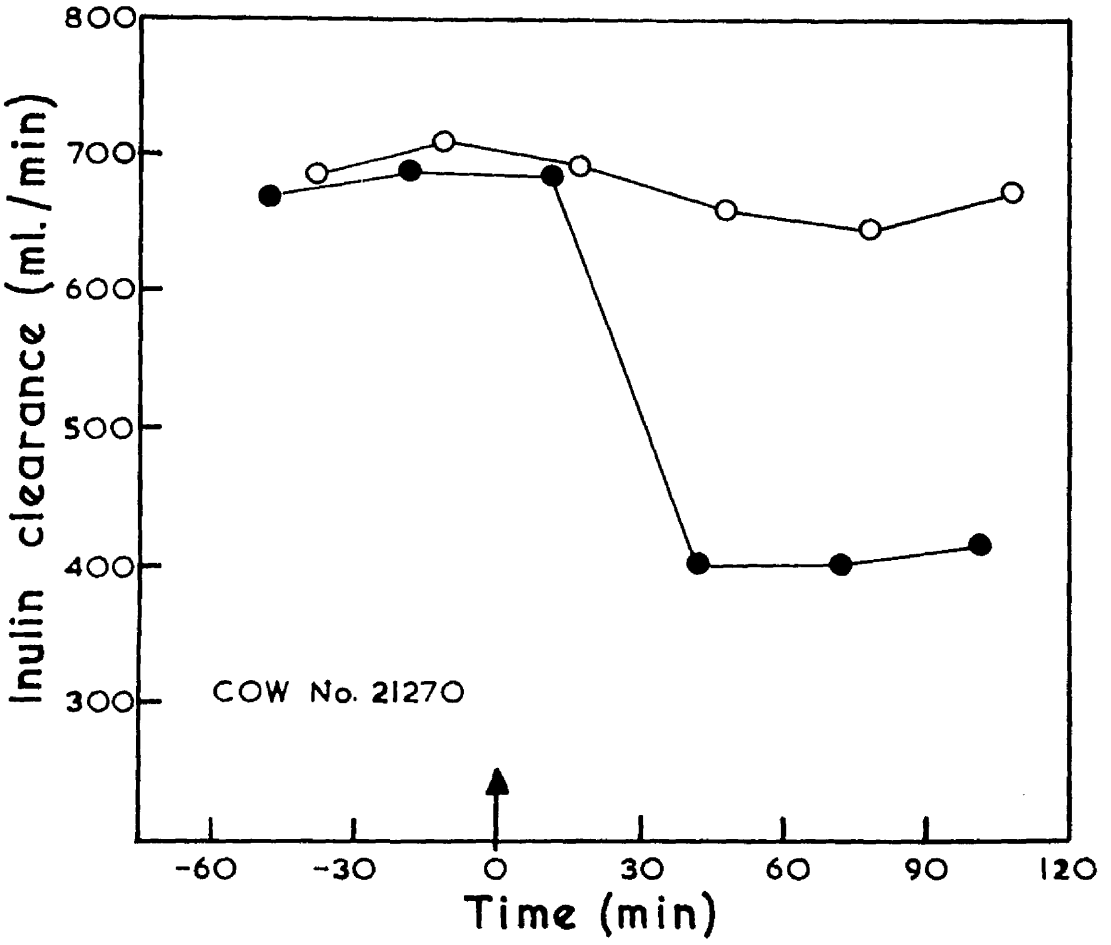

Fig. 27. Effect of intravenous acetazolamide (●——●) and intramuscular hydrochlorothiazide (o——o) on glomerular filtration rate. 2.0g acetazolamide and 250 mg hydrochlorothiazide, respectively, were injected at time 0.

Discussion

The increases in urine flow observed after administration of acetazolamide and hydrochlorothiazide were accompanied by little alteration in the total concentration of the main urinary electrolytes. The diuresis did not therefore represent increased losses of osmotically free water but involved increased solute losses consequent on reductions in the tubular reabsorption of solute with the increase in urine volume providing the vehicle for this increased solute excretion.

The increases in the rates of excretion of sodium and bicarbonate after administration of acetazolamide can be explained on the assumption that the drug inhibited carbonic anhydrase activity in renal tubules. The mechanism of bicarbonate reabsorption by renal tubules has been clearly described by Pitts (1963). Bicarbonate is believed to be reabsorbed indirectly by conversion to carbon dioxide within the tubular urine as a consequence of the tubular secretion of hydrogen ions which are made available by the hydration of carbon dioxide within the cells. This latter reaction is catalysed by carbonic anhydrase and the movement of hydrogen ions into the tubular urine is matched by sodium reabsorption. Thus, restriction of the supply of hydrogen ions, by inhibition of carbonic anhydrase, leads to reduced reabsorption (and increased excretion) of both bicarbonate and sodium.

In distal regions of the nephron hydrogen ions and potassium ions are believed to compete for secretion in exchange for reabsorbed sodium since in the dog and in man restriction of the supply of hydrogen ions for secretion has been shown to result in an increased excretion of potassium (Berliner et al., 1951; Counihan et al., 1954). The kaliuresis observed after aceta-

zolamide in the present work, whilst quantitatively much less pronounced than in the dog and in man, probably represents a qualitatively similar response, and the rise in urine pH noted after acetazolamide is attributable to this action. The quantitative difference in kaliuretic response presumably occurred because acidification of the urine in the cows used was proceeding at a low level before dosing, as indicated by the alkaline reaction of the urine, compared with the normally acidic urine of man and the dog. Although weak, the kaliuretic response to acetazolamide in the present work was apparently sufficient to account for the observed reductions in plasma concentrations of potassium, since the increases in potassium excretion were greater than theoretical increases required to lower potassium concentration in extracellular fluid to the plasma levels observed. These theoretical values were calculated assuming extracellular fluid to be approximately 20% of the body weight, and potassium concentration to be the same in interstitial fluid as in plasma, as illustrated in the example below:

Cow No. 21270 (381 kg) 2.0 g. acetazolamide intravenously followed by infusion of 67 mg/min for 60 min.

Observed fall in plasma K^+, 60 min after dosing:	0.75 m-equiv/l.
Volume of extracellular fluid (20% of body wt) :	76 l.
Theoretical loss of K to reduce e.c.f. concentration by 0.76 m-equiv/l. :	57 m-equiv
Observed increase in K excretion above pre-dosing rate during 60 min after dosing (calculated from graph of excretion rate against time) :	97 m-equiv

The present work showed hydrochlorothiazide to be effective as a natriuretic agent in the cow and the absence of alteration

of sodium and chloride concentrations in plasma suggests that the diuresis promoted by hydrochlorothiazide reduced the extracellular fluid volume in the same degree by which the excretion of sodium and chloride was increased. However, the mechanism of action of the drug is not clear. The absence of effects on the rates of excretion of bicarbonate and potassium indicated that, at the dosage used, the drug had little carbonic anhydrase-inhibiting activity. Beyer and Baer (1961) point out that inhibition of carbonic anhydrase by thiazides is readily demonstrable in vitro, but that a natriuretic response to these drugs occurs in vivo at a dosage inadequate to affect bicarbonate excretion. The suggestion that thiazide diuretics exert this natriuretic effect by an action on proximal convoluted tubules (Beyer, 1958) is supported by the findings of Kessler, Hierholzer, Gurd and Pitts (1959), and Vander, Malvin, Wilde and Sullivan (1959) in stop-flow experiments on dogs. These authors concluded that chlorothiazide depressed proximal reabsorption of sodium, leading to an iso-osmotic reduction in water reabsorption at that site. It may therefore be concluded that the diuresis observed after administration of hydrochlorothiazide in the present work resulted from reduced reabsorption in proximal tubules of the sodium moiety which is normally accompanied into the peritubular fluid by chloride ions, with no effect demonstrable on the normal tubular exchange of sodium ions with potassium ions.

Further studies on the effects of acetazolamide in normal cows

Seven experiments were carried out on two non-pregnant, non-lactating Ayrshire cows to extend the findings described in the preceding section, and in particular:

(1) To confirm that the hypokalaemia seen after administration of acetazolamide is the result of the slight kaliuresis promoted by the drug.

(2) To investigate whether metabolic acidosis might develop in the cow as a consequence of the reduction in urinary acidification seen after administration of the drug, as has been shown to occur in man during treatment with acetazolamide (Chart, Renzi, Barrett and Sheppard, 1959).

(3) To investigate whether the reduction in glomerular filtration rate observed after acetazolamide involves reduction in renal plasma flow or alteration of filtration fraction.

All experiments were conducted at the same time of day and followed an identical time schedule involving six 15 min clearance periods with 15 min between successive periods. Continuous collection of urine was begun as soon as the priming dose of inulin was given, the first clearance period commencing 105 min later. A single dose of 2.0 g of acetazolamide ('Diamox sodium (parenteral)', Lederle) was given 5 min before the start of the third clearance period. The inulin space was calculated by the difference method at the end of each clearance period as described on p. 82, and p-aminohippurate (sodium salt) was infused

with the inulin solution as described on p. 75 , to allow simultaneous determinations of inulin and PAH clearances.

During the second, fourth and sixth clearance periods, in two experiments on each animal, arterial blood samples were taken anaerobically from the coccygeal artery, as described on p. 68 , for the measurement of blood pH, and plasma total CO_2 and bicarbonate concentrations. Samples of arterial blood were not obtained in all the experiments because delays arising from the difficulty encountered in obtaining successive samples were not allowed to disrupt the experimental schedule.

Samples of venous blood were taken at the mid-point of each clearance period for determination of plasma concentrations of inulin, PAH, sodium, potassium and chloride. Samples of collected urine volumes were similarly analysed to permit calculation of excretion rates and clearance values. An additional urine sample was taken anaerobically at the midpoint of each clearance period for determination of urinary pH, total CO_2 and bicarbonate concentrations. Details of analytic procedures are given in earlier sections.

Results

Detailed results of all experiments are given in Tables 39 - 45. In all experiments peak diuresis occurred within 30 min of administration of the drug and averaged 300% of the mean pre-dosing rate of flow, before declining almost to pre-dosing levels in the following 90 min (Fig. 23). The rates of excretion of bicarbonate, sodium and potassium similarly reached peak values within 30 min of drug administration and then declined, although the mean rates of excretion of sodium and bicarbonate were still

TABLE 39

Cow No. 22349 (511 kg) 2.0 g acetazolamide i/v at time 0.

Parameter measured	Time (min)					
	- 55 to - 40	- 25 to - 10	5 -20	35 - 50	65 - 80	95 - 110
Plasma:						
Na (mM/l.)	138.0	138.0	140.0	138.0	138.0	138.0
K "	4.0	3.9	3.8	4.0	3.7	3.5
Cl "	112.0	110.5	111.5	111.5	114.5	110.0
art. HCO_3 "	-	27.9	-	25.0	-	24.9
art. blood pH	-	7.41	-	7.41	-	7.41
Urine:						
Na (mM/l.)	138.8	153.8	180.8	206.3	220.0	205.0
K "	161.3	180.0	83.0	90.5	107.5	151.3
Cl "	154.5	177.0	42.5	41.5	39.0	40.0
HCO_3 "	78.2	63.8	195.4	228.8	261.6	265.9
Vol (ml./min)	16.93	12.40	43.4	34.27	24.47	19.27
pH	7.64	7.54	7.77	7.85	7.84	7.85
Excretion:						
Na (mM/min)	2.350	1.907	7.812	7.070	5.339	3.950
K "	2.731	2.232	3.602	3.101	2.631	2.916
Cl "	2.616	2.915	1.845	1.422	0.954	0.771
HCO_3 "	1.324	0.791	8.480	7.841	6.401	5.124
G.F.R. (C_{IN})(ml./min)	1087	1138	664	841	884	1051
RP.F.(C_{PAH}) "	6725	7059	5805	5624	5260	5553
C_{IN}/C_{PAH}	0.16	0.16	0.11	0.15	0.17	0.19
Inulin space (l)	65	71	68	70	73	81

T A B L E 40

Cow No. 22349 (513 kg) 2.0 g acetazolamide i/v at time 0.

Parameter measured	Time (min)					
	- 55 to - 40	- 25 to - 10	5 - 20	35 - 50	65 - 80	95 - 110
Plasma:						
Na (mM/l.)6	146.3	147.5	150.0	147.5	147.5	147.5
K "	4.1	4.1	3.5	3.5	3.1	3.3
Cl "	110.0	110.0	106.5	111.0	112.0	114.0
art. HCO_3 "	-	27.2	-	26.3	-	26.1
art. blood pH	-	7.42	-	7.41	-	7.41
Urine:						
Na (mM/l.)	31.0	50.5	104.5	136.5	155.0	141.3
K "	120.0	220.0	109.0	129.0	147.5	172.5
Cl "	67.5	146.5	33.5	34.5	30.0	32.5
HCO_3 "	39.3	40.9	158.6	189.4	222.9	236.3
vol (ml./min)	37.20	17.67	58.40	36.60	26.60	19.00
pH	7.54	7.43	7.80	7.88	7.93	8.01
Excretion:						
Na (mM/min)	1.153	0.892	6.103	4.996	4.123	2.685
K "	4.464	3.887	6.366	4.721	3.924	3.278
Cl "	2.511	2.589	1.956	1.263	0.798	0.618
HCO_3 "	1.462	0.716	9.262	6.932	5.929	4.490
G.F.R. (C_{IN}) (ml./min)	1111	1153	867	841	893	905
R.P.F. (C_{PAH}) "	7142	6949	6205	5942	5899	5778
C_{IN} / C_{PAH}	0.16	0.17	0.14	0.14	0.15	0.16
Inulin space (l.)	48	52	51	46	49	48

TABLE 41

Cow No. 22349 (485 kg) 2.0 g acetazolamide i/v at time 0

Parameter measured	Time (min)					
	-55 to - 40	- 25 to - 10	5 - 20	35 - 50	65 - 80	95 - 110
Plasma:						
Na (mM/l.)	137.5	137.0	138.8	137.0	138.0	140.0
K "	3.5	3.5	3.4	3.3	3.3	3.3
Cl "	105.5	107.5	107.5	103.5	111.5	106.5
Urine:						
Na (mM/l.)	23.0	45.8	101.0	133.0	155.0	150.0
K "	63.0	137.5	93.5	94.0	140.0	150.0
Cl "	46.5	123.0	29.5	33.0	42.5	39.5
HCO_3 "	19.6	15.6	145.4	168.9	220.7	217.2
Vol (ml./min)	46.47	18.87	56.13	40.93	23.33	21.13
pH	7.19	6.97	7.71	7.88	7.88	7.90
Excretion:						
Na (mM/min)	1.069	0.864	5.669	5.444	3.616	3.170
K "	2.928	2.595	5.248	3.847	3.266	3.170
Cl "	2.161	2.321	1.656	1.351	0.992	0.835
HCO_3 "	0.911	0.294	8.161	6.913	5.149	4.589
G.F.R. (C_{IN}) (ml./min)	1020	993	832	740	766	957
R.P.F. (C_{PAH}) "	6940	7581	6654	5655	5923	6264
C_{IN}/C_{PAH}	0.15	0.13	0.13	0.13	0.13	0.15
Inulin space (l.)	55	63	63	59	58	65

TABLE 42

Cow No. 22349 (500 kg) 2.0 g acetazolamide i/v at time 0.

Parameter measured	Time (min)					
	-55 to -40	-25 to -10	5 - 20	35 - 50	65 - 80	95 - 110
Plasma:						
Na (mM/l.)	138.8	138.8	138.8	138.8	140.0	140.0
K "	3.4	3.4	3.4	3.3	3.2	3.3
Cl "	106.5	111.5	108.0	111.5	109.0	110.0
Urine:						
Na (mM/l.)	52.0	59.0	128.8	170.0	177.5	157.5
K "	160.0	177.5	76.5	104.0	127.5	90.0
Cl "	86.5	122.5	27.5	31.5	28.5	32.0
HCO_3 "	65.2	45.9	163.5	217.0	242.6	194.0
Vol (ml./min)	19.20	14.73	55.33	31.47	27.53	34.60
pH	7.60	7.38	7.68	7.73	7.72	7.70
Excretion:						
Na (mM/min)	0.998	0.869	7.127	5.350	4.887	5.450
K "	3.072	2.615	4.233	3.273	3.510	3.144
Cl "	1.661	1.804	1.522	0.991	0.785	1.107
HCO_3 "	1.252	0.676	9.046	6.839	6.679	6.712
G.F.R. (C_{IN}) (ml./min)	1268	1280	839	723	853	887
R.P.F. (C_{PAH}) (ml./min)	7580	7987	5805	5289	6544	6684
C_{IN}/C_{PAH}	0.17	0.16	0.15	0.14	0.13	0.13
Inulin space (l.)	85	86	85	86	99	101

TABLE 43

Cow No. 21271 (422 kg) 2.0 g acetazolamide i/v at time 0.

Parameter measured	Time (min)					
	- 55 to - 40	- 25 to - 5	5 - 20	35 - 50	65 - 80	95 - 110
Plasma:						
Na (mM/l.)	146.3	146.3	150.0	147.5	150.5	147.0
K "	2.6	2.6	2.4	2.5	2.5	2.4
Cl "	102.5	105.5	101.0	107.5	105.5	109.5
art. HCO_3 "	-	7.44	-	7.44	-	7.44
art. blood pH	-	35.2	-	34.2	-	33.0
Urine:						
Na (mM/l.)	100.0	101.3	112.0	145.0	168.8	168.8
K "	156.3	136.3	58.5	57.5	95.0	113.8
Cl "	96.5	87.5	31.5	42.5	30.0	26.5
HCO_3 "	52.9	56.2	126.2	145.6	191.4	210.9
Vol (ml./min)	14.13	14.27	61.80	42.80	25.07	21.87
pH	7.46	7.50	7.75	7.85	7.90	8.00
Excretion:						
Na (mM/min)	1.413	1.446	6.922	6.206	4.232	3.692
K "	2.202	1.945	3.615	2.461	2.382	2.489
Cl "	1.364	1.249	1.947	1.819	0.752	0.580
HCO_3 "	0.747	0.802	7.799	6.232	4.798	4.612
G.F.R. (C_{IN}) (ml./min)	1167	1158	638	695	699	637
R.P.F. (C_{PAH}) (ml./min)	8645	8548	7328	5671	6768	6054
C_{IN}/C_{PAH}	0.14	0.14	0.09	0.12	0.10	0.11
Inulin space (l.)	46	59	62	58	63	61

TABLE 44

Cow No. 21271 (427 kg) 2.0 g acetazolamide i/v at time 0

Parameter measured	Time (min)					
	- 55 to - 40	- 25 to - 10	5 - 20	35 - 50	65 - 80	95 - 110
Plasma:						
Na (mM/l.)	148.8	148.8	148.8	148.8	148.8	148.8
K "	3.3	3.1	2.9	2.7	2.8	2.6
Cl "	109.0	106.5	106.0	109.0	112.5	108.5
art. HCO_3 "	-	33.1	-	31.2	-	30.9
art. blood pH	-	7.44	-	7.44	-	7.44
Urine:						
Na (mM/l.)	55.0	71.0	117.0	136.0	143.8	141.3
K "	118.0	142.0	64.0	68.5	90.0	124.0
Cl "	80.5	90.5	32.5	40.0	45.0	35.5
HCO_3 "	49.9	57.0	141.6	151.0	157.4	196.7
Vol (ml./min)	22.67	16.13	59.67	45.60	32.33	18.67
pH	7.46	7.49	7.77	7.84	7.86	7.92
Excretion:						
Na (mM/min)	1.247	1.145	6.981	6.202	4.649	2.638
K "	2.675	2.290	3.819	3.124	2.910	2.315
Cl "	1.825	1.460	1.939	1.824	1.455	0.663
HCO_3 "	1.131	0.919	8.449	6.886	5.089	3.672
G.F.R. (C_{IN}) (ml./min)	1155	1006	847	678	731	708
R.P.F. (C_{PAH}) (ml./min)	6913	6387	5401	5107	5934	5073
C_{IN}/C_{PAH}	0.17	0.16	0.16	0.13	0.12	0.14
Inulin space (l.)	66	79	78	70	82	90

TABLE 45

Cow No. 21271 (405 kg) 2.0 g acetazolamide i/v at time 0

Parameter measured	Time (min)					
	- 55 to - 40	- 25 to - 10	5 - 20	35 - 50	65 - 80	95 - 110
Plasma:						
Na (mM/l.)	141.3	142.5	142.5	141.3	142.0	141.3
K "	2.8	2.8	2.7	2.6	2.4	2.4
Cl "	105.0	103.0	107.5	106.5	108.5	105.5
Urine:						
Na (mM/l.)	46.5	60.5	99.0	128.0	143.8	140.0
K "	136.3	157.5	73.0	71.5	125.0	135.0
Cl "	69.0	84.5	33.5	34.5	37.5	33.5
HCO_3 "	67.7	60.3	136.8	149.0	199.0	211.0
Vol (ml./min)	14.07	9.47	55.40	43.00	21.67	19.33
pH	7.58	7.53	7.54	7.72	7.79	7.80
Excretion:						
Na (mM/min)	0.654	0.573	5.485	5.504	3.116	2.706
K "	1.918	1.492	4.044	3.075	2.709	2.610
Cl "	0.971	0.800	1.856	1.484	0.824	0.648
HCO_3 "	0.953	0.570	7.579	6.407	4.312	4.079
G.F.R.(C_{IN}) (ml./min)	1111	1084	953	766	849	967
R.P.F.(C_{PAH}) (ml./min)	7136	7498	7761	6296	6736	7099
C_{IN}/C_{PAH}	0.16	0.15	0.12	0.12	0.13	0.14
Inulin space (l.)	46	45	44	41	51	55

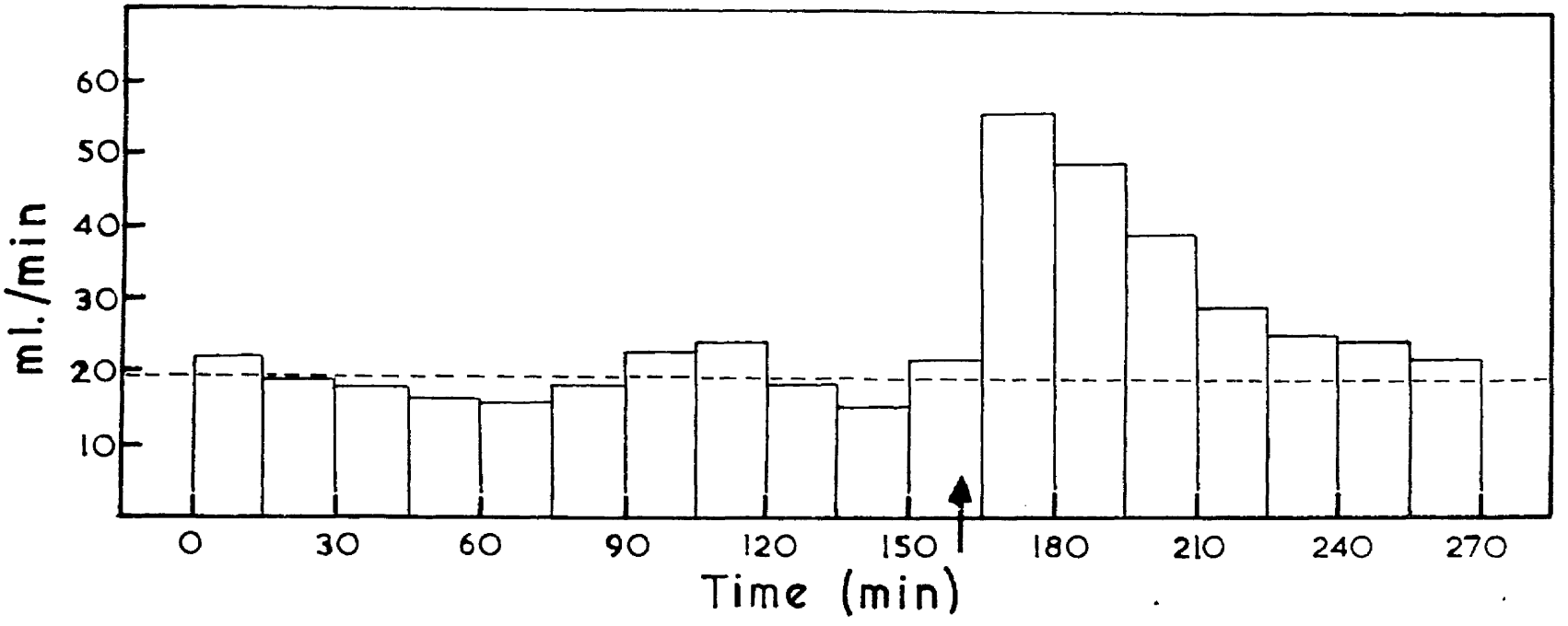

Fig. 28. Diuretic response to acetazolamide (Mean of seven experiments with an identical time schedule). 2.0 g of the drug were injected intravenously at the arrow. The horizontal broken line indicates the overall mean pre-dosing rate of urine flow (18.9 ml./min).

elevated at 200 - 300% of the pre-dosing rates in the last clearance period (Fig. 29). Mean values of potassium excretion showed a maximum increase to approximately twice the pre-dosing rate, while chloride excretion showed no increase, but fell to approximately half its pre-dosing rate (Fig. 29). Urine pH rose progressively after administration of the drug (Fig. 29).

Plasma potassium concentrations

No change in plasma sodium concentrations was detected after dosing but in all experiments plasma potassium fell slightly. Mean values, plotted separately for each animal, are shown in Fig. 30. The theoretical decline in plasma potassium concentrations, as predicted from the observed increases in potassium excretion and shown by the broken lines in Fig. 30 was greater than the actual decline. The calculation of these theoretical values assumed that for each animal the mean rate of excretion of potassium before administration of the drug represented a steady rate of loss from the extracellular fluid, equal to the rate of absorption of potassium from the gut. Cumulative losses of potassium in the urine in excess of the pre-dosing rate were measured from the graph of potassium excretion against time and were subtracted from the figure for total extracellular potassium, calculated as the product of the inulin space and the plasma potassium concentration before dosing. The figures so obtained for depleted total extracellular potassium were then divided by inulin space values to give theoretical concentrations of plasma potassium after dosing. Details of these calculations for one of the animals are given below.

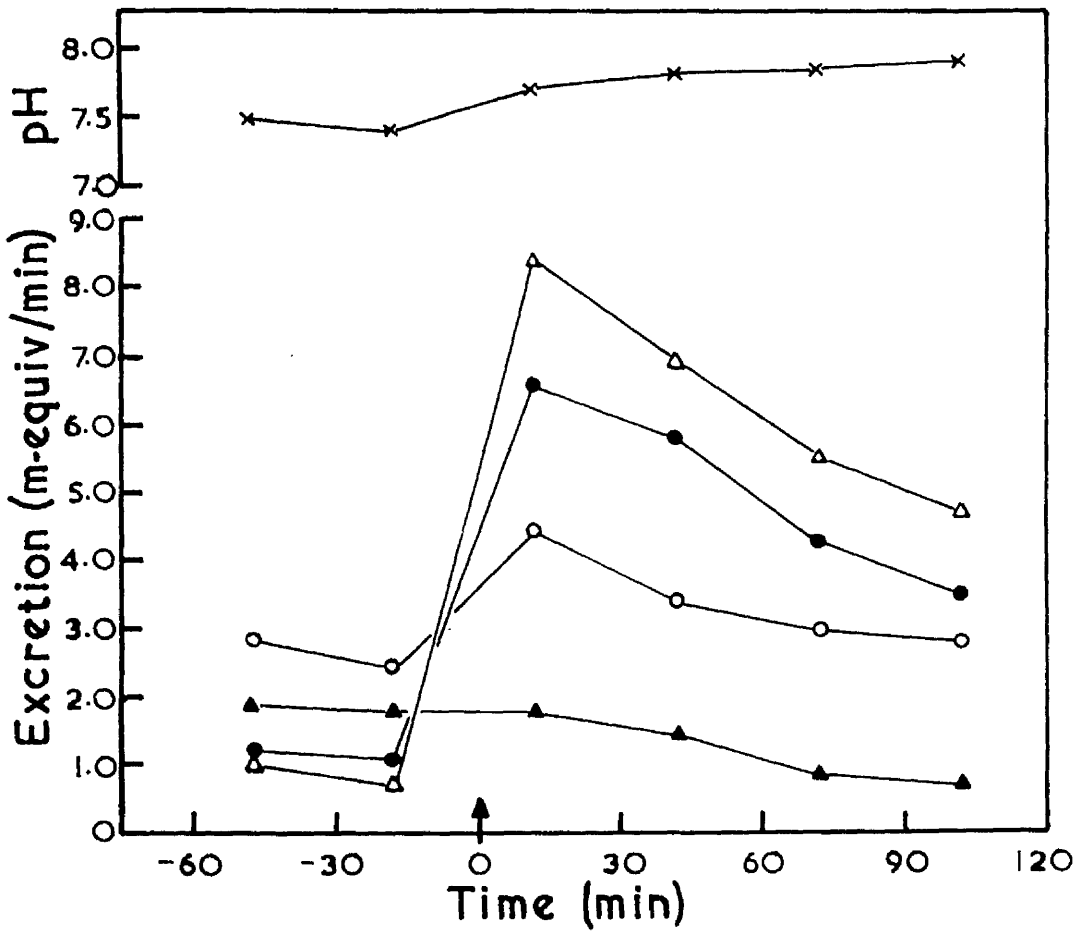

Fig. 29. Effect of acetazolamide on urinary pH (×——×), and on the rates of excretion of sodium (●——●), potassium (o——o), chloride (▲——▲) and bicarbonate (△——△): mean values from seven experiments with an identical time schedule. 2.0g acetazolamide were injected intravenously at time 0.

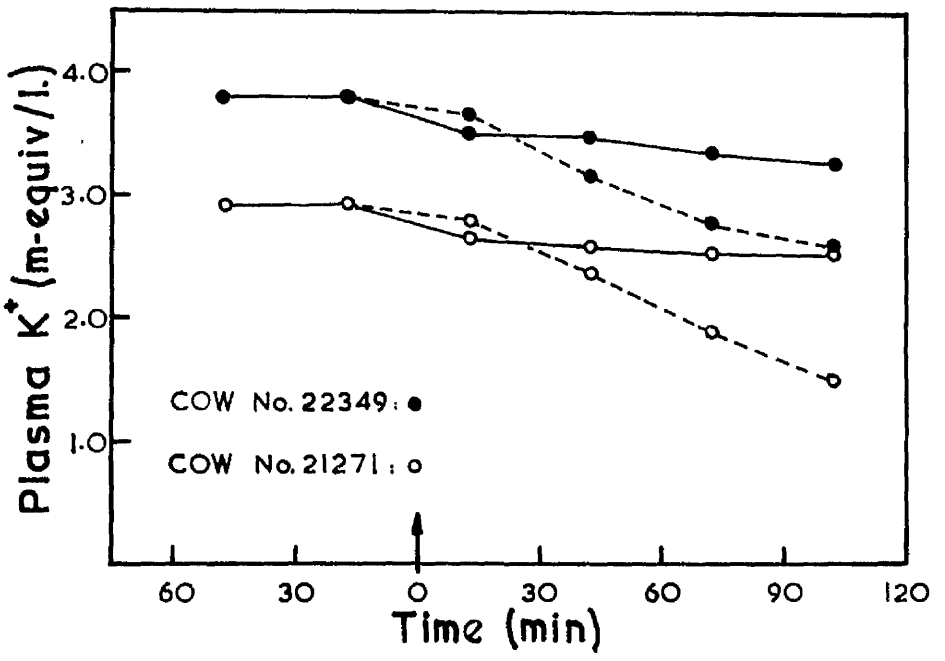

Fig. 30. Effect of acetazolamide on plasma potassium concentration (mean values). Points on the broken lines represent theoretical concentrations calculated from mean increases in potassium excretion observed after dosing (see text). 2.0 g acetazolamide were injected intravenously at time 0.

Calculation of theoretical plasma $[K^+]$: Cow No. 22349

Mean pre-dosing plasma $[K^+]$, P : 3.8 m-equiv/l.

Mean pre-dosing inulin space, S : 68 l.

Total extracellular K, before dosing,

PS : 258.4 m-equiv

Mean pre-dosing excretion rate : 3.066 m-equiv/min

Interval after dosing (min) :	20	50	80	110
Cumulative excretion above pre-dosing rate, E (m-equiv) :	11.2	47.9	61.7	66.5
Total extracellular K^+, PS-E (m-equiv) :	247.2	210.5	196.7	191.9
Inulin space, S (l.) :	67	65	70	74
Theoretical plasma $[K^+]$, $\frac{PS-E}{S}$ (m-equiv/l.) :	3.7	3.2	2.8	2.6

Inulin space

An overall mean value of 14.6 ± 3.6 l./100 kg body weight was found for the volume of distribution of inulin. Wide variations were noted, however, between values obtained for the same animals on different days, although the successive estimates made in each experiment showed much closer agreement. These findings are fully described elsewhere (p. 90). The mean successive values of inulin space for the two animals ranged from 63 to 74 l. and from 58 to 71 l. respectively (Fig. 31); these mean values were used in the calculations of theoretical concentrations of plasma potassium described above.

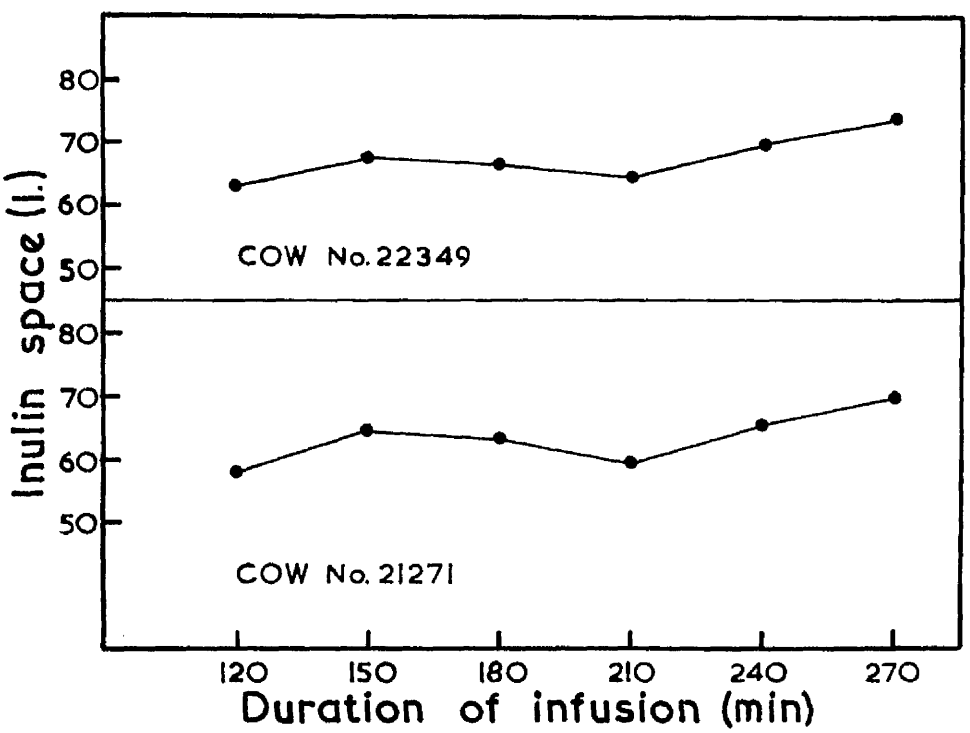

Fig. 31. Successive estimates of inulin space during constant infusion of inulin. For each animal are plotted mean values of four experiments with an identical time schedule.

pH and bicarbonate concentration of arterial blood

In the four experiments in which arterial blood samples were obtained, no change was detected in the pH of samples taken 45 min and 105 min after administration of the drug. The bicarbonate concentration of the arterial plasma, however, fell by 2 - 3 m-equiv/l. to 89 - 93% of the mean pre-dosing value (Table 46).

Glomerular filtration rate and renal plasma flow

Both inulin and PAH clearance values fell after administration of the drug. The maximum reductions, expressed as percentages of the pre-dosing value, ranged from 26% to 45% for inulin and from 14% to 34% for PAH. Mean values for each animal showed that maximal reductions occurred within 1 hr of dosing, with the reduction of inulin clearance 10 - 15% greater than that of PAH (Fig. 32). In one animal (No. 22349), however, this difference in the degree of depression was not sustained and during the last clearance period inulin and PAH clearances were both approximately 85% of the pre-dosing value (Fig. 32).

The filtration fraction (inulin clearance/PAH clearance) showed reductions which reflected the difference in the degrees of depression of these clearance values. In both animals filtration fraction fell from 0.15 - 0.16 to 0.12 - 0.13 within 15 min of dosing (Fig. 33). Whilst this depression persisted in one animal, in the other, filtration fraction returned to its pre-dosing value, since in this animal (No. 22349) the degree of depression of the two clearance values became equal as the experiment continued (Fig. 32).

TABLE 46

Effects of acetazolamide on arterial blood pH and plasma HCO_3

Sample	Arterial pH (at 38°C)	Total CO_2 (mM/l.)	HCO_3 (m-equiv/l.)
Cow No. 22349			
pre-dose	7.41	29.2	27.9
D + 45	7.41	26.2	25.0
D + 105	7.41	26.1	24.9
pre-dose	7.42	28.5	27.2
D + 45	7.41	27.6	26.3
D + 105	7.41	27.4	26.1
Cow No. 21271			
pre-dose	7.44	34.5	33.1
D + 45	7.44	32.4	31.2
D + 105	7.44	32.2	30.9
pre-dose	7.44	36.8	35.2
D + 45	7.44	35.7	34.2
D + 105	7.44	34.4	33.0

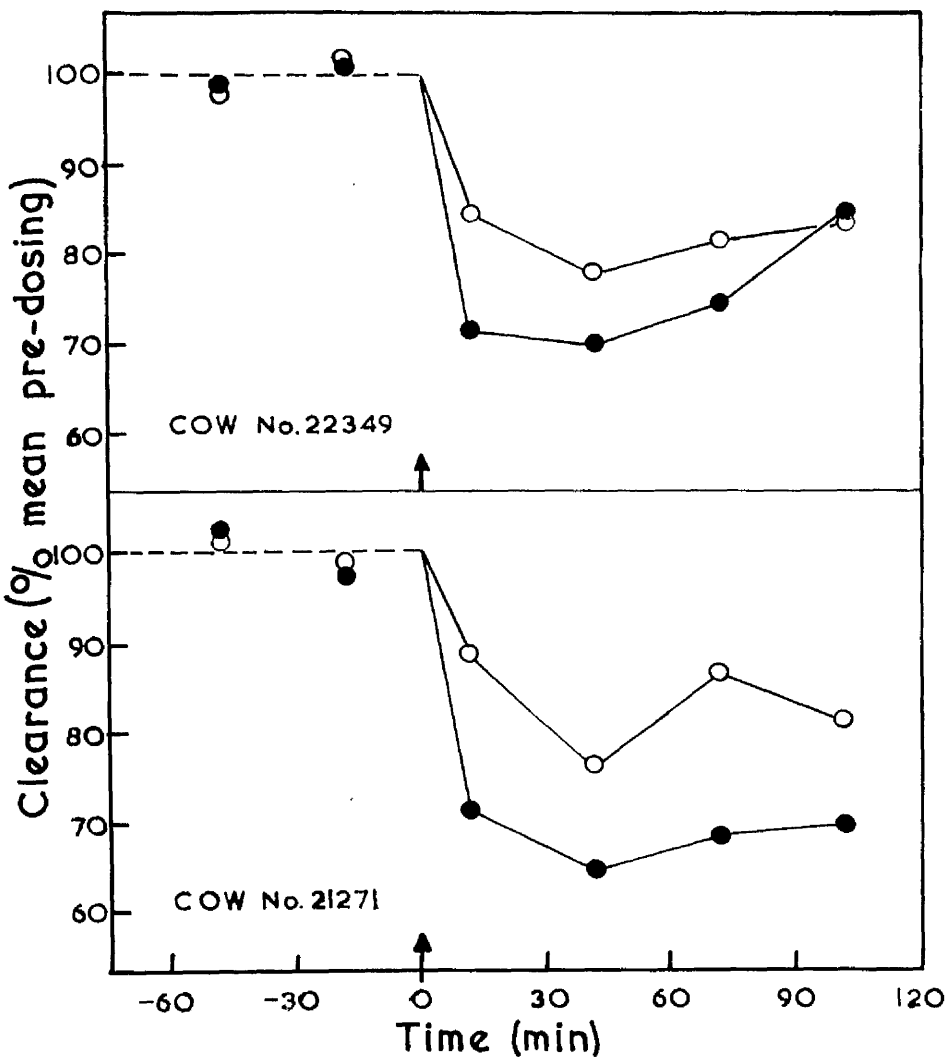

Fig. 32. Effect of acetazolamide on inulin (●——●) and PAH (o——o) clearances. The mean clearance values plotted are expressed as percentages of the respective mean pre-dosing figures. 2.0g acetazolamide were injected intravenously at time 0.

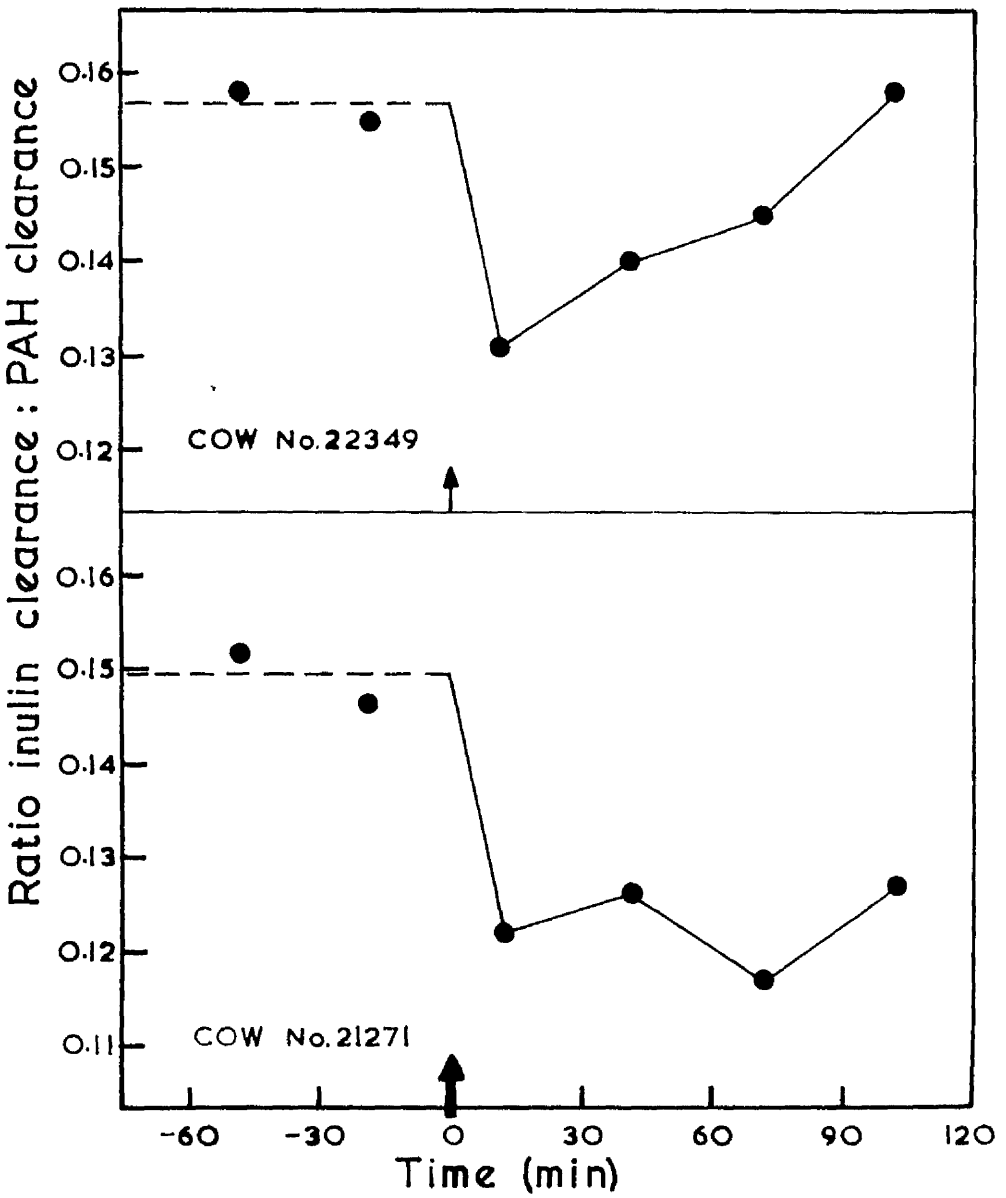

Fig. 33. Effect of acetazolamide on filtration fraction (mean values). 2.0 g acetazolamide were injected intravenously at time 0.

Discussion

The present findings confirm the effects of acetazolamide on the rate of urine flow and electrolyte excretion described in the preceding section. The values obtained for inulin space are discussed in full in an earlier section (p. 93). Although wide variations were found between determinations of the inulin space made on different days, the overall mean values for each animal were similar to those reported in cattle by other workers, and were used as a measure of the extracellular fluid volume in calculating theoretical reductions of plasma potassium from observed increases in the rate of excretion.

In these calculations potassium concentrations were assumed to be the same in interstitial fluid as in plasma. No allowance was made for lower concentrations in interstitial fluid due to the Donnan effect arising from the relative impermeability of the capillary walls to plasma protein. Assuming a normal concentration of plasma proteins, the concentration of a univalent cation in interstitial fluid may be calculated as 95% of its concentration in plasma water (Berliner, Kennedy and Hilton, 1950). However, this factor was not used in the present work since plasma volumes were not measured and, in calculating the total extracellular potassium, the extracellular volume was not partitioned into intra- and extra- vascular compartments. Furthermore, if allowance were made for the Donnan effect, although slightly lower estimates of total extracellular potassium would be obtained, theoretical plasma concentrations predicted from observed increases in potassium excretion would be little different from those plotted in Fig. 30 . These values showed that the hypokalaemia seen after administration of acetazolamide was

wholly accountable by the observed kaliuresis. In fact the observed falls in plasma potassium concentration were much less than were predicted from the increased excretion, which suggests that the increased losses of potassium from the extracellular fluid were in part replaced by an increased uptake, either from intracellular fluid, which would involve some redistribution of the total body potassium, or by increased absorption from the alimentary tract.

The great increase in bicarbonate excretion seen after administration of acetazolamide was accompanied by an increase in urinary pH, but, within 2 hr of dosing, no change was recorded in the pH of arterial blood; and, although the concentration of bicarbonate in arterial plasma fell by 2 - 3 m-equiv/l., the values found after dosing still lay within the normal range for cows of 22 - 33 m-equiv/l. (Anderson and Pickering, 1962). Thus no disturbance of acid-base equilibrium was demonstrable after a single dose of acetazolamide, but the possibility remains, in view of the effects of the drug, that metabolic acidosis and hypokalaemia might develop in the cow if treatment were prolonged, as has been reported in man (Chart et al., 1959).

A fall in glomerular filtration rate after the intravenous administration of acetazolamide has been described in man (Maren, Mayer and Wadsworth, 1954) and in the dog (Berliner, Kennedy and Orloff, 1951; Madsen, 1954). The fall observed in the present work involved some reduction in the filtration fraction since inulin clearance declined from between one sixth and one seventh to about one eighth of the PAH clearance. However, a concurrent reduction of PAH clearance (renal plasma flow) was also noted, which may be a more important factor. This is suggested by the results for one of the animals which showed that the

filtration fraction had returned to normal values, 2 hr after dosing (Fig. 33), although at this time PAH clearance was still depressed by 15%, as also was the glomerular filtration rate (Fig. 32). There is no evidence that carbonic anhydrase is involved in the tubular transport mechanism for PAH (Lotspeich, 1959) so that these observed reductions in PAH clearance were probably true indications of changes in renal plasma flow rather than a consequence of reduction in the percentage extraction of PAH caused by interference from acetazolamide in tubular transport mechanisms.

Madsen (1954), working with dogs, attributed the reduction in filtration rate seen after intravenous acetazolamide to an observed fall in arterial blood pressure. Such an extra-renal influence of the drug might account for the reductions of renal plasma flow and glomerular filtration rate observed in the present work. Further work is required to establish whether a fall in blood pressure occurs in the cow after acetazolamide, and, also, whether the autoregulatory activity of the kidney is affected by the drug. It has been shown in the dog that glomerular filtration rate and renal plasma flow are little affected by changes in perfusion pressure over a range of 80 - 180 mm Hg (Shipley and Study, 1951), whereas the fall in blood pressure noted by Madsen (1954) was only 10 - 15 mm Hg.

SUMMARY

AND

CONCLUSIONS

The scarcity of studies on bovine kidney function prompted the present work, in which the renal responses of the cow to various experimental procedures were studied and compared with those of other species. The excretion of sodium and potassium was studied in particular, in view of the differences in the dietary intake of these elements when compared with that of man and the dog.

The historical development of modern concepts of renal function has been reviewed in relation to the contributions made by glomerular filtration and renal tubular activity to the excretion of these elements.

The measurement of sodium and potassium concentrations in body fluids by flame photometry has been investigated and the need for correction of mutual interference effects emphasised by the finding that maximal interference could give rise to errors which were at least 5% of the true reading. Interference became maximal when the molar ratio of interfering to test element reached 8:1 for potassium readings and 4:1 for sodium readings, and was unaffected by further increases in these ratios. Allowance for these effects was made by using calibration curves plotted from readings obtained for the same range of concentrations of the test element in the presence of increasing proportions of the interfering element. The concentration of the test element in a sample undergoing analysis was read from the point on the pencil of calibration curves which corresponded to the concentration ratio (K : Na) of the sample, calculated directly from the instrument readings for potassium and sodium. This method of correcting for mutual interference was less tedious in application than those described by previous workers and effectively overcame the difficulties encountered in the analysis of urine in which, unlike plasma, the approximate concentration ratio

of interfering to test element cannot be predicted before analysis, and may alter considerably as a result of experimental procedures. The method will therefore be applied in the continuation of the studies begun in this thesis. Procedures described by previous workers were used in determinations of chloride, pH, total CO_2 and bicarbonate, inulin, p-amino hippurate, and plasma protein.

Methods of urine collection used in the cow have been reviewed and attention drawn to the difficulties encountered in making accurate collections over short periods of time. These had not been emphasised by previous workers and the technique developed for continuous collection using an indwelling urethral catheter under posterior epidural anaesthesia was therefore described in detail. The accurate collection of urine is a crucial pre-requisite of any investigation involving clearance procedures, and this technique will prove valuable in the further studies contemplated on bovine renal function. In addition the technique could readily be applied to the investigation of clinical conditions by clearance procedures. The need for careful control of the experimental conditions was shown during 10 min serial collection periods in which increases in urine flow and electrolyte excretion were observed, associated with disturbances in the experimental room or with signs of discomfort from the presence of the urethral catheter.

The measurement of glomerular filtration rate and renal plasma flow have been discussed and the procedures described which were developed for the determination of clearances of inulin and p-amino hippurate. The scarcity of information on these procedures in cattle was reflected in the long time spent on this part of the work, but the information given by these procedures is of such importance to studies of kidney function that the techniques developed will

certainly be used in further investigations in cattle, and possibly in other farm animals. Their use in special examinations of clinical cases is also clearly feasible.

In 103 determinations on seven cows, inulin clearance was 1,100 ± 236 ml./min/500 kg. The repeatability of the method was found to be within the accepted limits of accuracy for renal clearance studies, but occasional day-to-day variations in the filtration rate of the same individual were recorded. In 14 determinations on two cows, PAH clearance was 7,984 ± 1,210 ml./min/500 kg. The filtration fraction calculated from simultaneous determinations of PAH and inulin clearance in these two animals had a mean value of 0.16. This was in close agreement with the figure calculated from clearances of diodrast and inulin (Poulsen, 1957) and from values of PAH and inulin clearance given by Vogel (1959) and Ketz (1960). It was pointed out, however, that Vogel (1959) and Ketz (1960) expressed PAH and inulin clearance values as ml./min/sq m body surface, which, when recalculated as ml./min/500 kg body weight, were less than half of the clearance values found in the present work. It was suggested that this difference might have arisen in part through the use of different formulae to relate body weight to surface area, since the authors did not specify how the surface area of these animals had been calculated. Unfortunately, repeated attempts to contact these authors and so determine what method was used, were unsuccessful, so that further clarification of this anomaly was not possible.

Methods available for the measurement of extracellular fluid volume have been discussed and in eight experiments on two cows, 48 determinations of the volume of distribution of inulin were made by the 'difference method'. The overall mean value found for each animal was 13.5 ± 3.5% and 15.7 ± 4.0% respectively (l./100 kg body weight), which were similar to those reported by previous workers

using single injection techniques.

The values of inulin space found in the same animal on different days, however, showed much greater variation than did the successive values obtained in each experiment. After discussing possible causes of variation it was concluded that the differences between days appeared to represent true day-to-day differences in the volume of distribution of inulin, perhaps arising from day-to-day differences in the patency of some capillary beds in the animals studied. It was thought unlikely that these large variations reflected similar changes in the volume of extracellular fluid. The results of experiments using more readily diffusible marker substances, such as sucrose, mannitol, thiosulphate or radiosulphate would be of interest in view of the poor repeatability shown by the estimations of inulin space.

Analysis of human and bovine urine samples showed that the potassium:sodium concentration ratio was on average twelve times greater in bovine than in human urine as a result of presumed differences in, primarily, the dietary intake of these elements. The capacity of the bovine kidney to maintain high excretory rates of potassium has been investigated by clearance studies carried out on three cows before and during intravenous infusion of potassium chloride. During infusion, plasma potassium concentrations increased by 1 - 2 m-equiv/l. and then were maintained around this level. The rate of excretion of potassium rose to equal, approximately, the rate of infusion, and in all cases the clearance of potassium rose to exceed that of inulin. At the same time the sodium excretion and the rate of urine flow showed a parallel increase which declined as infusion continued but persisted above pre-infusion rates.

The possible renal mechanisms involved in these responses were

discussed and, in comparing these responses with those described in the dog by previous workers, it was pointed out that the dog showed a similar ability to tolerate intravenous loading with potassium only if a supplement of a potassium salt had been fed over a period before the intravenous infusion; and that this ability to tolerate intravenous loading appeared to involve the secretory capacity of the distal tubules. However the physiological mechanism initiating and maintaining the tolerant state was not clear. Although no evidence was available which indicated that adrenocortical activity was increased, this possibility merits investigation in view of the known effects of aldosterone and other mineralocorticoids on potassium and sodium excretion. The renal responses of the cow to drugs such as S U 4885 ('Metopirone', CIBA) which block steroid synthesis, or to those which block the effects of steroids on the kidney, such as spirolactones, might provide relevant information.

The renal responses to acetazolamide and hydrochlorothiazide have been compared in twelve experiments on three cows. Both drugs increased the rate of urine flow and electrolyte excretion. Hydrochlorothiazide promoted increased urinary losses of sodium and chloride while acetazolamide increased the excretion of sodium and bicarbonate. Potassium excretion was unaffected by hydrochlorothiazide but was slightly augmented by acetazolamide. Urine pH rose after acetazolamide and fell after hydrochlorothiazide. No change of electrolyte concentrations in plasma was seen after hydrochlorothiazide but potassium levels fell slightly after acetazolamide. Inulin clearance values fell by about 40% after this drug but were unaltered by hydrochlorothiazide. Maximal effects were obtained within 30 min of a single dose of either drug, then declined rapidly, but responses were still apparent 2 hr after

dosing. Infusion of acetazolamide did not sustain the initial response.

The diuretic action of both compounds apparently resulted from reduction in the tubular reabsorption of electrolytes. It was concluded that hydrochlorothiazide caused reductions in the reabsorption of sodium and chloride in proximal tubules, and had no effect on excretory mechanisms for potassium. The effects of acetazolamide on electrolyte excretion were attributed to the restriction of the supply of hydrogen ions in renal tubular cells resulting from inhibition of carbonic anhydrase. In contrast to its reported effects in man and the dog this drug did not give rise to large increases in potassium excretion - an observation which illustrated a difference in emphasis of mammalian renal mechanisms in the cow. Acidification of the urine is thought to occur in distal regions of the nephrons, where potassium and hydrogen ions are believed to compete for secretion; before dosing, acidification of the urine in the cow was not so pronounced as in man and the dog. However, this weak kaliuretic response to acetazolamide was apparently sufficient to account for the observed reductions in plasma potassium concentration since the increases in potassium excretion were greater than theoretical increases calculated to be required to lower the potassium concentrations to the plasma levels observed.

The responses to a single dose of acetazolamide were further studied in seven experiments on two cows. The effects of observed increases of potassium excretion on potassium concentrations in plasma were calculated using values of inulin space as a measure of the extracellular fluid volume. The calculations showed that the hypokalaemia seen after administration of acetazolamide was wholly accountable by the observed kaliuresis since the observed falls in plasma potassium concentration were less than those pre-

dicted from the increased excretion.

No change was recorded in the pH of arterial blood samples taken within 2 hr of dosing, and although the bicarbonate concentration of arterial plasma fell by 2 - 3 m-equiv/l., the values found still lay within the normal range for cattle described by earlier workers. Thus no disturbance of acid-base equilibrium was demonstrable after a single dose of the drug, but, in view of the effects shown, it was concluded that metabolic acidosis and hypokalaemia might nevertheless develop in the cow if treatment with acetazolamide were prolonged.

Measurement of the clearance of p-amino hippurate and inulin showed that the fall in glomerular filtration rate seen after administration of acetazolamide involved reductions of both filtration fraction and renal plasma flow. Since a fall in systemic arterial blood pressure has been reported in the dog after acetazolamide administration, it was concluded that further work should determine whether this effect occurred in cattle, and investigate its possible contribution to the observed reductions in glomerular filtration rate and renal plasma flow.

Further work proposed to extend the findings described in this thesis falls into two main categories.

First, clarification of the results of the potassium-loading experiments will be sought by a study of the responses of the cow to administration of the mineralocorticoid antagonist spironolactone. It has been suggested that this drug exerts its diuretic effect in man and the dog by inhibiting the sodium-retaining action of aldosterone on the renal tubules (Liddle, 1958), but it is more probable that other steroids which affect electrolyte excretion are also inhibited since the sodium-retaining action of cortisol has been shown to be blocked nearly as effectively as that of

aldosterone (Mills, 1962).

Investigation of the responses of normal cattle to this drug, in experiments similar to those in which the responses to acetazolamide and hydrochlorothiazide were studied should demonstrate any tonic influence of mineralocorticoids on potassium and sodium excretion. Comparison of the findings with those reported in man and the dog may provide evidence of relative differences in mineralocorticoid activity which arise from the differing requirements of these species for sodium conservation and potassium excretion. The effects of prior administration of spironolactone on the renal response to intravenous infusion of potassium chloride will also be studied in order to establish whether increased activity of the adrenal cortex is involved in the phenomenon of 'potassium tolerance'.

Second, the clearance techniques developed in the present work will be utilised to investigate the influence of differences in the dietary intake of protein and of salt on the urinary excretion of urea.

The classical view of the mechanism of urea excretion by the kidneys is based essentially on a presumed indifference of the renal tubules to urea presented in the glomerular filtrate. The reduction in the volume of the filtrate which occurs during its transformation into urine results in the urea concentration becoming greatly increased. In consequence some urea diffuses from the tubular urine back into the blood stream so that urea clearance values are always less than inulin clearance values. This reabsorption is presumed to be a passive process so that the fraction of filtered urea which is excreted is mainly influenced by the rate of urine flow since this determines the concentration gradient promoting the back diffusion. This theory, however, has been questioned by

Schmidt-Nielsen (1958) in a review describing various anomalies and inconsistencies in a variety of mammalian species. In particular the fraction of filtered urea which appeared in the urine was seen in most cases to be reduced when comparing animals on a low protein diet, but with a similar rate of urine flow, to those on a high protein diet. This suggests that the renal tubules either actively reabsorb urea, or vary their permeability to urea in a highly selective manner. In the ruminants studied (the sheep and camel) this effect of the dietary intake of protein on the fraction of filtered urea excreted was qualitatively the same as in simple-stomached animals but was quantitatively much more dramatic.

This finding has special significance when the ability of ruminal micro-organisms to utilise urea in the synthesis of bacterial protein is considered. It is well known that urea enters the rumen both by diffusing from the blood stream across the ruminal epithelium and also in the large volumes of saliva secreted by the ruminant. Thus, by returning a proportion of its own nitrogenous waste to the rumen for utilisation in microbial protein synthesis the dietary intake of the animal receives a supplement of protein of high biological value when ruminal micro-organisms pass on and are digested in the abomasum and small intestine. The studies on urea excretion (Schmidt-Nielsen, 1958) suggest that this recycling of urea is facilitated or encouraged by renal tubular reabsorption restricting urinary losses of urea, especially when the dietary intake of protein is low. This feature of kidney function presumably has become more pronounced in ruminants in the process of evolution since the reconstitution of nitrogenous waste to protein by microbial synthesis in the rumen has an obvious survival value when food intake is of poor quality.

The intake of sodium chloride can also influence the excretion of urea since the addition of salt to a low protein diet has been shown in rats to result in greater conservation of urea than occurred on the low protein diet alone (Truniger and Schmidt-Nielsen, 1964).

There have been no reports of the effects on urea excretion of diets of differing protein content in cattle, and investigations in sheep (Schmidt-Nielsen and Osaki, 1958) did not attempt to demonstrate any influence of the salt intake. The clearance techniques of the present study will therefore be used to define the renal responses of the cow to these influences by determining the fraction of filtered urea which is excreted whilst feeding diets which provide different intakes of nitrogen and sodium chloride. The responses to water diuresis and to intravenous infusion of urea will also be studied.

The results of these experiments will be of interest not only for the emphasis they might give to the need for revision of the classical concepts of urea excretion, but also by defining quantitatively how renal conservation of urea might contribute to the nitrogen economy of the cow.

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